Influence of Hemorrhage on Adrenal Secretion, Blood Glucose and Serum Insulin in the Awake Pig

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A study was performed to quantitate the adrenal medullary and cortical response to hemorrhage in awake animals bled at different rates and to relate these responses to simultaneous changes in blood glucose and serum insulin. A series of awake pigs were bled either slowly or rapidly of 30% of their calculated blood volume. Infusions of exogenous epinephrine were performed in an additional series of unbled animals and infusions of epinephrine plus hydrocortisone were similarly performed in an additional series. Increase in blood glucose and epinephrine secretion rate following hemorrhage were found to be significantly dependent upon the rate of initial hemorrhage. Cortisol secretion was found to rise significantly during and following hemorrhage in both rapidly and slowly bled animals. Serum insulin levels remained at baseline levels during shock, despite the presence of significant hyperglycemia. In unbled animals infused with epinephrine at rates comparable to those measured in shock, elevations in blood glucose were markedly lower, shifting to the right of the dose-response curve during hemorrhage. Simultaneous infusions of cortisol and epinephrine resulted in a dose-response curve which did not differ significantly from that following infusion of epinephrine alone.

POSTHEMORRHAGIC HYPERGLYCEMIA was first described by Claude Bernard in 1877[5] and has since been well documented both in animal experiments and in clinical studies. Evidence has recently been presented that elevation of blood glucose is significantly related to the rate of initial blood loss, being much higher following rapid hemorrhage.[9] Although direct proof is lacking, the etiology of shock hyperglycemia has been attributed to an increased secretion of catecholamines during blood loss. The mechanism is thought to be principally the effect of epinephrine on hepatic glycogen breakdown as well as its more recently demonstrated suppressive effect on pancreatic insulin secretion.

The present study was performed to quantitate the adrenal medullary and cortical response to hemorrhage in awake animals bled at different rates and to relate these responses to simultaneous changes in blood glucose and serum insulin. A series of awake pigs were bled either slowly or rapidly of 30% of their calculated blood volume. The baseline adrenal epinephrine secretion rate (10.9 ± 1.6 ng/kg/min) obtained with the experimental model agreed closely with previously measured values. Changes in blood glucose correlated closely with epinephrine secretion rate ($r = 0.86$, $P < 0.001$), and both were higher in rapidly bled animals (80 ± 26 mg % vs 18 ± 13 mg %). The rate of adrenal corticosteroid secretion was increased more rapidly than epinephrine secretion during hemorrhage and its magnitude was not dependent on rate of initial hemorrhage.

In a second series of animals infused with epinephrine at rates similar to those found in shock, less hyperglycemia occurred, suggesting other contributing factors. Results were not altered by simultaneous infusion of hydrocortisone. No significant increase in insulin secretion occurred in response to hyperglycemia following blood loss. This is felt to represent suppression of insulin secretion by elevated levels of catecholamines.

Methods

Hemorrhage Study

All studies were performed upon young, unanesthetized, cross-bred pigs weighing 10 to 18 kg. All

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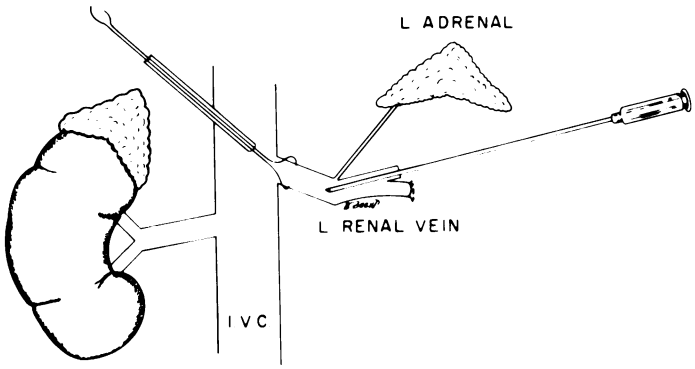

FIG. 1. Diagram of experimental preparation allowing intermittent determination of adrenal secretion rates.

animals were trained to tolerate suspension in a Pavlov stand without excitement. Twelve animals were anesthetized on the day prior to study and underwent a left nephrectomy with ligation of the left renal vein just distal to its orifice (Fig. 1). Although the right adrenal vein frequently was found to enter directly into the vena cava, the left adrenal vein was found to consistently enter into the left renal vein. A siliconized catheter was then placed in the renal vein stump and a silk choker was placed around the left renal vein at its junction with the inferior vena cava. Any tributaries of the renal vein other than the left adrenal vein were ligated and divided. The position of the choker and the catheter was carefully checked so that no obstruction to the adrenal vein was present when the choker was released. Additional catheters were placed in the inferior vena cava and aorta through the femoral vessels. The choker and all catheters were then brought through the skin of the back and taped in place. Allowed only water during the 10 hours preceding study, the pigs were fed after recovery from anesthesia.

On the day of the study, the experimental animal was placed in the Pavlov stand. After thirty minutes, baseline samples were obtained and the animals were then bled through the venous line of 30% of the calculated blood volume, either rapidly (30 min) or slowly (80 min). During each of three periods of 10% hemorrhage, the choker was tightened, allowing the entire left adrenal venous drainage to be collected and the time required to take the sample accurately recorded. Peripheral venous samples were drawn at the same time. Similar samples were drawn at 30, 60, and 90 minutes following the end of hemorrhage. Samples drawn after the initial hemorrhage accounted for an additional loss of 15% of the calculated blood volume.

In all animals vena cava glucose and insulin levels were measured during the sample periods. In 6 of the animals, adrenal epinephrine secretion rate and peripheral venous epinephrine and norepinephrine levels were also measured. In a second group of 6 pigs, adrenal corticosteroid secretion rates were determined. Autopsies were performed upon all animals and it was verified that catheter and choker position had not changed.

Blood volumes were calculated, employing a standard formula for pigs using T-1824[32], which has been checked frequently in our laboratory and has been found to be consistently accurate.

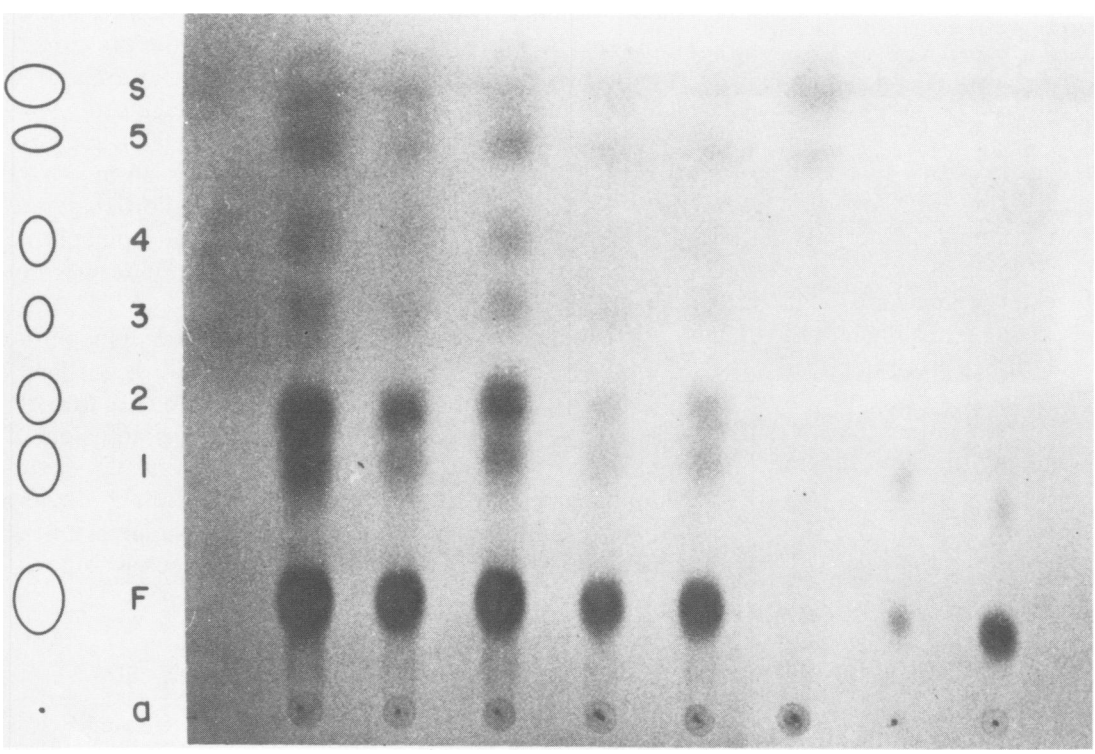

FIG. 2. A thin-layer chromatogram from one experiment. Reading from right to left the columns represent a 2 μg cortisol and 2 μg corticosterone standard; the pig adrenal venous plasma extract at the beginning of the experiment during initial 10% hemorrhage; and levels of the remaining extracts concluding with the 90-minute posthemorrhage extract on the far left. a = point of application, F = cortisol, 1 = corticosterone and cortisone, 2 = cortexolone (Reichstein's substance S), 3 = 11-B-hydroxyandrostenedione, 4 = cortexone (desoxycorticosterone) and 17-alpha-hydroxyprogesterone, 5 = progesterone, and S = unknown substance migrating with the solvent front.

Infusions in Unbled Animals

Infusions of exogenous epinephrine (8 to 1024 ng/kg/min) were performed in 12 animals which were awake and immobilized in a Pavlov stand. The epinephrine solution was infused through a catheter which had been placed in the inferior vena cava via a femoral cutdown on the day before the study. A Harvard Apparatus infusion pump was used for control of infusion rate, and oxidation of epinephrine was prevented by the addition of small amounts of ascorbic acid.

Infusions of epinephrine plus hydrocortisone (1 mg/kg/min) were similarly performed in an additional 12 animals.

Chemical Assays

Glucose determinations were performed using the O-toluidine reaction.[18] Insulin levels were measured using a standard immunoassay technique.[29] Plasma catecholamine concentrations were determined by a fluorometric method described elsewhere.[27] Intermittently, excitation and fluorescence spectra were checked, using a Farrand Mark I spectrophotofluorometer.

The total epinephrine secretion rate (TESR) was obtained by multiplying the left adrenal vein epinephrine concentration (microgram/L) by the left adrenal vein plasma flow (ml/min), dividing by the body weight (kg), and multiplying the last figure by 2, on the assumption that the right adrenal was secreting as much epinephrine as the left adrenal.

Adrenal vein plasma cortisol concentrations were determined by a method suggested by the work of Uetwiller and Keller.[30] Approximately 15 ml of plasma were placed in a 50 ml centrifuge tube which was filled with methane. After 15 seconds of shaking, the mixture was centrifuged at 1500 rpm for 5 minutes. (This step removed phenolic substances, especially estrogens.) The dichloromethane layer was aspirated and evaporated to dryness. The residue was spotted on a precoated silica-gel G plate impregnated with a 254 mμ phosphor; 10 μg and 2 μg standards of hydrocortisone were spotted on each plate. The plates were developed in 95:5 chloroform methanol for a distance of 13 to 15 cm, requiring about 60 to 90 minutes. The appearance of a typical thin-layer plate viewed under a 254 mμ hand lamp is shown in Fig. 2. The air-dried

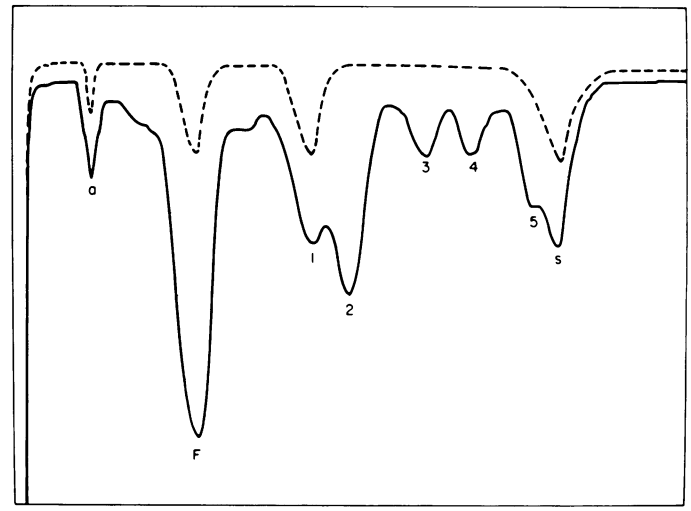

FIG. 3. Superimposed scans of two columns from a typical thin-layer plate. Abbreviations are the same as in Fig. 2. The top of the plate is on the right. The solid line is an adrenal plasma extract and the dotted line is from 2 μg cortisol and 1 μg corticosterone standards.

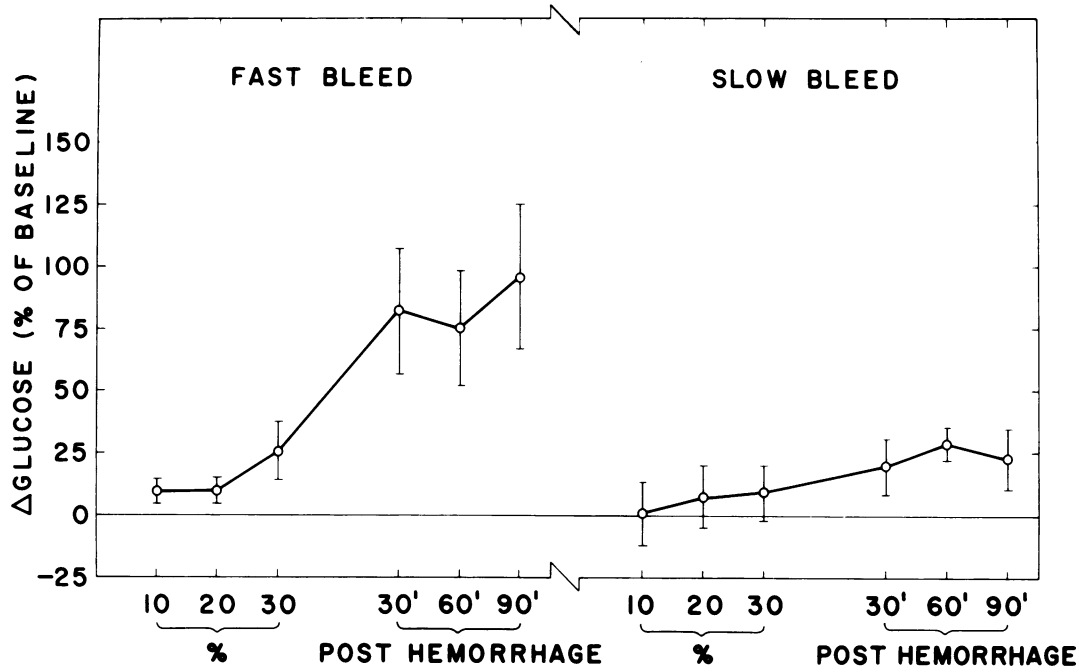

FIG. 4. Changes in blood glucose levels (± S.E.M.) during and following 30% hemorrhage in slowly and rapidly bled animals.

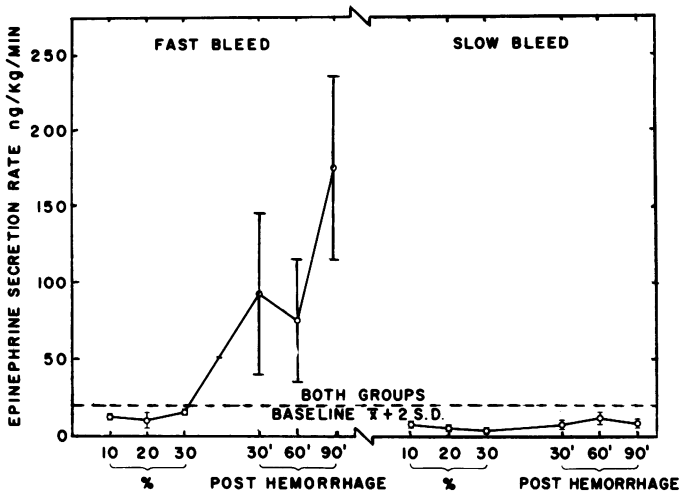

FIG. 5. Epinephrine secretion rates ($\bar{x} \pm 2$ S.D.) in rapidly bled and slowly bled animals during and following 30% hemorrhage.

plates were then placed in the thin-layer chromatogram scanning attachment of a Ferrand Mark I spectrophotofluorometer connected to a chart recorder. The excitation was set for 254 mμ with emission at 270 mμ. All slits were 5 mμ and the widest aperture was used. The quench profiles obtained from a sample and a standard are shown in Fig. 3.

The lower limit of detection of the method described was approximately 200 ng of cortisol with the instrument set to record at range 1. For less concentrated samples, the scale deflection could be increased 40 to 50% by setting the emission monochromator at 265 mμ and/or could be increased 3.3-fold by setting the range at 0.3.

The major glucocorticoid of pig adrenal venous plasma was found to be cortisol. Smaller quantities of cortexolone (Reichstein's substance S) were also identified. A third, faint spot represented the overlap between very small amounts of cortisone and corticosterone. All four of these substances were identified in extracts of pig adrenal. A fourth spot, also faint, represented the overlap between 17-α-OH-progesterone and cortexone (desoxycorticosterone). In preliminary studies it was found that the quenching intensity was always proportional to the logarithm of the weight. Accordingly, for each plate a curve of the two standards was plotted on semilog paper and the weight of cortisol in each dichloromethane extract was read from it. Although only cortisol was quantitated, it was apparent from inspection of the plates that whenever the concentration of cortisol increased, the concentrations of most of the other steroids also increased (Fig. 3). Cortisol concentrations were converted to total cortisol secretion rates by the same calculations described from TESR (vs).

Results

Hemorrhage Study

Increase in blood glucose following hemorrhage was found to be significantly dependent upon the rate of initial hemorrhage. Glucose values in rapidly bled animals had increased approximately 80 ± 26 (S.E.) mg% at 30 minutes posthemorrhage compared to an increase of 18 ± 13 mg% in the slowly bled group ($P = 0.05$) (Fig. 4). Similar significant differences were also found at the end of 60 and 90 minutes. Changes in blood pressure, expressed as per cent fall from baseline, were not significantly different between the rapidly and slowly bled animals.

Epinephrine secretion rate in the rapidly bled animals was not found to rise significantly during hemorrhage but was elevated at 30, 60, and 90 minutes following hemorrhage (Fig. 5). These rates are probably not miximal (see appendix). In contrast, no increase in the epinephrine secretion rate was demonstrated in slowly bled animals despite the loss of the same relative volumes of blood. The differences in epinephrine secretion response between the two groups is comparable to the previously described differences in blood glucose elevation.

Epinephrine secretion rates correlated closely with changes in blood glucose values ($r = .86$, $P < 0.001$). Venous epinephrine and norepinephrine levels correlated closely with adrenal secretion rates as would be expected ($r = .813$, $P < 0.001$) (Table 1). A more marked rise in peripheral norepinephrine levels was found in the rapidly bled animals. However, this experimental technique provided no direct measurement of the norepinephrine secretion rate, since in the pig, as in most animals, significant amounts of norepinephrine are secreted in extra-adrenal sites.

TABLE 1. *Mean Values (S.E.) of Peripheral Venous Epinephrine and Norepinephrine (ng/ml) During and Following Hemmorrhage*

	10%	20%	30%	10 min	20 min	30 min
Rapidly Bled						
Epinephrine	0.41 ± .26	2.45 ± .48	10.18 ± 4.74	8.50 ± 5.18	13.12 ± 6.46	26.76 ± 16.27
Norepinephrine	0.26 ± .14	0.28 ± .13	1.50 ± .44	1.65 ± .32	1.55 ± .32	24.91 ± 13.15
Slowly Bled						
Epinephrine	0.62 ± .07	0.77 ± .16	0.95 ± .45	2.98 ± .31	0.52 ± .30	0.07 ± .02
Norepinephrine	0.41 ± .25	0.08 ± .02	0.05	0.05	1.08 ± .11	0.67 ± .04

Cortisol secretion was found to rise significantly during and following the end of hemorrhage in both rapidly and slowly bled animals (Fig. 6).

No increase in serum insulin levels occurred in response to the hyperglycemia following blood loss (Fig. 7). Serum insulin levels remained at baseline levels (33.8 ± 8.0 μμ/ml) during shock, despite the presence of significant hyperglycemia.

Infusion Study

When unbled animals were infused with epinephrine at varying dose levels and the subsequent changes in blood glucose were graphed, a typical dose-response curve was obtained (Fig. 8). The curve, however, was shifted significantly to the right of that obtained during the hemorrhage study, and it was suspected that increased levels of glucocorticoids following hemorrhage might add to the hyperglycemic effect of epinephrine. For this reason, simultaneous infusions of cortisol and epinephrine were performed in an additional series of 12 animals to determine the effect of high infusion rates of cortisol on the epineprhine-glucose dose-response curve. The dose-response curve so obtained, however, did not differ significantly from that following infusion of epinephrine alone.

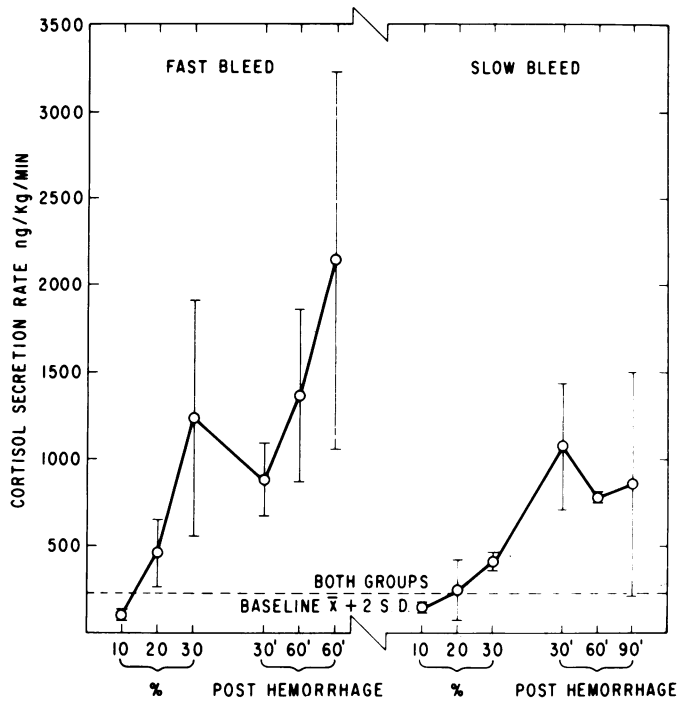

FIG. 6. Cortisol secretion rates (x̄ ± 2 S.D.) in rapidly bled and slowly bled animals during and following 30% hemorrhage.

Discussion

Baseline Secretion Rate

The experimental model described appears to furnish a valid and relatively simple method of studying adrenal response in an awake animal, yielding results similar to previously reported methods. The baseline adrenal epinephrine secretion rate was calculated as 10.9 + 1.6 ng/kg/min. Relatively little variation in the baseline values was found and this is felt to be due to the relatively long sampling period of 10 minutes. Similar baseline values in experimental animals have been reported: Walker et al. measured mean secretion rates of 11.1 and 5.9 ng/kg/min in two groups of unanesthetized dogs,[33] and Ikeda reported a basal adrenal epinephrine secretion rate varying from 4 to 11 ng/kg/min during two consecutive control periods in dogs.[19] The baseline secretion rate was also similar to that predicted in man to be 8 ng/kg/min by Vendsalu[31] and 10 ng/kg/min by Cohen et al.[11] more recently. A directly measured value in man of 8.7 ng/kg/min has been obtained by Sapira and Bron,[26] using a direct adrenal vein catheterization technique.

Epinephrine Secretion Rate

Blood Glucose Levels. Increase in blood glucose levels was found to be significantly dependent upon the rate of initial hemorrhage. This agrees with the previous report of Carey and Wallack in which a similar relationship between rate of hemorrhage and hyperglycemic response was found in anesthetized pigs.[9] Increase in epinephrine secretion rate was found to be similarly dependent upon initial rate of hemorrhage. It is significant that slowly bled animals developed only a minimal rise in epinephrine secretion rate despite the loss of approximately 42% of calculated blood volume. Fall in blood pressure was not significantly different between the two groups. This suggests that the stimulus for epinephrine secretion is dependent on rate of decrease of volume. Recently it has been recognized that the rate of plasma refill is considerably more rapid than previously believed.[8] Although this could not be calculated from data obtained in the present study, it is possible that plasma refill may have occurred rapidly enough in the slowly bled animals that the stimulus for epinephrine secretion was minimal. In a study of hemorrhagic shock performed later in this laboratory on awake pigs bled rapidly of 45% of calculated blood volume, plasma refill calculated from chages in hematocrit following hemorrhage was estimated to restore 25% of original bled volume within one hour. An additional explanation is suggested by a recent clinical study of variation in hemodynamic responses following hemorrhage over a relatively long (>4 hrs) or short (<4 hrs) period.[2] Higher cardiac outputs, arterial pressures, and central blood volumes were found in patients who had bled slowly. This was felt to represent the effects of a

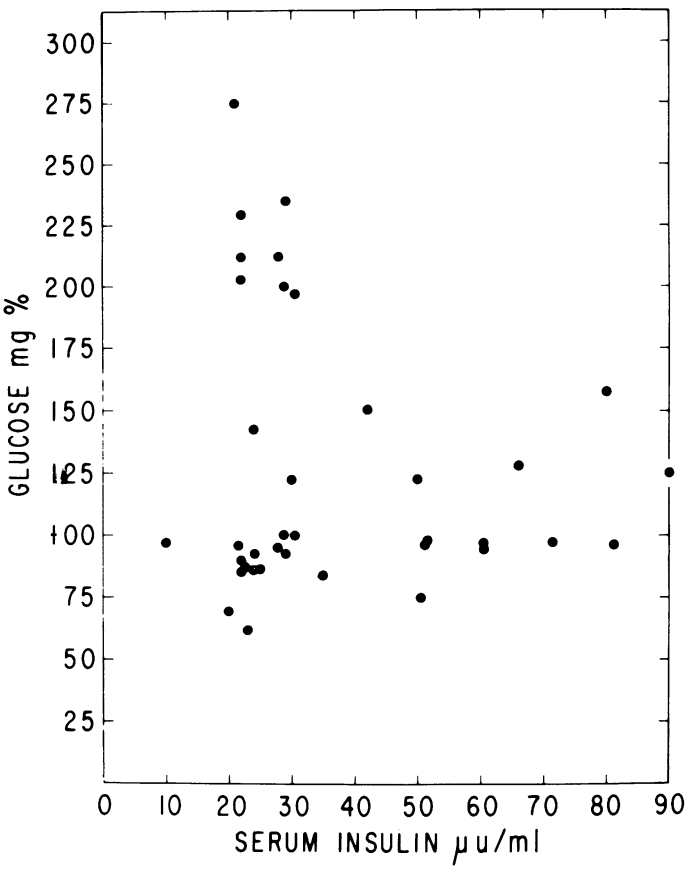

FIG. 7. Blood glucose (mg%) versus serum insulin (μu/ml) determined during and following 30% hemorrhage.

redistribution of blood volume into the central pool of blood.

The adrenal epinephrine secretion rate correlated closely with blood glucose levels following hemorrhage. If epinephrine secretion was primarily responsible for hyperglycemia, as was suspected, it was felt that similar levels of hyperglycemia would be achieved by infusion of epinephrine at similar rates into nonbled animals. When nonbled animals were infused with epinephrine at rates comparable to those measured in shock, elevations in blood glucose were markedly lower, shifting to the right, the dose-response curve during hemorrhage (Fig. 8). The presence of the adrenal medulla has been previously shown to be necessary for the occurrence of the hyperglycemic response.[14,23] The present data, however, indicate that additional humoral factors or peripheral metabolic changes significantly alter or contribute to the effect of epinephrine on carbohydrate metabolism during hemorrhagic shock.

Cortisol Secretion

Steroids are known to modify the kinetics of catecholsensitive reactions.[1] It is also well established that adrenal cortisol secretion increases following hemorrhage, although the exact mechanism triggering this release is incompletely understood. Because of the possible contributions of elevated cortisol to hyperglycemia following hemorrhage, adrenal cortisol secretion was found to increase in response to hemorrhage. The adrenal cortical response was found, however, to differ from the medullary response in several important respects.

A marked difference was found between the rapidity with which the adrenal medulla and the cortex responded to the stimulus of hemorrhage. In most animals a significant increase in cortisol secretion was found during the second 10-minute period of hemorrhage (while blood loss was approaching 20% of blood volume). This response differed from that of adrenal epinephrine secretion, which was only first significantly increased in samples taken 30 minutes following hemorrhage. Although the chemical methods used did not allow simultaneous measurement of both epinephrine and cortisol in the same animals, results indicate that cortisol secretion occurs much more rapidly than epinephrine secretion in response to hemorrhage. This is in agreement with studies reported by Gann and Egdahl who found that adrenal vein levels of 17-OH corticosteroids in dogs were elevated in samples drawn 15 minutes following hemorrhage.[18]

No relationship was found between cortisol secretion and rate of hemorrhage. Even in slowly bled animals, cortisol secretion was elevated before hypotension occurred. These results agree with previous studies

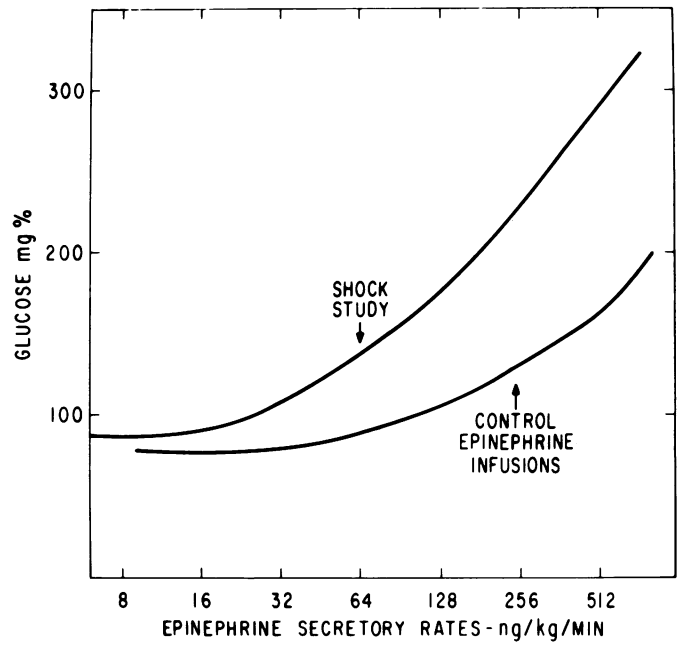

FIG. 8. Glucose-epinephrine response curves determined during adrenal secretion response to hemorrhage and during control epinephrine infusions.

demonstrating that the stimulus for adrenal cortical secretion is not dependent upon hypotension alone.[1,15]

Lack of correlation between glucose and cortisol secretion, although a significant part of the physiologic response to hemorrhage, seems to indicate that increased cortisol secretion does not significantly contribute toward hyperglycemia following hemorrhage. It was in order to verify this that unbled animals were "infused" with both epinephrine and cortisol. The glucose-epinephrine dose-response curve obtained by infusion of unbled animals with epinephrine alone was not significantly shifted by the additional infusion of cortisol.

Assuming, for the above reasons, that elevated levels of cortisol do not significantly alter or increase effects of epinephrine during shock, additional hyperglycemic factors following hemorrhage are poorly defined. Growth hormone is known to increase following injury and blood loss.[28] This was previously felt to be a somewhat delayed reaction; however, in a study of Vietnam War casualties, levels of both glucose and GH were elevated on admission to the hospital.[6] But the relationship between growth hormone and hyperglycemia is poorly defined. In the pig, growth hormone has been shown to cause a minimal rise in blood glucose[15] and it is doubtful that this alone would account for the unexplained hyperglycemia.

Peripheral glucose utilization is an additional factor which must be considered. The decreased cellular perfusion associated with hemorrhage and shock causes a shift of cellular metabolism from aerobic to anaerobic. Because of this, and because of epinephrine's effect of suppressing insulin secretion, it is likely that glucose utilization is decreased. Peripheral glucose changes during hemorrhage have been extremely difficult to quantitate in vivo and at the present time these are incompletely understood. However, if glucose utilization is decreased during hemorrhagic shock, this would offer a possible mechanism to account for the hyperglycemia noted in this study.

Insulin Secretion

With the availability of accurate insulin immunoassay techniques, investigation of change in insulin secretion during various shock states has begun. Comparison between different experimental shock studies in this area is difficult however, because of varying responses in different animal species as well as significantly different shock preparations. A rise in both insulin secretion and plasma insulin levels has been demonstrated in dogs. However, some evidence was found for a partial inhibition of insulin release when intravenous glucose tolerance tests were performed on dogs in hemorrhagic shock.[23] Recent studied in subhuman primates have failed to demonstrate rises in serum insulin in response to hemorrhage.[10,12] In fact, during the first hour in a hypotensive state, insulin values fell significantly below control levels, returning during the second hour to control levels. In addition, decreased response to tolbutamide was demonstrated following hemorrhage. Failure of insulin response to hyperglycemia has been reported in man during hypothermia,[4] cardiogenic shock,[13] and hemorrhagic shock.[7,20] In the latter study, insulin values were found to rise in response to hyperglycemia following initiation of resuscitative measures. The high levels of epinephrine found in shock are felt to be the predominant factor contributing to the observed failure of plasma insulin rise. This inhibitory effect of epinephrine has been demonstrated in humans,[25] in pigs,[17] and in isolated pancreatic slices, and appears to be predominantly an alpha receptor effect.[24] In the present study no evidence for increased insulin response to hyperglycemia was found. This suggests significant suppression of insulin secretion, as plasma insulin levels have been previously demonstrated to increase in pigs (although relatively less than in man) in response to hyperglycemia.[21] Findings were consistent with epinephrine effect, as hyperglycemia was almost always associated with elevated epinephrine secretion rates. Mean values of insulin decreased slightly during the study but this was not statistically significant. Changes in pancreatic blood flow and peripheral insulin breakdown are also of significance but are poorly understood at present.

Appendix

A 15-kg pig with a left adrenal weighing 1.671 gm containing 140 μg of epinephrine per gm has total body stores of epinephrine of approximately 470 μg. At 15-kg pig at a basal total epinephrine secretion rate of 10 ng/kg min would require only 9 μg of epinephrine during an hour. With the epinephrine secretion rate at 100 ng/kg/min for 100 minutes, only 150 μg would be required, or less than a third of the available. None of our animals reached or maintained a 300 ng/kg/min total epinephrine secretion rate for 100 minutes, which would have required 450 μg of epinephrine, i.e. over 95% of that available.

If the elevated total epinephrine secretion rate of 100 ng/kg/min were maintained for 300 minutes, 450 μg of epinephrine would be required. However, this would not be greater than 95% of the total available for the following reason: At a basal secretion rate of 10 ng/kg/min a 15-kg pig would have to synthesize 150 ng/min to maintain steady states. With a total body store of 470 μg this would mean that 03% of the total body store is synthesized per minute, or 9.6% of the original total body store would have been newly synthesized during the 300 minutes.

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