Effect of Dehydration and Hyperosmolarity on Glucose, Free Fatty Acid and Ketone Body Metabolism in the Rat

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SUMMARY

The effect of water deprivation was studied in rats fasted twenty-four, forty-eight and seventy-two hours. Glucose tolerance was impaired and insulin levels were lower one hour after an intraperitoneal glucose load. Ketone bodies (KB) and free fatty acid (FFA) levels were significantly lower. FFA rise after epinephrine was reduced. Hypertonic mannitol (3 M) administered subcutaneously to fasted rats also resulted in glucose intolerance and decreased levels of FFA and KB. In vitro hyperosmolarity (50, 100 mOsm. per liter mannitol) reduced FFA release from rat epididymal fat pads and impaired pancreatic insulin response to glucose. No effect on ketone body production by perfused rat liver was found. It is postulated that the above metabolic effects of dehydration and hyperosmolarity may be involved in the pathogenesis of hyperosmolar nonketotic coma in patients. DIABETES *22*:264-71, April, 1973.

The clinical features of hyperosmolar nonketotic coma (HNC) have been well documented.[1-7] However, despite studies regarding levels of plasma insulin,[1,8-12] growth hormone,[1,8,9,13] cortisol,[1,8,9,13] free fatty acid,[1,8,9,14] and ketone bodies,[14] the pathogenesis of this condition remains unclear.[1,2,7]

Marked dehydration and striking elevation of plasma osmolarity are invariably associated with HNC.[1-7] Several investigations suggest that these might be important factors in the exaggerated hyperglycemia and lack of ketosis in this syndrome. In vitro studies of Kuzuya et al.[15] and Clausen[16] indicate that hyperosmolar solutions inhibit release of free fatty acids from adipose tissue and impede insulin-stimulated glucose uptake by muscle tissue in the rat. Moreover, Nitzan and Zelmanovsky[17] have induced glucose intolerance in the rat by administration of hypertonic saline. In human subjects, Passmore and Johnson[18] found that dehydration diminished both nutritional and postexercise ketosis. More recently, Bavli and Gordon[19] reported dehydration to be a prerequisite for production of extreme hyperglycemia without ketosis in cortisol-treated, mildly diabetic rats.

These studies suggested that it would be of interest to examine further the effects of dehydration and hyperosmolarity on carbohydrate and ketone body metabolism in seeking an explanation for the pathogenesis of HNC. Two types of experiments were performed: (1) in vivo studies examining the effect of dehydration on glucose tolerance, free fatty acid levels and ketone body metabolism during fasting; and (2) in vitro studies examining the effects of hypertonic mannitol on the metabolism of rat adipose tissue, liver and pancreas.

METHODS AND MATERIALS

In Vivo Studies

Effect of dehydration and fasting on glucose tolerance, plasma free fatty acids (FFA) and ketone bodies (KB) and epinephrine-mediated lipolysis. Male Sprague-Dawley rats weighing 250 gm. were utilized. The rats were weighed, randomly divided into two groups and fasted for periods of 24, 48 or 72 hours. Group A consisted of experimental rats that were deprived of water; Group B consisted of control rats allowed access to water ad libitum. At the end of each fasting period, rats from both groups were anesthetized with sodium pentobarbital (4 mg./100 gm. body weight), reweighed and exsanguinated via the abdominal aorta for determination of blood glucose,[20] plasma insulin, IRI,[21] FFA,[22] KB[23] and electrolytes (Na^+, K^+, Cl, CO_2, BUN).[24] Glucose tolerance tests were performed on rats from each group after twenty-four hour fasts; 3 cc. of 20 per cent glucose was injected intraperitoneally and blood was obtained via the tail vein for glucose tests at 0, 60 and 120 minutes. IRI determinations were carried

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out in separate groups of animals at zero and sixty minutes after glucose loads. Epinephrine (100 μg. per kilogram) was administered intramuscularly to rats of each group (fasted twenty-four hours), and blood was obtained via the tail vein at zero and sixty minutes for determination of glucose. FFA determinations were carried out in additional groups of epinephrine-treated animals at zero and sixty minutes.

Effect of hypertonic mannitol-induced dehydration on glucose tolerance, plasma FFA and ketone bodies. Rats were weighed and randomly divided into two groups. Group A (twelve rats serving as experimental animals) received 6 cc. of 3 M mannitol administered subcutaneously in two doses separated by five hours. Group B (twelve rats serving as controls) received 6 cc. of isotonic (0.3 M) mannitol in a similar manner. Food and water were withheld from both groups for twenty-four hours. Subsequently rats were reweighed, anesthetized and subjected to intraperitoneal glucose tolerance tests as described above. In addition, rats from both groups were exsanguinated via the abdominal aorta for plasma FFA, ketone bodies, electrolytes and hematocrit determinations.

In Vitro Studies

Effects of hyperosmolarity on FFA release. Paired epididymal fat pads from anesthetized, overnight fasted rats were incubated in Krebs-Henseleit buffer containing 3 per cent bovine albumin (KBA) under conditions previously described.[25] Control fat pads were incubated in the above buffer while contralateral fat pads were incubated in KBA buffer containing mannitol at concentrations of 25, 50 and 100 mOsm. per liter. After one hour, FFA released into the medium was determined.

Effects of hyperosmolarity on pancreatic insulin release. Two or three pieces of pancreas weighing approximately 40 mg. each were obtained from anesthetized rats deprived of food overnight. The specimens were incubated in 25 ml. flasks containing 2 ml. KBA buffer, trasylol (1,000 μ per milliliter) and 5 mM glucose. Three successive twenty minute incubations were performed as previously described by Edgar et al.[26] Mannitol at either 50 mOsm. per liter or 100 mOsm. per liter was added to the medium of the second incubation period in the experimental series while controls were incubated in its absence. Aliquots of medium were immediately frozen for IRI determination.

Effects of hyperosmolarity on hepatic ketogenesis. Isolated liver perfusions were performed according to the method of Penhos,[27] using livers from rats fasted overnight, and their pooled heparinized whole blood as perfusate. After a forty-five minute control period, mannitol to a concentration of 100 mOsm. per liter was introduced into the perfusate. Glucose, BUN and ketone bodies were determined from aliquots of perfusate taken at 0, 30, 45, 65, 75, 95 and 120 min.

RESULTS

In Vivo Studies

Effects of dehydration and fasting. As shown in table 1, rats deprived of water progressively lost more weight than controls during the seventy-two hour fast. The experimental group also developed significantly higher plasma levels of Na^+, Cl^+ and BUN. No difference was observed in the plasma bicarbonate levels during the fast. In the control group, however, seventy-two hour values were significantly lower than initial twenty-four hour values. Plasma K^+ rose similarly in both groups during the fast.

Plasma FFA and ketone bodies (table 2) increased throughout the fast in both groups but were significantly lower in rats deprived of water. The blood glucose patterns differed markedly. Whereas values progressively declined in controls, the blood glucose of rats deprived of water rose after twenty-four hours to levels 28 and 33 mg./100 ml. above those of the controls. Despite these differences, plasma IRI diminished similarly in both groups although the values for rats deprived of water were consistently but not significantly lower.

Results of glucose tolerance tests are shown in figure 1. Experimental rats had significantly higher blood glucose levels at sixty and 120 minutes and lower plasma IRI levels at sixty minutes. Initial blood glucose and plasma IRI values were similar in both groups.

The effects of twenty-four hours of dehydration on blood glucose and plasma FFA responses to epinephrine are shown in figure 2. Both groups had similar elevations of blood glucose levels. However, the FFA of rats deprived of water were initially lower and showed smaller increments than those of controls.

Effects of hypertonic mannitol-induced dehydration. As shown in table 3, rats which had received hypertonic mannitol lost more weight than controls. There was no difference in plasma electrolytes or hematocrit. However, the plasma FFA and ketone bodies of rats given hypertonic mannitol were significantly lower than controls. Figure 3 shows the results of glucose tolerance tests. The experimental group had significantly higher blood glucose levels at sixty and 120 minutes. No differences were observed in fasting blood glucose levels or in IRI values at zero and sixty minutes.

TABLE 1

Sequential effects of dehydration on weight loss and plasma electrolytes in rats

Hours fasted	24			48			72			P 24 hr. vs. 72 hr.	
	$+H_2O$	$-H_2O$	P	$+H_2O$	$-H_2O$	P	$+H_2O$	$-H_2O$	P	$+H_2O$	$-H_2O$
Weight loss (%)	10 ± .5 N=18	11.7 ± .8 N=22	<.05	14.7 ± .6 N=8	16.6 ± .2 N=10	<.005	16.1 ± .9 N=9	20.5 ±1.4 N=10	<.02	<.001	<.001
Na (mEq. per liter)	151 ± .8 N=10	152 ±1.6 N=11	NS	152 ± .9 N=22	155.7 ±2.1 N=10	NS	149.3 ±1.6 N=17	153.7 ±1.0 N=18	<.02	NS	NS
Cl (mEq. per liter)	99.8 ±1 N=10	100.1 ± .8 N=11	NS	97.6 ± .8 N=22	103.9 ± .7 N=10	<.001	100.8 ±1.2 N=17	106.6 ± .7 N=18	<.001	NS	<.001
K (mEq. per liter)	3.4 ± .1 N=10	3.4 ± .07 N=11	NS	3.4 ± .1 N=22	3.5 ± .1 N=10	NS	3.7 ± .1 N=17	3.7 ± .1 N=18	NS	<.02	<.02
CO_2 (mEq. per liter)	21.8 ±1 N=10	20.5 ±1.5 N=11	NS	21.0 ± .4 N=22	19.8 ± .7 N=10	NS	18.3 ± .7 N=17	19.3 ± .6 N=18	NS	<.005	NS
BUN (mEq. per liter)	17.9 ±1.1 N=10	16.0 ±1.7 N=11	NS	15.9 ±1.2 N=22	21.0 ±1.8 N=10	<.02	18.5 ± .9 N=17	26.9 ± .6 N=18	<.005	NS	<.001

N=Number of animals.
Mean values ± S.E.M. and p values for the effect of dehydration are shown.

In Vitro Studies

Effect of hypertonic mannitol on FFA release. FFA release from rat epididymal fat pads (figure 4) was significantly inhibited by all concentrations of mannitol used. The degree of inhibition increased with increasing concentrations of mannitol.

Effect of hypertonic mannitol on pancreatic insulin release. Pancreatic insulin release (figure 5) was significantly decreased in the presence of both 50 and 100 mM mannitol. When pieces of pancreas were reincubated in buffer without mannitol, insulin release returned towards normal levels, indicating the reversibility of the mannitol-induced inhibition. Both concentrations of mannitol employed appeared to exert similar degrees of inhibition.

Effect of hypertonic mannitol on hepatic ketogenesis. No change was observed in the rate of accumulation of ketone bodies, glucose and urea (figure 6) in the perfusate of isolated liver perfusions after the introduction of mannitol. The values shown are the means (± S.E.M.) of four separate perfusions. Control perfusions without mannitol addition have been previously reported and show no significant differences from those with mannitol.[27]

TABLE 2

Changes in plasma FFA, ketone bodies, blood glucose, and IRI in rats during seventy-two hour fast

Hours fasted	24			48			72		
	$+H_2O$	$-H_2O$	P	$+H_2O$	$-H_2O$	P	$+H_2O$	$-H_2O$	P
FFA (μEq. per liter)	688 ±54 N=19	531 ±26 N=17	<.01	812 ±45 N=11	689 ±32 N=13	<.02	892 ±32 N=13	715 ±19 N=14	<.001
Ketone bodies (mg./100 ml.)	18.1 ±1.9 N=16	12.1 ±1.1 N=19	<.01	23.7 ±1.9 N=9	18.1 ±1.5 N=12	<.02	30.6 ±1.2 N=7	22.1 ±1.2 N=8	<.0005
Glucose (mg./100 ml.)	107 ±4 N=9	104 ±5 N=9	NS	101 ±4 N=17	129 ±9 N=10	<.001	94 ±4 N=17	127 ±6 N=18	<.0005
IRI (μunit per milliliter)	73 ±8 N=15	66 ±6 N=16	NS	61 ±7 N=19	51 ±10 N=10	NS	59 ±11 N=11	47 ±10 N=15	NS

N=Number of animals.

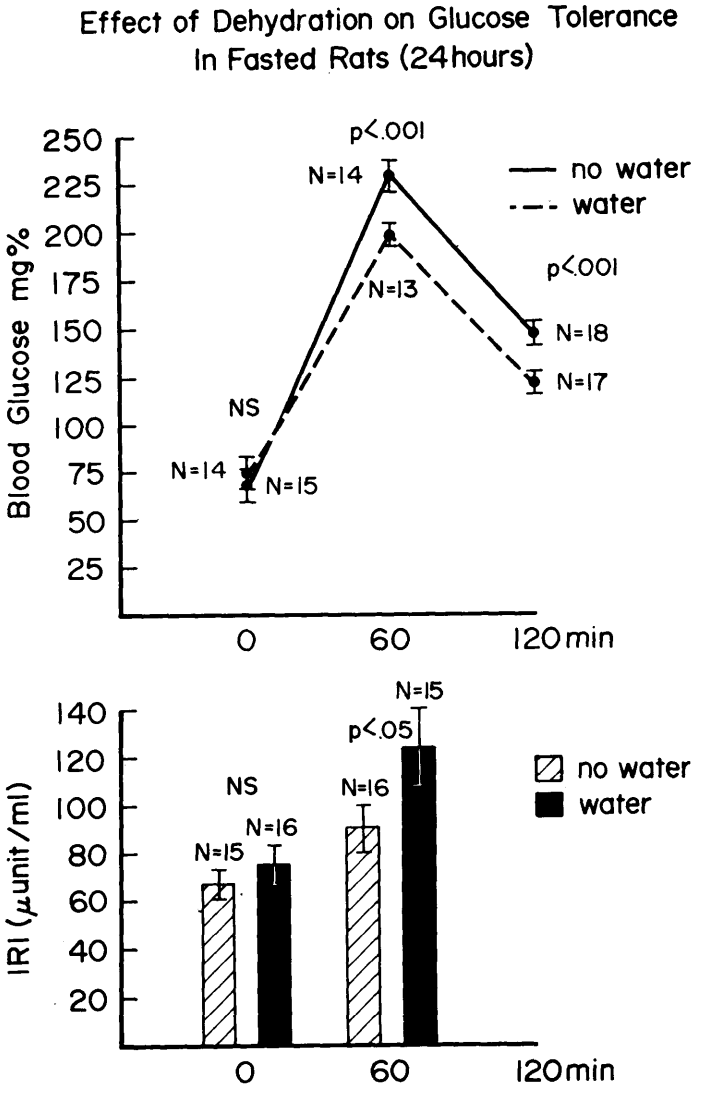

FIGURE 1

Glucose tolerance was significantly impaired by water restriction in rats fasted twenty-four hours. Effects on insulin release were noted at the sixty minute sample. (See text for experimental details.)

DISCUSSION

The results of these experiments indicate that in vivo dehydration induced by water restriction results in significant metabolic derangements in the rat. The major changes found were decreased plasma levels of free fatty acids and ketone bodies in response to fasting, as well as impairment in free fatty acid elevation following epinephrine administration. In addition, evidence for impaired glucose tolerance without increased insulin levels was seen. A similar metabolic pattern was also observed in fasted rats receiving hyperosmolar mannitol. Free fatty acid levels and ketone bodies were lower and glucose levels were elevated when compared with fasted controls.

In vitro studies with hyperosmolar mannitol indicated that free fatty acid release from epididymal fat pads was impaired and that the higher the osmolarity, the greater the impairment. Incubation of rat pancreas pieces with hyperosmolar mannitol revealed that insulin release in response to glucose was significantly decreased and that the impairment was reversible when tissues were placed in physiologic buffers. Finally, normal ketone body production was found in livers perfused with hyperosmolar mannitol. A variety of metabolic effects of hyperosmolarity on the liver have previously been reported,[15-19,28-33] including increased glycogenolysis[16] and gluconeogenesis.[33]

Whether these in vivo and in vitro observations were mediated through a single mechanism can not be answered by the present study, since the experimental de-

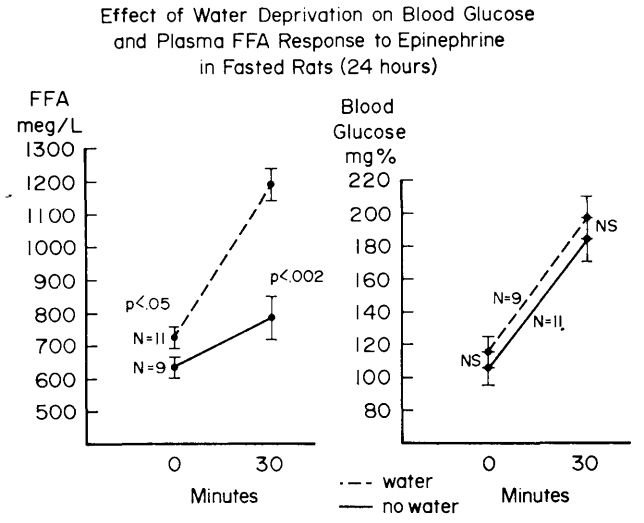

FIG. 2. FFA response to epinephrine was impaired in rats deprived of water. No effect of dehydration on blood glucose response to epinephrine was found.

TABLE 3

Effects of hypertonic mannitol diuresis in rats fasted and deprived of water for twenty-four hours

	3 M Mannitol	Control (0.3 M Mannitol)	P
Weight loss (%)	10.2 ± .3	6.5±.3	<.001
Hematocrit	43 ±2	45±2	NS
Na (mEq. per liter)	141±1	144±2	NS
Cl (mEq. per liter)	102	102	NS
K (mEq. per liter)	4.3	4.3	NS
CO_2 (mEq. per liter)	23	23	NS
BUN (mg./100 ml.)	22±2	19±2	NS
Fasting FFA (mEq. per liter)	572±52	788±47	<.05
Fasting Ketones (mg./100 ml.)	18±2	28±3	<.05
Number of rats	12	12	

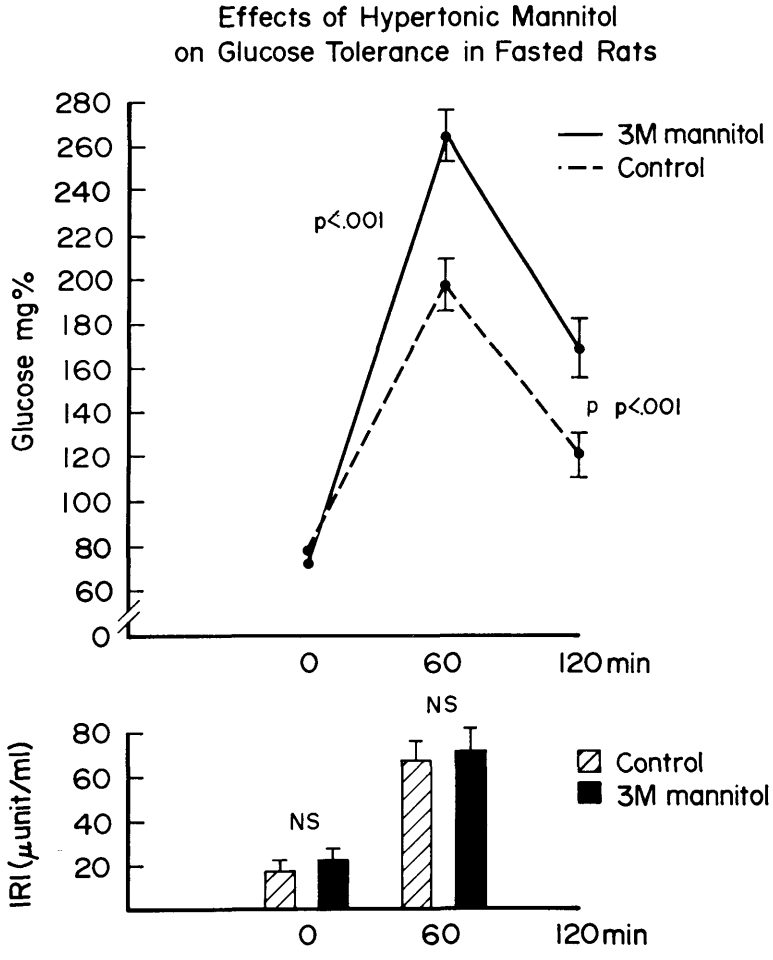

FIGURE 3

Twelve control rats received 0.3 M mannitol, while twelve experimental animals received 3 M mannitol. Glucose tolerance was significantly impaired at sixty and 120 minutes after glucose. No alteration in insulin levels was found.

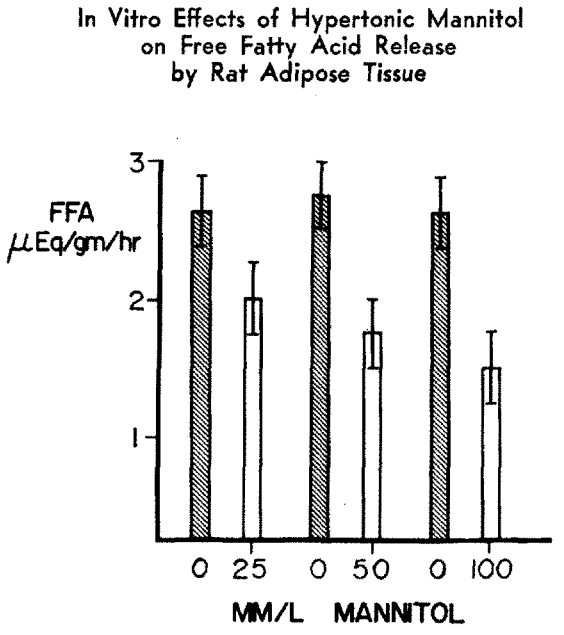

FIG. 4. Twenty-four pairs of tissue were tested at 25, 50 and 100 mM per liter mannitol. Control tissue incubated without mannitol. $P < .01$ for the difference between all control and experimental incubations.

sign employed did not permit clear-cut isolation of hyperosmolarity from dehydration and vice versa. For example, although water loss as reflected by body weight change was greater in water restricted rats, their calculated serum osmolarity was also greater than controls (332 vs. 317 mOsm./per liter). On the other hand, the in vivo mannitol experiments do suggest that the results may have been due to dehydration since in these studies greater dehydration as reflected by weight loss occurred without change in plasma electrolytes, calculated serum osmolarity or hematocrit readings. The observed weight loss can be attributed to water deficit since such changes in weight over a short period of time (less than twenty-four hours) usually represent alterations in water balance. In addition features of catabolism such as elevated BUN, plasma FFA and ketone bodies were not seen. Thus, impaired carbohydrate tolerance and impaired FFA mobilization occurred with isotonic dehydration in these latter studies.

The "in vitro" effects of hyperosmolarity with concomitant intracellular dehydration may explain certain of the "in vivo" consequences of water deprivation. Carbohydrate intolerance may have resulted from diminished insulin secretion, insulin insensitivity[16] and enhanced glycogenolysis[16] and gluconeogenesis.[33] The blunted response of plasma FFA to fasting and epinephrine administration may be ascribed to impaired lipolysis. This would also explain the diminished ketosis since hepatic production of ketone bodies is a function of the availability of FFA as precursors.[34] Moreover, while there are no studies concerning the effects of hyperosmolarity on FFA or ketone body *utilization*, hepatic ketone body production was shown not to be affected by hyperosmolarity.

The findings of the present study have obvious implications concerning the pathogenesis of hyperosmolar nonketotic coma in man. Although it is generally thought that dehydration and hyperosmolarity in this syndrome result from an osmotic diuresis due to uncontrolled glycosuria,[2-7,35-37] there is clinical evidence[1,19] as well as experimental evidence[15-19,28,29,32,33] that dehydration and hyperosmolarity may not be mere consequences but rather important factors in the genesis of hyperglycemia. Most patients with this syndrome have pre-existing conditions associated with dehydration[1-3] and most are mild diabetics,[1-3] a situation analogous to that described in the rat by Bavli and Gordon.[19] The findings of the present study suggest that in susceptible persons, i.e., mild diabetics, dehydration or hyperosmolarity impair insulin secretion to such an extent that severe hyperglycemia develops. Subsequent inhibition of lipolysis by hyperosmolarity and hyperglycemia[38] finally result in the full-blown clinical picture of hyperosmolar nonketotic coma.

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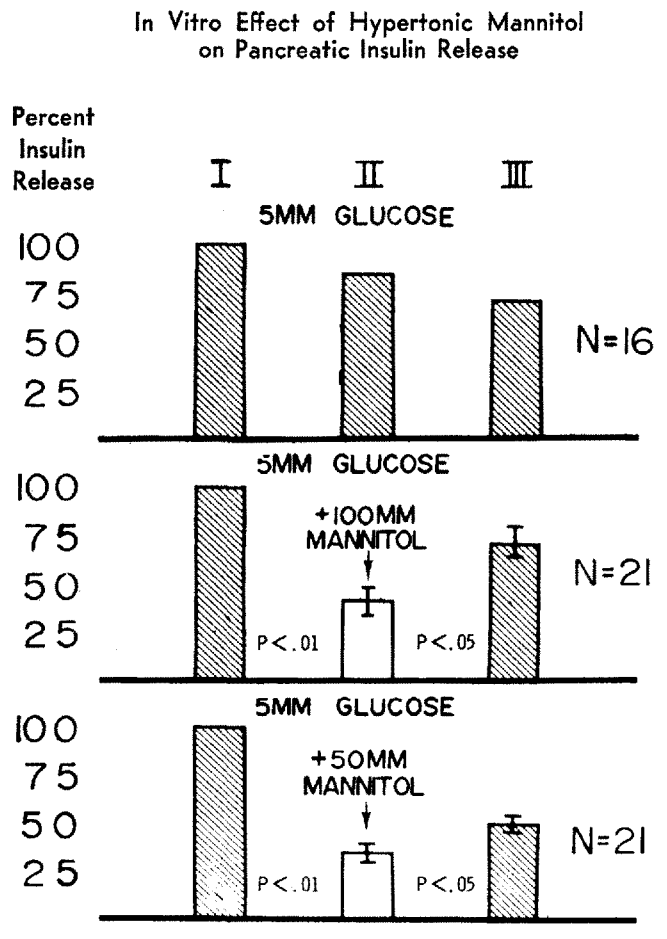

FIGURE 5

Pieces of pancreas were incubated in 5 mM per liter glucose for three time periods, phase I, II, III (see text for details). Insulin release is shown as per cent of control, namely phase I, for each experiment. Significant reduction of insulin release occurs with hypertonic mannitol (phase II). Reversibility of the process is seen in phase III after mannitol.

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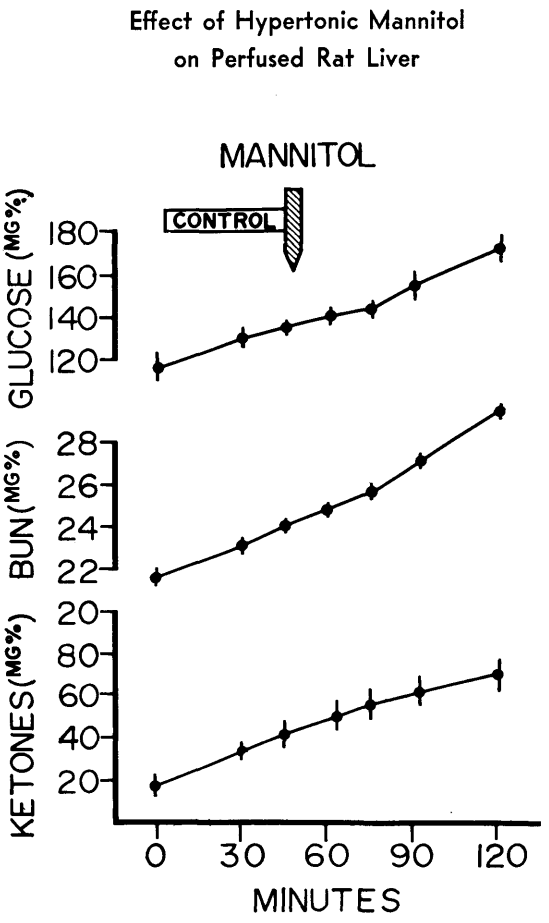

FIG. 6. Four isolated rat livers from overnight fasted rats were perfused for sixty minutes during which time glucose, urea nitrogen and ketone bodies in the perfusate were measured. Hypertonic mannitol was then introduced into the perfusate. As can be seen, no alteration of the rates of ketone body formation was seen in the succeeding one hour of perfusion. Rates of ketone body, urea and glucose production in control experiments showed similar results to the above and have been published elsewhere.[27]

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