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EFFECTS ON MONOMETHYLHYDRAZINE ON BLOOD AND CEREBROSPINAL FLUID GLUCOSE IN ANESTHETIZED MONKEYS

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The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," 1965 prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

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FOREWORD

This report presents research performed by the Toxicology Branch, of Toxic Hazards Division, of the Aerospace Medical Research Laboratory. The research was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202, "Diagnostic and Therapeutic Procedures Protection Against Exposure to Air Force Toxic Materials. "The study was presented, in part, at the Tenth Annual Meeting of the Society for Toxicology, Washington, D. C., March 12, 1971. The authors acknowledge the advice and assistance of Major Richard Bradbury, DVM, and Lieutenant Colonel Dale Boyd, DVM, of the Veterinary Medicine Division.

This technical report has been reviewed and is approved.

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INTRODUCTION

Although the propellant hydrazines have been extensively investigated in recent years, the relationship between their effects on glucose metabolism and their ability to produce convulsions remain controversial. This is particularly true for monomethylhydrazine (MMH). When MMH was administered to anesthetized dogs (Fortney and Clark, 1967), blood glucose decreased and convulsions appeared to occur simultaneously with the hypoglycemia. This suggested that the convulsions following MMH are due to the hypoglycemia with deprivation of substrate glucose to the brain, similar to that occurring, for example, in insulin shock.

If this were the case, the resulting decrease in brain glucose should also be reflected in cerebrospinal fluid since this is a better representative of brain extracellular fluid than is blood (Davson, 1967). We, therefore, carried out the following experiments in anesthetized rhesus monkeys in an attempt to correlate the convulsigenic action of MMH with its effect on cerebral glucose metabolism.

METHOD

Animals. Juvenile female rhesus monkeys 1 1/2 - 2 years of age weighing 2.5-3 kg were utilized. Food, but not water, was withheld 18 hours prior to use. Temperature was controlled with a heating blanket between 37-37.5C.
MMH. Dilute solutions of monomethylhydrazine were prepared at the time of use by diluting reagent grade MMH with isotonic saline (Matheson, Coleman and Bell). The usual dose (25 mg/kg) was 0.75 - 1 ml of a 1/10 solution.

EXPERIMENTAL PROCEDURE

The monkeys were anesthetized with sodium thiamylal (30 mg/kg) and maintained at a plane of surgical anesthesia with pentobarbital. Catheters were placed in peripheral arm and leg veins and urinary bladder. Endotracheal intubation was performed also at this time. The monkeys were then placed in a stereotaxic apparatus (Baltimore Company) with their necks flexed 45° and a 20 gage spinal needle inserted into the cisterna magna. Samples of blood and CSF were obtained over a control period of 30 min - 1 hour and MMH was given intravenously over a 5-minute period. Blood and CSF samples were subsequently obtained at half hour intervals.

VENTRICULO-CISTERNAL PERFUSION

In 6 animals, ventriculocisternal perfusion was performed as described previously in cats (Shaywitz et al., 1969). The perfusate consisted of a simulated CSF containing appropriate salt concentrations (Merlis, 1940). By addition of 40 mg percent inulin to the perfusate, the bulk formation and reabsorption of CSF were measured.

ANALYTIC PROCEDURES

Heparinized blood was immediately centrifuged at 4C, the plasma removed and frozen. Samples of CSF were frozen immediately after they were collected and weighed. Glucose in plasma and CSF was determined by the glucose oxidase method (Meites, 1965).

CALCULATIONS

Formation of cerebrospinal fluid was calculated from drainage rates. In later experiments, inulin dilution using ventriculocisternal perfusion was utilized. Formation of CSF was calculated as follows: $Vf = Vi \frac{Ci-Co}{Co}$

Where Vf = formation of CSF in ml/min
Vi = inflow rate of artificial CSF
Ci = concentration of inulin in inflow syringe
Co = concentration of inulin in cisternal effluent

RESULTS

Fasting blood and CSF glucose were obtained from 20 rhesus monkeys, and experiments utilizing MMH were performed on 12 of those animals. Ventriculocisternal perfusions to determine CSF formation were carried out on an additional 6 monkeys.

Fasting blood and CSF glucose values for a group of 20 anesthetized monkeys are shown in figure 1. Blood glucose averaged 48.8 ± 2.5 (S.E.) mg % and CSF glucose averaged 45.1 ± 2.9 mg %. Of interest is the relative similarity between blood and CSF glucose concentrations in the monkey. This ratio of CSF/blood glucose of 0.923 is similar to that found in dogs but differs from that seen in humans (see discussion).

A typical experiment is shown in figure 2. After a baseline period, MMH was given at time 0. In this particular experiment, convulsions occurred approximately two hours after administration of MMH, at a time when blood and CSF concentrations were above fasting levels.

Maximum blood and CSF glucose concentrations after administration of MMH are shown in figure 3. Average blood glucose increased from 48.8 to a maximum of 90.6 ± 8.0 mg %, while average CSF glucose increased from 45.1 to 63.8 ± 5.5 mg %. The CSF/blood ratio decreased from 0.923 before MMH to 0.704 after the intravenous administration of MMH. The effect of MMH on blood and CSF glucose in eight monkeys is shown in figures 4-6. The increase in both blood and CSF glucose is statistically significant ($p < 0.01$) using Wilcox-White rank test (White, 1952). The results are represented as plus of minus the fasting glucose levels in figure 7. It is apparent that most values are above fasting levels. The determinations that are less than fasting levels are relatively few in number and are not markedly below baseline.

Convulsions occurred in 4 of 8 monkeys given MMH. These occurred 120 minutes after the i.v. administration of MMH at a time when both blood and CSF glucose were above fasting levels (table 1).

Formation of cerebrospinal fluid averaged 0.028 ml/min before and 0.035 ml/min after the MMH administration (fig. 8). These formation rates were calculated by drainage and compare well with a rate of 0.034 mi/min obtained in 6 normal monkeys using inulin dilution and ventriculocisternal perfusion. Bulk flow of CSF appears to be relatively unchanged by MMH during the experimental period.

INFUSION EXPERIMENT

In an effort to standardize the endogenous glucose concentration an additional group of experiments were performed. A typical experiment is shown in figure 9. In this procedure, a glucose load (1.5 g/kg) was given intravenously for one hour. Following a control period, MMH was given intravenously. Results of blood glucose in 4 monkeys are shown in figure 10. It is apparent that MMH had an effect similar to that seen in the previously described experiments with a slight increase in blood glucose evident.

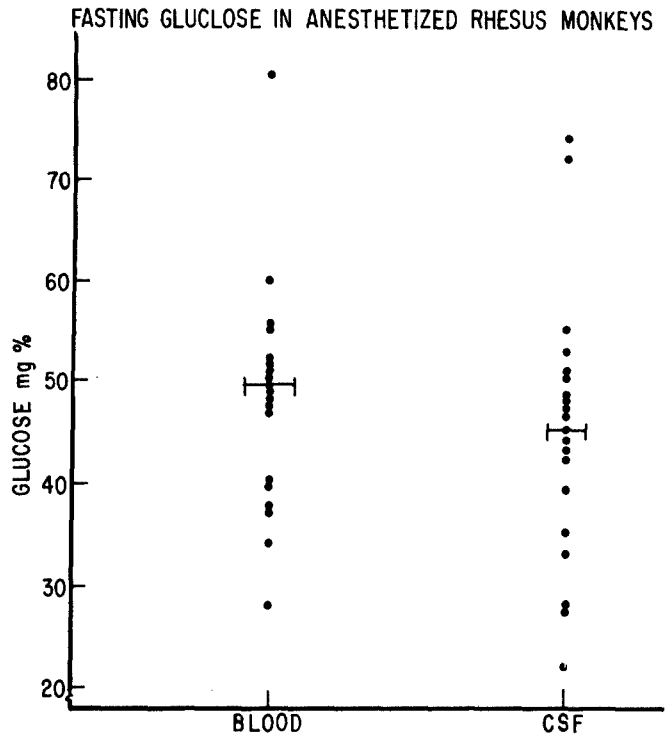

Figure 1. Fasting blood and CSF glucose in anesthetized rhesus monkeys. Each dot represents one animal. Horizontal line is mean.

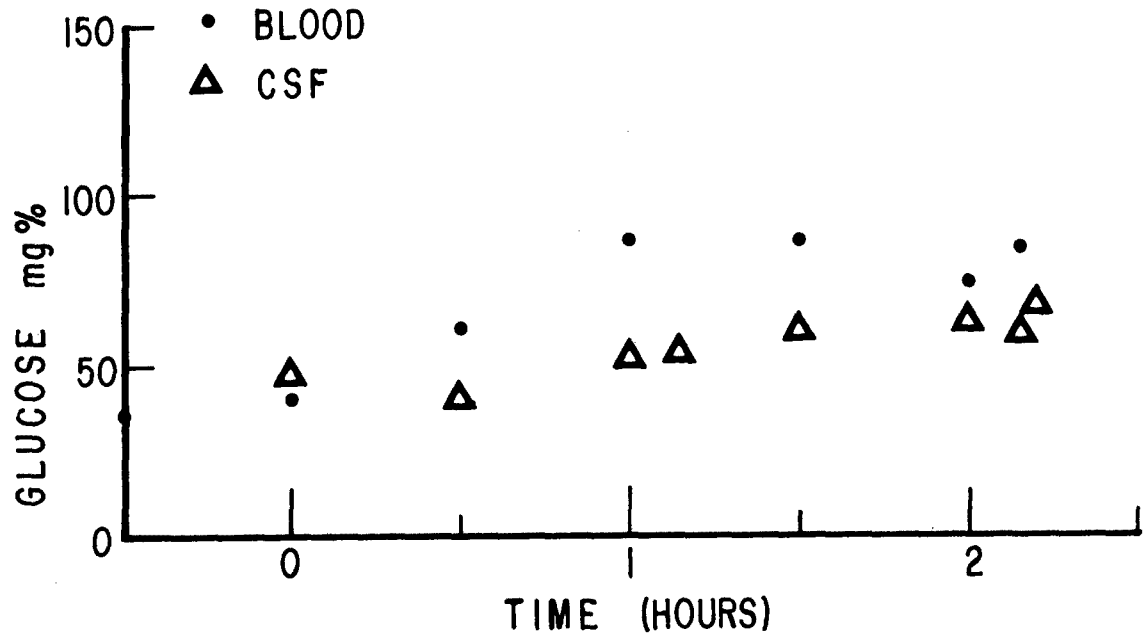

Figure 2. A typical experiment showing blood and CSF glucose after MMH. At 0 time, MMH 25 mg/kg was administered.

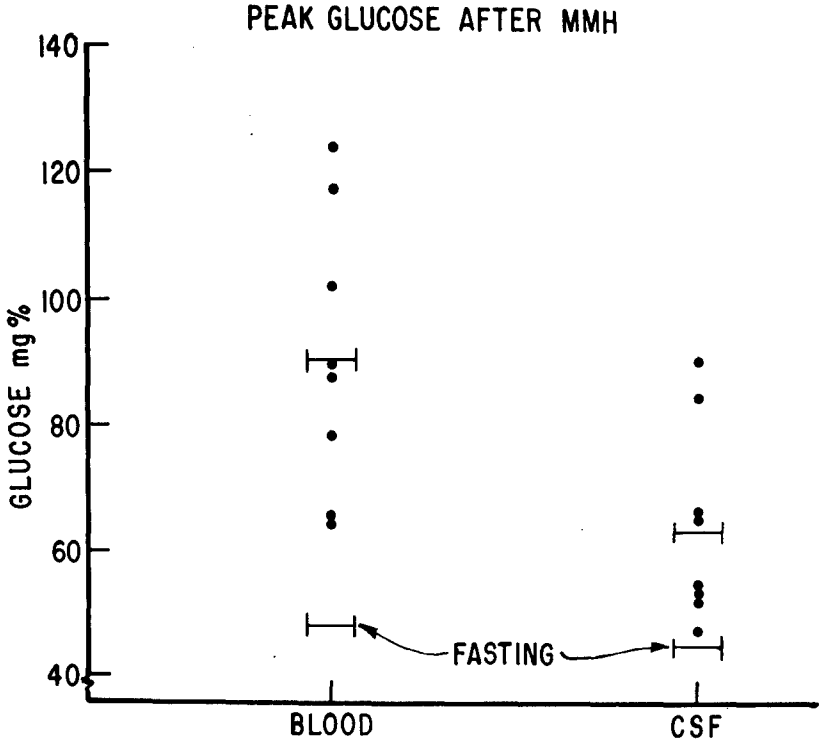

Figure 3. Maximum glucose values obtained in blood and CSF after MMH. Each dot represents a single animal. Horizontal line is mean. "Fasting" represents values from Figure 1.

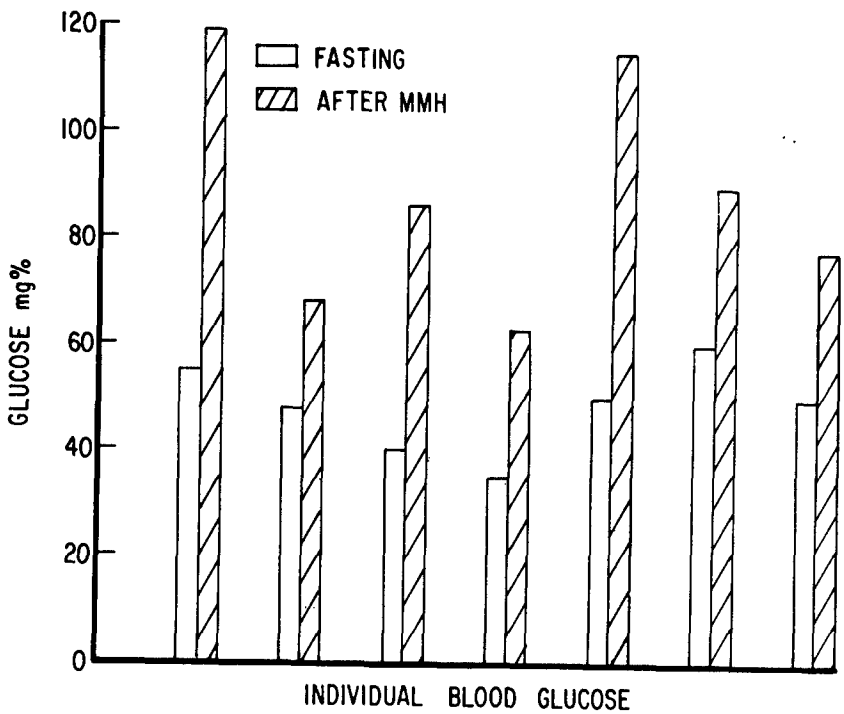

Figure 4. Individual blood glucose before and after MMH.

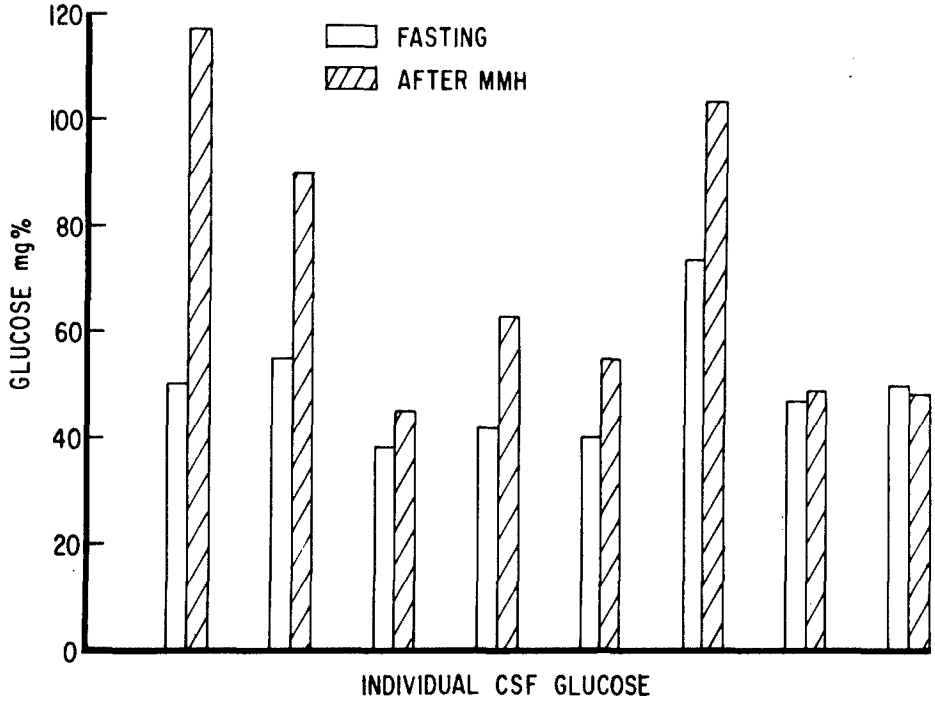

Figure 5. Individual CSF glucose before and after MMH.

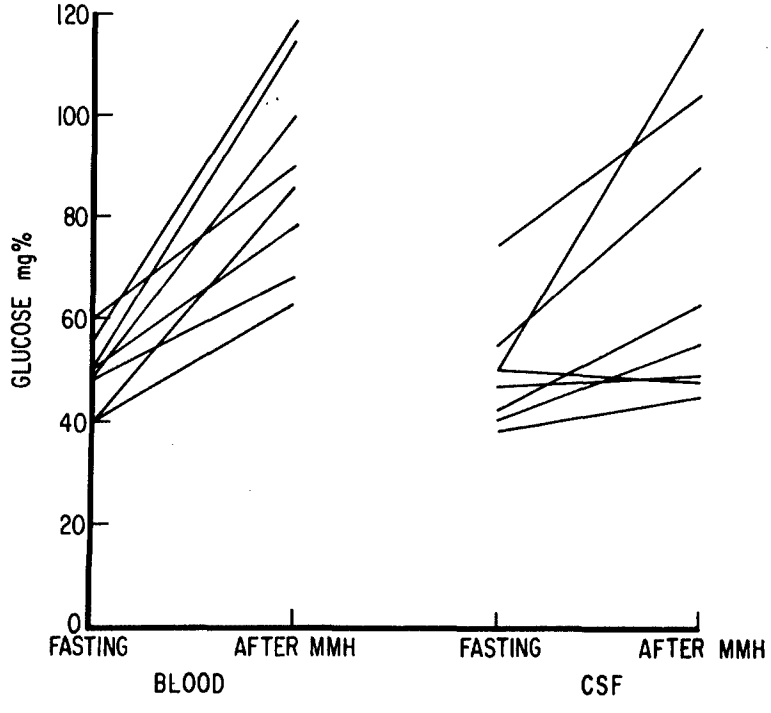

Figure 6. Individual blood and CSF glucose before and after MMH.

DISCUSSION

BASELINE VALUES

Fasting blood glucose values are somewhat lower than have been reported previously (Hall, 1970; Rao and Shipley, 1970). Care was taken to quickly separate plasma from red cells and freeze plasma and CSF thus avoiding artificial lowering of glucose due to in vitro glycolysis. It should be noted that all experiments were begun approximately 90 minutes from the time the monkeys were caught, anesthetized, brought to our laboratory and prepared for the experiment. We followed serial blood glucose values in several animals and noted values of 60-70 mg% before anesthesia which rose within 15-30 minutes after surital and pentobarbital. Coincident with beginning the experiment, the blood glucose had fallen to values of 50 mg% reported here. In several monkeys, chloralose anesthesia was used and similar effects on blood glucose were obtained.

The fasting CSF glucose value of 45 mg% is also lower than reported by Fankhauser (1954). However, this author reported lumbar CSF rather than cisternal CSF studied by us. In dogs, cisternal CSF has lower glucose levels than does lumbar CSF (Fishman, 1964) and this difference in CSF sampling should be noted. The most likely explanation for the slightly lower fasting CSF glucose levels probably reflects the slightly lower fasting plasma glucose levels.

In humans, CSF glucose is approximately 60% of plasma glucose (Merritt and Fremont-Smith, 1937). This presumably reflects additional mechanisms for removal of glucose from CSF then for glucose entry into CSF. This is not the case for the monkey where approximately equal values for fasting CSF and blood glucose were found. Fishman has also noted similar fasting blood and CSF glucose values in anesthetized dogs (Fishman, 1964). The explanation for this apparent species difference between humans on one hand and dogs and monkeys on the other is unknown. However, it may reflect a more complex system for glucose entry and exit in the human CSF system than in other animals.

EFFECTS OF MMH ON GLUCOSE

The effects of MMH on blood glucose concentration have been controversial. Fortney and Clark noted a decrease in glucose in anesthetized dogs occurring simultaneously with convulsions. Dost et al. (1971) studied effects of MMH in anesthetized rats and noted a general increase in blood glucose similar to that noted in rats given much larger amounts of MMH (O'Brien et al., 1964).

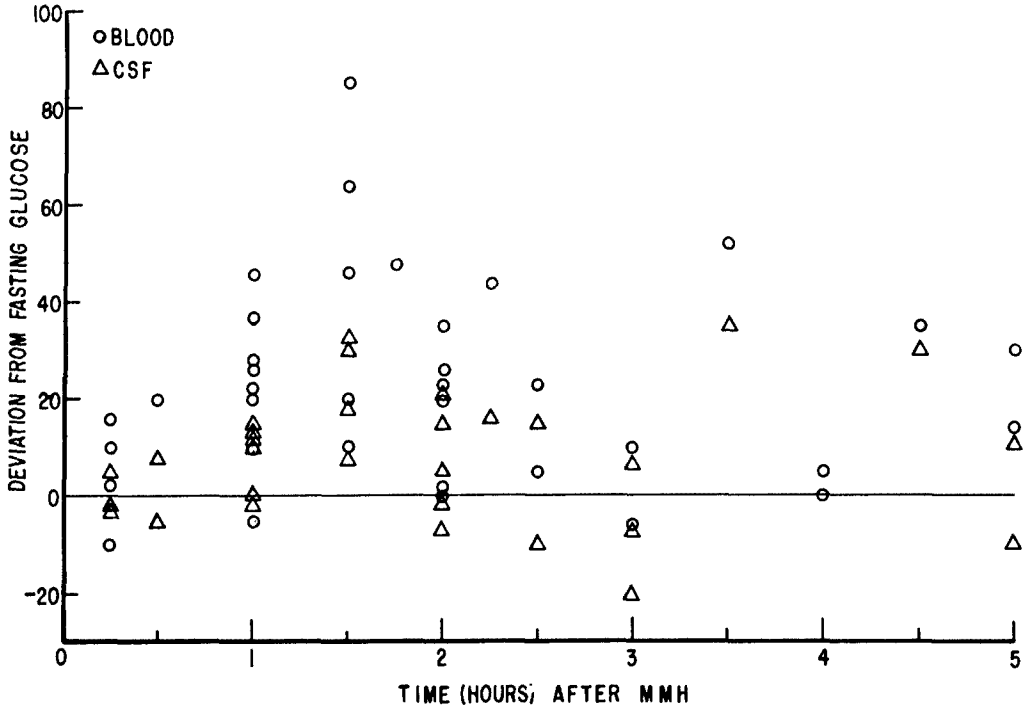

Figure 7. Blood and CSF values after MMH represented as ± fasting glucose in mg %.

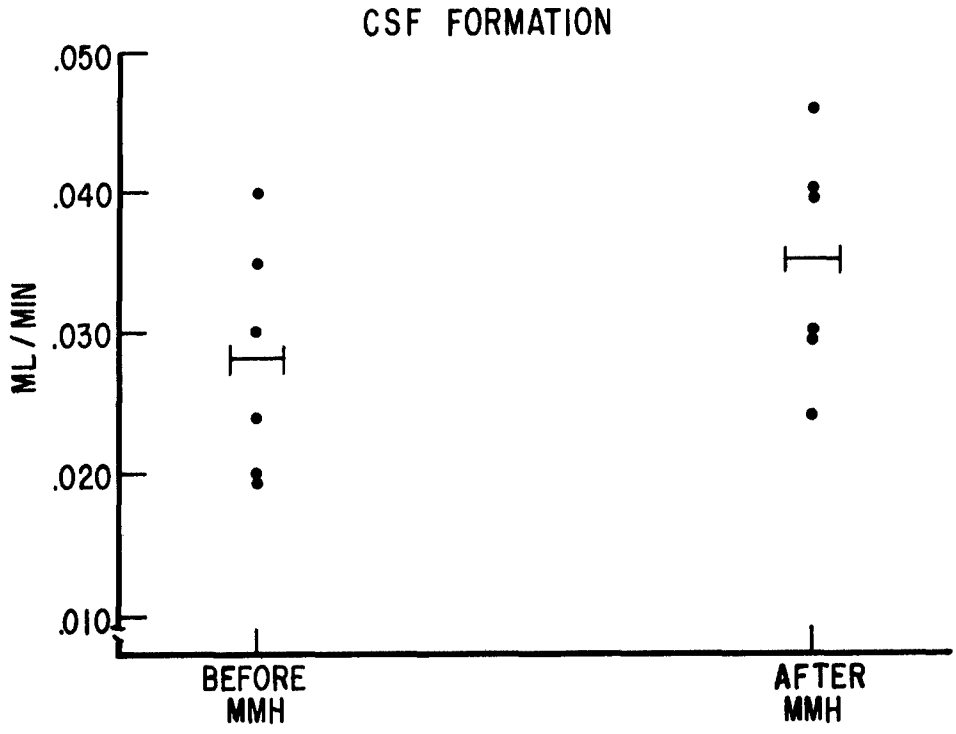

Figure 8. CSF formation before and after MMH as determined by drainage.

TABLE 1

	Control	MMH
Blood Glucose mg %	(20) 48.8 ± 2.5	(8) 90.6 ± 8.0
CSF Glucose mg %	(20) 45.1 ± 2.9	(8) 63.8 ± 5.5
CSF/Blood	(8) 0.923	(8) 0.704
Duration to Convulsion (minutes)		(4) 120 ± 17
Duration to Peak Blood Glucose (minutes)		(8) 111 ± 21
Duration to Peak CSF Glucose (minutes)		(8) 104 ± 17
CSF Formation (Drainage) ml/min	(8) 0.028 ± .003	(8) 0.035 ± .003
CSF Formation (Inulin) ml/min	0.034 ± .003	

mean ± S.E.
(n) = number of animals

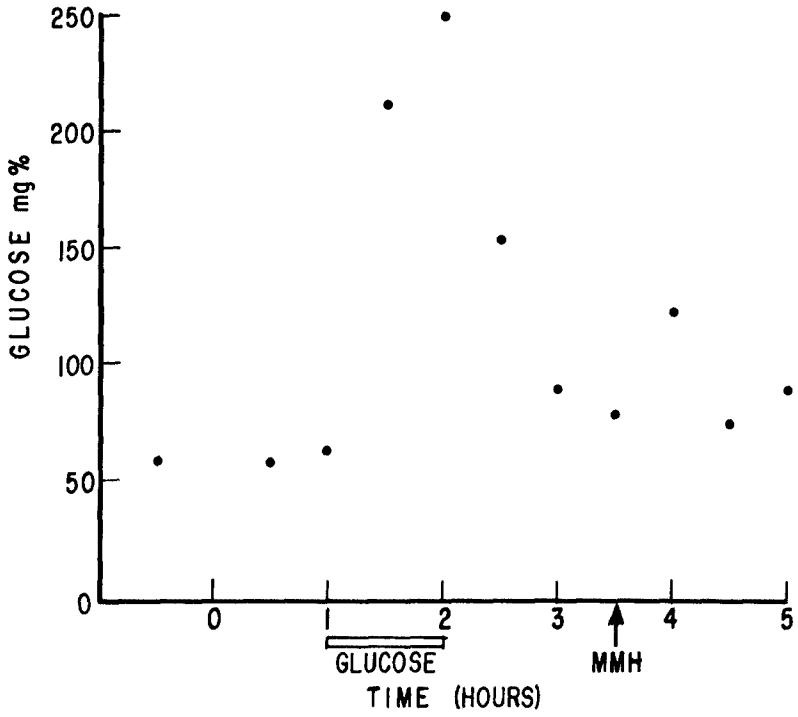

Figure 9. A typical experiment demonstrating the effect of MMH on blood glucose in a monkey primed with glucose. An infusion of glucose 1.5 g/kg i.v. was given between 1-2 hours. Ninety minutes later, MMH 25 mg/kg, was given intravenously.

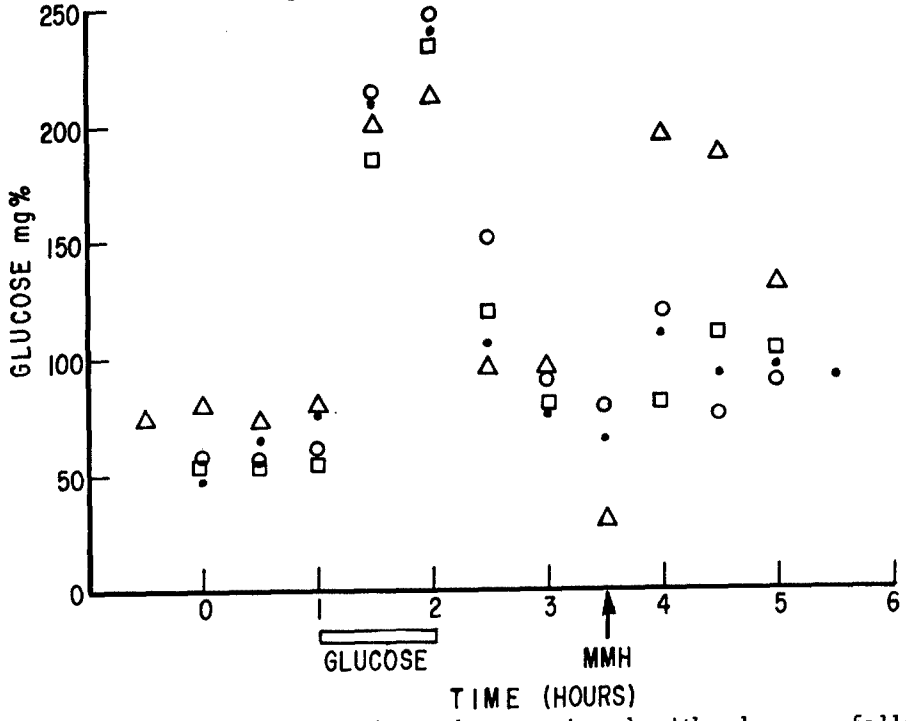

Figure 10. Blood glucose in 4 monkeys primed with glucose followed by MMH. Each animal is represented by a separate character.

Previous investigations from our laboratory indicated slight increases in blood glucose after chronic administration of MMH (Back and Pinkerton, 1967), but blood sampling was infrequent and examination of CSF glucose was not included. However, CSF is a better representative of brain extracellular fluid than is plasma and could reflect alterations in cerebral metabolism of glucose, perhaps with greater accuracy than blood glucose.

The control of CSF glucose concentration is complex and is believed to be regulated by several mechanisms. It has been shown by Fishman (1964) and by Bronsted (1970) that glucose is transported both from blood to CSF and from CSF to blood and brain. This transport is both by simple diffusion and by carrier mediated transport. In addition, the efflux of glucose from CSF is further complicated by comprising ouabain sensitive as well as ouabain insensitive carrier mediated transport system (Bronsted, 1970).

Our results suggest that none of these mechanisms are significantly altered by MMH in the doses given.

CSF FORMATION

CSF is formed by the choroid plexus of both lateral ventricles and after passage through the ventricular system exits by bulk absorption through valve like villi in the arachnoid granulations of the cerebral subarachnoid space (Davson, 1967).

It might be argued that MMH could reduce CSF glucose levels; but if it also reduced CSF formation and bulk flow, the concentration in CSF would appear to remain unchanged. This possibility is excluded by demonstrating that MMH had no effect on CSF production, which suggests that MMH does not affect the choroid plexus system concerned with CSF production. The significance of this awaits further investigation.

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