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Effects of Vasopressin on Carbohydrate Metabolism in Hepatocytes from Dehydrated Rats (41137)

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Abstract. Hepatocytes isolated from rats deprived of water for 48 hr (dehydrated) were compared with hepatocytes from control rats with respect to four effects of vasopressin: the inhibition of glucose-promoted glycogen synthesis; the stimulation of glycogenolysis in the absence of exogenous glucose; glucose production; and pyruvate decarboxylation. Vasopressin (ADH) prevented net glycogen synthesis in hepatocytes from both control and dehydrated rats. The inhibitory effect of ADH on hepatocyte glycogen synthesis disappeared within 45 min of washing the cells with hormone-free medium. Vasopressin stimulated glycogenolysis by 60% and glucose output by 80% in hepatocytes from either control or dehydrated rats. In 24-hr-fasted rats, prior dehydration had no effect on either basal or ADH-stimulated glucose production or pyruvate decarboxylation. Thus no evidence was found that water deprivation and the associated prolonged exposure to elevated plasma ADH led to adaptive changes in carbohydrate metabolism or in the ability of hepatocytes to respond to ADH.

Vasopressin (ADH) causes hyperglycemia in the intact rat (1) and promotes glycogenolysis in rat liver slices (2), perfused liver (3), and isolated hepatocytes (4, 5). It also causes a moderate stimulation of gluconeogenesis in both perfused liver and isolated hepatocytes (3, 6, 7) and prevents glycogen accumulation *in vivo* and in perfused liver (3). These acute hepatic effects of vasopressin are maximal within 1 to 20 min (3, 8). Hyperglycemia after a single intraperitoneal dose of ADH in the fed rat was transient and was followed by hypoglycemia within 30 min, whereas epinephrine-induced hyperglycemia lasted 90 min (1).

At least two questions about the hepatic effects of vasopressin arise in this regard. One concerns the time course of the disappearance of the hormone's acute effects after its removal from the medium. This has not been closely examined in isolated liver preparations, where, as opposed to whole-animal studies, the effects of other tissues and hormones are eliminated. In the studies reported here, this question was investigated using isolated hepatocytes exposed to vasopressin *in vitro* and then washed by several changes of medium.

The second question is whether prolonged exposure to vasopressin has long-term, possibly adaptive, effects on hepatic carbohydrate metabolism. During dehydration, plasma vasopressin concentrations in rats can rise 5- to 10-fold over basal levels and remain high for days (9, 10). Such chronic hormone exposure might lead to adaptive alterations in enzyme levels, as occur in response to fasting or to feeding carbohydrate- or protein-rich diets (11). That a profound change in some aspect of mitochondrial function occurs as a result of dehydration is indicated by progressive mitochondrial swelling which was reversed within 10 min after drinking (12). Chronic exposure to a hormone may also lead to alterations in the responsiveness of various tissues to the hormone. For example, hyperinsulinemia is associated with insulin-resistant states *in vivo* (13), and chronic, but not acute, exposure of cultured cells to high insulin levels leads to loss of insulin receptors (14). Similar loss of hormone responsiveness of target tissues after chronic hormone exposure has been demonstrated for many other hormones including catecholamines (15), growth hormone (16), and luteinizing hormone (17). To test whether chronic vasopressin exposure in rats during dehydration might lead to adaptive changes in liver enzyme levels or to loss of the hormonal response, basal and

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arginine vasopressin-mediated carbohydrate metabolism were studied in hepatocytes from dehydrated and control rats.

Materials and Methods. Isolated hepatocytes were prepared from the livers of male Sprague–Dawley rats, weighing 200 to 350 g, by the method of Berry and Friend (18). The rats were fed Purina Rat Chow *ad libitum* or were fasted for 24 hr, as indicated. "Dehydrated" rats had no access to drinking water for 48 hr. Perfusion and routine preincubation medium contained 20 m*M* glucose to preserve hepatocyte glycogen content (5). Hepatocyte viability, judged by trypan blue exclusion, was consistently greater than 85%. Hepatocytes (10–20 mg protein per flask) were incubated in 50-ml-capacity polycarbonate Erlenmeyer flasks in a total volume of 2.5 ml Krebs–Ringer bicarbonate buffer containing 5 m*M* Hepes, 1.5 m*M* $CaCl_2$, and 0.3% (w/v) dialyzed bovine serum albumin. Vasopressin and substrates were present as indicated in the figure and table legends. The flasks were gassed for 20 sec with 95% O_2/5% CO_2, capped, and incubated at 37°C with gentle shaking.

Glucose content of cells plus medium was determined enzymatically (with Worthington Automated Glucose Reagent) after deproteinization with 0.5 vol 13% perchloric acid, neutralization with potassium hydroxide, and centrifugation. Glucose production is expressed as the difference between final and initial glucose concentrations.

Glycogen content was measured essentially by the method of Connett and Blum (19). Glycogenolysis is expressed as the difference between initial and final glycogen content of the hepatocytes.

$^{14}CO_2$ collection. After injection of 0.3 ml 13% perchloric acid into the capped hepatocyte incubation flasks, 0.2 ml Hyamine was added to their plastic center wells and CO_2 collected during a 1-hr incubation at 37° with shaking. Radioactivity of the well plus Hyamine was determined in glass counting vials containing 10 ml ACS scintillant, using Packard Tri-Carb spectrometer with external standard for quench correction.

Protein was measured by the method of Lowry *et al.* (20), using bovine serum albumin as a standard.

Hematocrit and plasma osmolality. For these experiments rats were anesthetized with diethyl ether and heparinized blood samples taken from the inferior vena cava. Hematocrit was determined by centrifugation, and osmolality of the resulting plasma was measured with a vapor pressure osmometer (Wescor Inc., Model 5100B).

Materials. [1-^{14}C]pyruvate and ACS scintillant were from Amersham Corp. Hyamine was from New England Nuclear. Collagenase and automated glucose reagent were from Worthington. Synthetic arginine vasopressin (grade VIII) was from Sigma.

Results. *Time course of reversal of the inhibition of glycogen synthesis by vasopressin.* The studies of Hems and Whitton (3) showed that ADH prevented net glycogen synthesis in the presence of 30 m*M* glucose in perfused rat liver and in liver of intact rats. It is well known that the presence of high concentrations of glucose will lead to glycogen deposition in liver (21) and in hepatocytes from fasted rats (22), and it was therefore of interest to ascertain whether ADH would inhibit glycogen synthesis in isolated hepatocytes. When incubated with 30 m*M* glucose, 5 m*M* lactate, 0.5 m*M* pyruvate, and 0.6 m*M* acetate, hepatocytes from 24-hr-fasted rats synthesized glycogen, and vasopressin inhibited this synthesis (Fig. 1). The effect of vasopressin was discernable at 10^{-10} *M*, and maximal inhibition was reached by 2×10^{-8} *M* (Fig. 1), even when the ADH was added only at the beginning of the 80-min incubation period and without any inhibitors of hormone degradation.

Inhibition of glycogen synthesis is thus a fairly sensitive acute hepatic response to ADH, and was therefore monitored in studies of the time course of disappearance of the hormone's effects (Fig. 2). In these studies, hepatocytes from a fasted rat were preincubated for 20 min in the absence or presence of 2×10^{-8} *M* ADH. This concentration was found to inhibit glycogen synthesis from the standard substrate mix by at least 85% whether the glucose concentration was 30 m*M* (Fig. 1) or 40 m*M* (data not shown). After preincubation, the

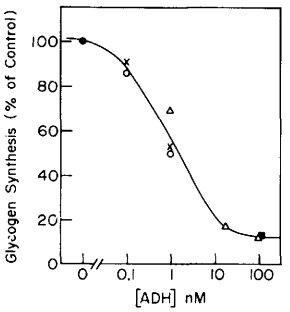

FIG. 1. Inhibition of glycogen synthesis by vasopressin. Hepatocytes from 24-hr-fasted rats were incubated with 30 m*M* glucose, 5 m*M* lactate, 0.5 m*M* pyruvate, and 0.6 m*M* acetate, with varying concentrations of vasopressin. Glycogen synthesis during 80 min was measured in duplicate as described under Materials and Methods, and is expressed as percentage of control. The average rate of glycogen synthesis in hormone-free control samples was 0.5 nmole/min·mg protein. (X, ○, △) Each represent the mean of a separate experiment, in which four determinations per point varied less than 5% from the mean. (■) Represents the average of eight experiments.

cells were washed (for about 15 min) by several changes of medium, resuspended in ADH-free medium, and incubated for 0, 20, or 45 min. At these times, aliquots were taken for determination of hepatocyte glycogen content and of ability to synthesize glycogen during a subsequent standard 80-min incubation with and without ADH. Results from one such experiment are shown in Fig. 2. Immediately after washing, the ADH-pretreated cells, incubated without added ADH, showed an appreciable (~25%) inhibition of glycogen synthesis as compared with control cells that had not been exposed to ADH. By 20 min after washing, glycogen synthesis by ADH-pretreated cells was inhibited by only 12%, and by 45 min there was no residual inhibition of glycogen synthesis. At each time point, both control and pretreated hepatocytes responded to added ADH by complete inhibition of glycogen synthesis (Fig. 2), thus establishing that neither the pretreatment nor the washing interfered with the response to ADH. Thus the ability of ADH to inhibit glycogen synthesis by hepatocytes is quickly reversed after removal of the hormone.

Carbohydrate metabolism in hepatocytes from dehydrated rats. The question of whether chronic vasopressin exposure during dehydration affects hepatic carbohydrate metabolism was investigated using hepatocytes from 48-hr-dehydrated rats and from control rats. Four effects of vasopressin were studied: the stimulation (in low-glucose medium) of glucose output, glycogenolysis, and pyruvate decarboxylation, and the inhibition (in high-glucose medium) of net glycogen synthesis.

Glycogenolysis and glucose production. Hepatocytes from rats deprived of water for 48 hr and from control rats were incubated in the absence (basal) and presence of 10^{-7} *M* ADH. When the vasopressin effect is computed as a percentage of the basal response, there is no difference between control and 48-hr-dehydrated animals (Fig. 3). For example, in the absence of added substrate, vasopressin stimulated glucose output by 79(±29) and 76(±12)% in hepatocytes from control and dehydrated rats, respectively (mean ± SEM); the stimulation

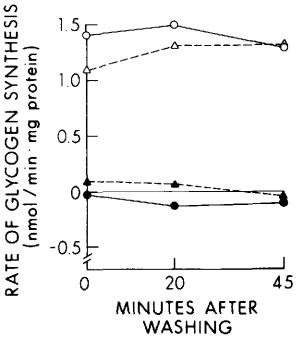

FIG. 2. Reversibility of vasopressin inhibition of hepatocyte glycogen synthesis. Hepatocytes were prepared from a 24-hr-fasted rat as described under Materials and Methods, were divided in two portions, and were incubated for 20 min at 37° with 20 m*M* glucose in the absence (○, ●, control) or presence (△, ▲) of 2×10^{-8} *M* vasopressin. Each group of cells was washed by centrifugation three times in 30 vol of buffer, resuspended in buffer with 20 m*M* glucose, and again incubated for 0, 20, or 45 min. At these times, aliquots were taken for determination of glycogen content and for incubation for 80 min with 38 m*M* glucose, 5 m*M* lactate, 0.5 m*M* pyruvate, and 0.6 m*M* acetate without (open symbols) or with (closed symbols) 10^{-7} *M* vasopressin. The experiment shown is representative of three. Each point is the mean of quadruplicate determinations which varied less than 5%.

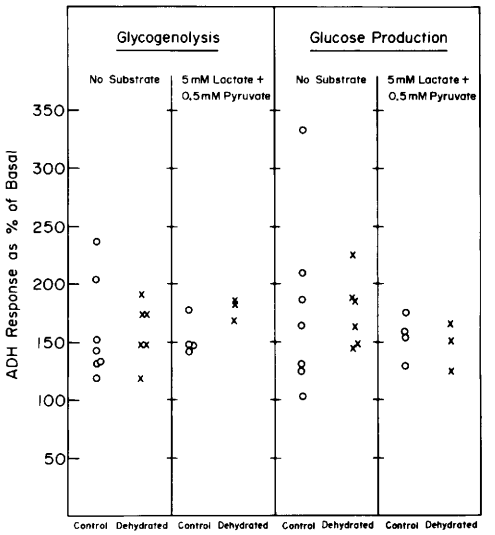

FIG. 3. Stimulation of glycogenolysis and of glucose production by ADH in hepatocytes from control and dehydrated rats. Hepatocytes were incubated for 30 min with or without lactate plus pyruvate as indicated and in the absence (basal) or presence of 10^{-7} *M* ADH. Glycogenolysis and glucose production were measured as described under Materials and Methods. Results are expressed as percentage of basal values. (O) Fed *ad lib;* (X) dehydrated 48 hr and fed *ad lib.*

of glycogenolysis under the same conditions was 59(±17) and 59(±11)%, respectively (from Fig. 3). Thus *in vivo* exposure to ADH during 48 hr of water deprivation does not cause loss of ability of subsequently isolated hepatocytes to respond to vasopressin.

The absolute values of basal glycogenolysis and glucose release in these experiments are presented in Table 1. Clearly, glucose production by hepatocytes from fed and control rats (Group A) is higher than in those from fed rats deprived of water (Group B). It can also be seen that: (i) hepatocytes from fed dehydrated rats (Group B) had 80% less initial glycogen than hepatocytes from fed control rats (Group A); (ii) in the absence of added substrate, glycogenolysis was sufficient to account for glucose released in both groups A and B; (iii) in the presence of lactate plus pyruvate, glucose output from fed dehydrated (but not fed control) rats exceeded the amount of glycogen breakdown, and therefore must contain a contribution from gluconeogenesis.

The probable major cause of liver glycogen depletion during dehydration was found by observation of the feeding behavior of rats during water deprivation; dehydrated rats ate less than half as much as control rats (Table I). Food consumption decreased from 6 ± 0.5% of body weight on Day 1 of dehydration to 3 ± 0.3% on Day 2, yielding an average food consumption of 4.5% of body weight for the water-deprived rats as compared to 10% for the controls (Table I); as a result of the reduced food and water intake, the dehydrated rats lost about 9% of body weight per day, compared with a gain of 4%/day for control rats (Table I). Decreased food intake by rats during water deprivation was also observed by Dicker and Nunn (23).

Because of the early onset of caloric restriction, it seemed likely that the decreased glucose output by hepatocytes from dehydrated fed rats as compared with those from fed control rats (Table I) was largely a reflection of their decreased glycogen content, rather than an effect of dehydration per se. The finding that glucose output from dehydrated rats' hepatocytes includes a component of gluconeogenesis further complicates investigations of the effects of dehydration on liver carbohydrate metabolism, since comparison of glucose output by Group A *versus* Group B hepatocytes compares primarily glycogenolysis in the first case to glycogenolysis plus gluconeogenesis in the second.

These problems were addressed by comparing 24-hr-fasted rats (Group C, Table I) with 48-hr-dehydrated rats which were fasted for the second 24 hr (Group D). This 24-hr fasting schedule eliminated the differences in glycogen content between the water-deprived and control rats (reducing hepatocyte glycogen to low levels in both), and abolished the differences in hepatocyte glucose output in the presence of lactate plus pyruvate (Table I). Under these conditions, glucose production results mainly from gluconeogenesis. In two experiments for each group, ADH stimulated glucose

TABLE I. EFFECTS OF DEHYDRATION ON SOME PHYSIOLOGICAL PARAMETERS

	Rat treatment			
	Fed *ad lib* (A)	Dehydrated 48 hr, fed *ad lib* (B)	Fasted 24 hr (C)	Dehydrated 48 hr, fasted final 24 hr (D)
Hepatocyte glucose production (nmol/mg protein·30 min)				
Lactate + pyruvate[a]	241 ± 42 (4)	149 ± 9 (3)	98 ± 6 (5)	101 ± 11 (3)
No substrate	213 ± 41[b] (4)[c]	88 ± 8 (3)	N.M.[d]	N.M.
Hepatocyte glycogenolysis (nmol glucose/mg protein·30 min)				
Lactate + pyruvate	282 ± 30 (4)	74 ± 29 (3)	N.M.	N.M.
No substrate	319 ± 29 (4)	74 ± 26 (3)	N.M.	N.M.
Initial glycogen content of hepatocytes (nmol glucose/mg protein)	873 ± 105 (4)	175 ± 78 (3)	16 ± 3 (18)	27 (2)
Food consumption (% of body wt per 24 hr)	10 ± 1 (6)	4.5 ± 0.2 (6)	0	0
Weight change (% of body wt per 24 hr)	+4 ± 1 (6)	−9 ± 0.6 (6)	−4 ± 0.8 (4)	−8 ± 0.4 (4)
Plasma osmolality (mOs/kg)	N.M.	N.M.	290 ± 1.5 (4)	304 ± 1.4 (4)
Hematocrit (%)	N.M.	N.M.	47 ± 0.6 (4)	50 ± 0.6 (4)

Note. Glucose production and glycogen content were measured in hepatocytes prepared from rats treated as indicated. Other groups of rats were studied for food consumption and weight change, and for plasma osmolality and hematocrit, as described under Materials and Methods.

[a] 0.5 m*M* pyruvate + 5.0 m*M* lactate.

[b] Mean ± SEM.

[c] Number of animals in parentheses.

[d] N.M., not measured.

output by 12 and 16% in group C, and by 4 and 20% in group D (average of two to four separate determinations, which varied less than 7% of the mean). Thus dehydration had no effect on hepatocyte basal or vasopressin-stimulated glucose production under conditions which controlled for differences in initial glycogen content.

Both plasma osmolality and hematocrit were increased after 48 hr of water deprivation (Table I).

Release of $^{14}CO_2$ from [1-^{14}C]pyruvate. The results so far presented show no change in response of rat hepatocytes to ADH stimulation of glucose production as a result of water deprivation. This conclusion might be vitiated, however, if ADH caused a large increase in flux through pyruvate dehydrogenase or a large increase in the release of lactate into the medium. Hems *et al.* have shown that ADH does not increase the output of lactate from hepatocytes of fed rats (4) and we have confirmed this in hepatocytes from dehydrated rats as well (not shown). Hems *et al.* (24) found that the activity of pyruvate dehydrogenase in homogenates of perfused liver from fed rats was increased up to 50% by perfusion with ADH. Since decarboxylation of [1-^{14}C]-pyruvate provides a direct assay for flux

through pyruvate dehydrogenase,[1] it was possible to measure this flux in intact hepatocytes. The results (Table II) showed that ADH stimulates flux through pyruvate dehydrogenase at most 12% in the intact hepatocytes and that there was no difference in response between hepatocytes from control and dehydrated rats.

Glycogen synthesis in hepatocytes from dehydrated rats. Having established the essential features of the inhibitory effect of ADH on glycogen synthesis in hepatocytes from rats fasted for 24 hr (Fig. 1), we next examined this effect of ADH on hepatocytes from 48-hr-dehydrated rats. Again it was noted that hepatocytes from dehydrated rats with access to food *ad libitium* had an initial glycogen content between those of fed and fasted rats, while hepatocytes from dehydrated fasted rats had glycogen levels comparable to those of control, fasted rats (Fig. 4B and Table I). Hepatocytes from dehydrated rats (Fig. 4B) attained rates of glycogen synthesis within the range of values from fasted control rats (Fig. 4A), and responded to ADH by glycogenolysis and inhibition of net glycogen synthesis, as seen in hepatocytes from controls.

Discussion. The reported minimum effective concentration (0.1 p*M*) and half-maximal effective dose (~10 p*M*) of vasopressin for hepatocyte phosphorylase activation (4) are well within the range of plasma levels of ADH in control (2–4 p*M*) and 48-hr-dehydrated (17 p*M*) rats (9, 10).[2] The acute hepatic effects of vasopressin have been well documented (2–5). However, neither the time course of the reversal of these acute hormone effects nor the possible hepatic effect of chronic vasopressin exposure has previously been investigated in detail.

TABLE II. EFFECTS OF VASOPRESSIN AND DEHYDRATION ON PRODUCTION OF ^{14}C-CO_2 FROM [1-^{14}C]PYRUVATE

Rat treatment	$^{14}CO_2$ from pyruvate: Control (nmole/hr·mg protein)	$^{14}CO_2$ from pyruvate: ADH effect as % of control
Fasted 24 hr	9.7	108
	10.5	106
Dehydrated 48 hr, fasted 24 hr	9.9	110
	8.6	112

Note. Hepatocytes were incubated for 60 min with 5 m*M* lactate, 0.5 m*M* pyruvate with tracer [1-^{14}C]-pyruvate (sp act 0.1 Ci/mole), and 0.6 m*M* acetate, in the absence or presence of 3×10^{-9} *M* vasopressin. Higher hormone concentrations had no further effect (data not shown). $^{14}CO_2$ production was assayed as described under Materials and Methods. Each value is the mean of measurements on two or four separate incubation flasks which varied by less than 6% of the mean. Radioactivity recovered as CO_2 ranged from 13 to 27% of the amount added.

In the studies reported here, the acute inhibitory effect of vasopressin on glycogen synthesis (Fig. 1) was found to be quickly reversed after removal of the hormone from the medium (Fig. 2). These results suggest that, if hepatic glycogen synthesis is affected by ADH levels *in vivo*, this regulation is a short-term response. Therefore, it is not surprising that cells from dehydrated rats showed no inhibition of glycogen synthesis (Fig. 4), since the time required for hepatocyte preparation was over 1 hr. In contrast to the rapid reversal of the effect of vasopressin after removal of the hormone from the medium (Fig. 2), when excess vasopressin is present throughout the hepatocyte incubation, its effect persists (Figs. 2 and 4).

Possible hepatic effects of chronic vasopressin exposure *in vivo* were investigated by comparing basal and vasopressin-mediated carbohydrate metabolism in hepatocytes from control and dehydrated rats. In these studies, 48 hr of water deprivation caused the expected increases in plasma osmolality and hematocrit (Table I), previously shown to be associated with 5- to 10-fold elevated levels of plasma vaso-

[1] Although appreciable $^{14}CO_2$ may be fixed via pyruvate carboxylase, most of that CO_2 will be released as $^{14}CO_2$ by the action of *p*-enolpyruvate carboxykinase on the [4-^{14}C]oxaloacetate formed in the fixation step.

[2] Approximately half-maximal phosphorylase activation in isolated hepatocytes by vasopressin has been observed in our laboratory at the lowest concentration tested, 30 p*M* (submitted for publication).

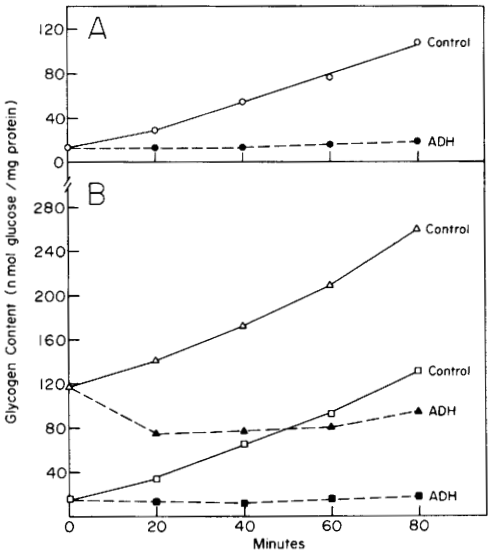

FIG. 4. Glycogen synthesis in hepatocytes from fasted (A) or dehydrated (B) rats. Hepatocytes were incubated with 30 m*M* glucose, 5 m*M* lactate, 0.5 m*M* pyruvate, and 0.6 m*M* acetate, in the absence (open symbols) and presence (closed symbols) of 10^{-7} *M* vasopressin, and glycogen was measured. A: (○, ●) 24-hr-fasted rats; each point represents the mean of two separate experiments which differed by less than 10%. B: 48-hr-dehydrated rats—(△, ▲) fed *ad lib;* (□, ■) fasted final 24 hr; each point is the mean of four measurements which varied less than 10%, on a single hepatocyte preparation.

pressin (9). Both basal and ADH-stimulated glucose production and glycogenolysis by hepatocytes from dehydrated, fed rats were much lower than from control, fed rats (Table I, columns A and B). When glycogen is abundant in hepatocytes, glycogenolysis is more than sufficient to account for glucose release [Table I, columns A and (4)]. Because water deprivation leads to a reduced intake of food, hepatocytes from dehydrated rats have low glycogen content and therefore produce a reduced amount of glucose. However, the fractions of their initial glycogen broken down by hepatocytes from fed, control rats and fed, dehydrated rats were comparable: about 37 and 42%, respectively (Table I). The decrease in glucose output after dehydration is therefore probably a reflection of the decreased initial glycogen levels (due to decreased feeding) rather than an effect of dehydration per se. Furthermore, when the ADH-mediated stimulation of glucose output is calculated as a percentage of the basal response no differences are seen between hepatocytes from control and dehydrated rats (Fig. 3). Under conditions in which glucose output is mostly due to gluconeogenesis there were also no differences in basal or ADH-stimulated glucose release in hepatocytes from control *versus* dehydrated animals (Table I, columns C and D).

The finding that oxygen consumption of perfused liver was increased by ADH (4) led Hems *et al.* (24) to examine pyruvate dehydrogenase activity. That this activity increased 25–50% in response to ADH in the perfused liver of fed rats suggested that ADH activation of this enzyme might account for the increase in Q_{O_2}. A direct assay of flux through the enzyme in the intact cell (Table II), however, showed that ADH caused only a very small increase. Both basal and ADH-stimulated pyruvate decarboxylation were unaffected by prior dehydration. The small or negligible changes in flux through pyruvate dehydrogenase caused by ADH and/or dehydration suggest that alteration in activity of this enzyme is responsible for neither the ADH-enhanced Q_{O_2} observed by Hems *et al.* (4) nor the reduced Q_{O_2} observed by Bartok *et al.* (25) in livers from 6-day-dehydrated rats. The slight stimulation of pyruvate decarboxylation by ADH contrasts with the 10 to 20% inhibition by glucagon in isolated hepatocytes (26), and offers a rare example of opposing hepatic effects of these two glycogenolytic and gluconeogenic hormones.

As noted above, dehydration of the rat did not decrease glycogen synthesis by the isolated hepatocytes (Fig. 4). Thus in all four aspects of carbohydrate metabolism studied, no evidence was found that chronic vasopressin exposure during water deprivation caused any lasting changes in hepatocyte enzyme activities or in hepatocyte response to vasopressin. This accords well with the rapid reversal of the acute effects of ADH here demonstrated.

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