an inhibitory activity; if the strain is resistant to one of the pair of antibiotics, its presence in a high but ineffective concentration may not influence the development of resistance to the second agent.

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Fatty Acid and Ketone Metabolism during Hemorrhage and Shock in the Rat.* (20434)

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Although there is now available considerable information concerning protein and carbohydrate metabolism during hemorrhagic shock in the rat, little or nothing is known about fat metabolism in this syndrome(1-3). Since hepatic metabolism appears to be particularly disturbed during shock, it seemed pertinent to investigate some aspects of ketone body metabolism since the liver is the chief site of synthesis of these substances. It has been shown that the oxygen consumption and the rates of a variety of oxidative reactions by liver slices from shocked rats may be profoundly depressed, but the degree of depression of oxygen consumption cannot be accounted for by the metabolic disturbances so far demonstrated in the liver. Since, during fasting, fat oxidation and ketone production may account for a very substantial part of the oxygen requirements of the liver, this study was initiated in an attempt to determine whether fatty acid and ketone metabolism might be sufficiently disturbed during hemorrhage and shock in the rat to contribute to the observed lowered hepatic oxygen consumption.

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Materials and methods. Male albino rats of the Vanderbilt strain, weighing between 200 and 270 g, were fasted 24 hours prior to bleeding. Shock was induced under light nembutal anesthesia by bleeding from the cut tail a volume of blood equivalent to 2.5% of the body weight in one hour. Blood samples for ketone and amino nitrogen determinations were withdrawn at appropriate intervals, and the animals observed for 3 to 5 hours and then sacrificed for other studies(4,5). In one group of rats, adrenalectomies were performed 4 to 5 days prior to bleeding, the animals being maintained with 0.5 mg of desoxycorticosterone acetate intramuscularly daily and saline in their drinking bottles. Ketone levels were determined on 0.4 ml samples of heparinized whole blood by a modification of the method of Greenberg and Lester(6,7) and amino nitrogen in plasma by the method of Frame *et al.*(8).

Results. The effects of hemorrhage and shock on blood ketone levels are depicted in Fig. 1. The control rats, which had blood samples drawn just for analyses every hour for 7 hours, exhibited a slow progressive rise in ketone levels, as might be anticipated during a continuing fast. The bled rats were divided into 2 groups, designated as "sublethal hemorrhage" and "hemorrhagic shock"

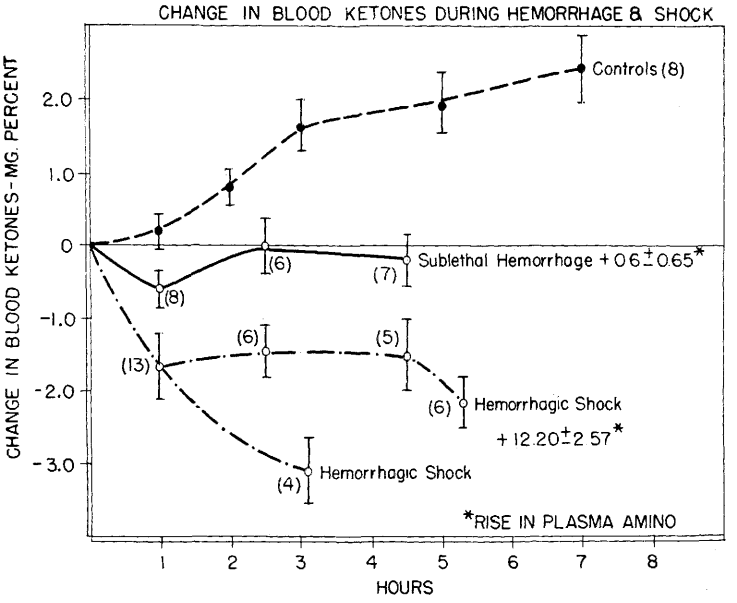

FIG. 1. Mean change in mg % ± S.E. in blood ketone levels in rats subjected to hemorrhage and shock. The numbers in parentheses refer to the number of observations at each point.

in terms of their clinical condition and degree of elevation in plasma amino nitrogen. Previous studies have demonstrated that the rate and degree of rise in plasma amino nitrogen following hemorrhage is a reliable indication of the severity of shock in the rat(9,10). On the basis of these data, all animals which had shown less than 3 mg % rise in amino nitrogen 3 to 4 hours after bleeding were classified as "sublethal hemorrhage", while those with greater than 3 mg % rise were considered to be in irreversible shock. Actual survival could not be determined since the animals were sacrificed at the end of the experiment for other studies. Note that the "sublethal hemorrhage" group had a mean plasma amino nitrogen elevation of 0.6 ± 0.65† mg % and that the usual rise in blood ketone levels was abolished. The hemorrhagic shock animals were divided into 2 groups in terms of their being obviously preterminal at approximately 3 and 5½ hours, respectively. In both groups, there was a very significant depression of ketone levels, the degree of depression varying with the rapidity with which shock developed. Plasma amino nitrogen levels rose progressively, with the mean final levels for all animals being +12.20 ± 2.57 mg %.

In a group of 6 adrenalectomized rats bled 2.0 and 2.5% of body weight, essentially similar results were found. Ketone levels dropped 1.21 ± 0.45 mg % at the end of bleeding and 2.16 ± 0.47 mg % by 3½ hours, plasma amino nitrogen increasing by 9.50 ± 2.08 mg %.

In an attempt to seek an explanation for the hypoketonemia of hemorrhage and shock, the effect of an intravenous infusion of sodium octanoate on ketone levels during hemorrhage and shock was investigated. Sodium octanoate, a fatty acid which is a well known ketone precursor, was infused over a 30-minute period into the external saphenous vein in a dose of 1.5 ml of a sterile 2% solution per 100 g body weight, beginning 2½ hours after bleeding the rats 2.5% of their body weight. The results are seen in Fig. 2, where they are plotted as mean change in blood ketone levels from the beginning to the end of the infusion. Ketone levels at the end of the infusion in normal animals increased by 4.40 ± 0.16 mg %, infusion of an equal volume of saline having no measurable effect. The ketone levels reach their peak at the end of the infusion and thereafter rapidly decline. In rats in mild shock,

† All values expressed as mean ± standard error.

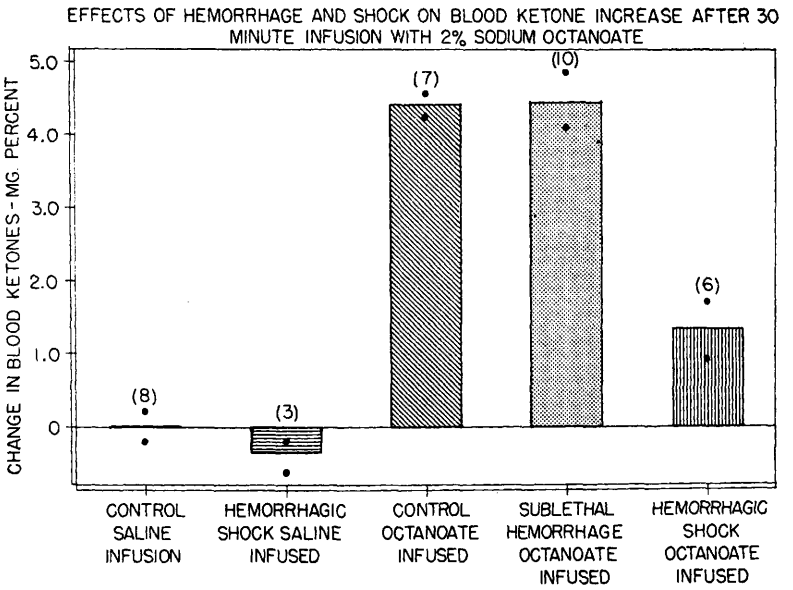

FIG. 2. Mean change in mg % ± S.E. in blood ketone levels during a 30-min. intravenous infusion of 1.5 ml of 2% sodium octanoate per 100 g body wt in normal and bled rats.

as indicated by a mean rise in plasma amino nitrogen of only 1.02 ± 0.20 mg %, the response to octanoate infusion was identical with the controls, *i.e.*, a rise of 4.45 ± 0.43 mg % in 30 minutes. In contrast, rats in severe shock, with amino nitrogen elevations of 6.08 ± 1.77 mg % prior to infusion, exhibited a significantly smaller rise in ketones after octanoate infusion, amounting to 1.35 ± 0.46 mg %. Compared with the sublethal hemorrhage group, these animals tolerated the infusion poorly, usually dying shortly after its termination with amino nitrogen levels rising to 13.81 ± 3.16 mg % above the starting levels. Comparable shocked rats, receiving a similar volume of saline, showed an insignificant fall in blood ketones. In this group, plasma amino nitrogen had risen 4.05 ± 0.16% prior to infusion and 8.95 ± 2.18 mg % at the end of the infusion, with death following shortly thereafter. Blood ketone levels exhibited the same decline in 2½ hours after hemorrhage and during shock as in the previous series.

Discussion. The most striking finding of this study was the ease with which blood ketone levels were depressed in the rat by a hemorrhage which was not sufficient to produce clinical evidences of shock or elevate plasma amino nitrogen levels. Aside from the early rise in blood sugar, attributable to epinephrine discharge, and the initial fall in plasma amino nitrogen, occurring immediately after bleeding(2), no other metabolic responses to hemorrhage have been recorded as appearing so promptly. This high sensitivity of blood ketone levels to minor disturbances in the circulation must be reckoned with in any study of ketonemia in the rat. Our experience suggests that it may be a factor of importance in any studies on ketosis in the adrenalectomized rat, since this preparation is very likely to be suffering from incipient circulatory failure(11).

The mechanism of the hypoketonemia of hemorrhage and shock is not immediately apparent from these studies. Since it occurs with equal rapidity in the absence of the adrenal glands, epinephrine discharge cannot be held responsible, even though epinephrine injection may cause a transitory depression followed by a rise in blood ketone levels in normal rats(11). By the same token, adrenal cortical discharge can be eliminated as a factor, although cortisone treatment suppresses both fasting and stress ketosis(11,12).

The lowered ketone levels might be attributed to either a depressed hepatic production

of ketone bodies or an accelerated utilization by the peripheral tissues, or both. The data on octanoate infusion in "sublethal" hemorrhage suggest that ketone production from an exogenous fatty acid is not disturbed under these conditions, but do not throw any light on possible changes in the rates of fat catabolism or ketone production from endogenous fatty acids in the liver or on possible alterations in the rate of ketone utilization. As shock progresses, however, suggestive evidence for an impairment in hepatic ketone production from infused octanoate becomes apparent in the lesser rise in ketone levels. This could be due to either decreased delivery of octanoate to the liver or to impaired oxidation of fatty acids by the liver. Since the oxygen supply to the liver apparently decreases as shock progresses(13) and since fat oxidation and ketone production demand a considerable amount of oxygen, a suppression of ketone production from fatty acids by the liver during shock would not be unexpected.

Less is known about the possibility that ketone utilization might be increased during hemorrhage and shock. Further studies are planned on the rate of disappearance of ketone bodies infused into normal and hepatectomized rats during hemorrhage and shock.

The present observations were made on rats under nembutal anesthesia with body temperature maintained by exposure to the heat of an electric lamp. Recent studies have indicated that under these conditions ketosis is greater during fasting in normal rats than in unanesthetized rats not exposed to an external source of heat(11). Further observations are necessary to demonstrate whether the above results still apply to unanesthetized rats subjected to hemorrhage. Preliminary data suggest that they do.

Summary. 1. Sublethal hemorrhage in the normal fasted rat without a significant rise in plasma amino nitrogen is followed by a prompt suppression of fasting ketosis but is without effect on the increase in blood ketones consequent to a 30-minute intravenous infusion of sodium octanoate. 2. Hemorrhagic shock with rising plasma amino nitrogen levels is associated with a significant and progressive hypoketonemia and an inhibition of the rise in blood ketones ordinarily seen after sodium octanoate infusion. 3. Adrenalectomized rats exhibit the same decline in blood ketone levels during hemorrhage and shock as do normal rats. 4. The significance of these results with respect to effects of hemorrhage and shock on ketone production and utilization in the rat is discussed.

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