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The absence of plasma free fatty acid response to epinephrine in vitamin-C-deprived guinea pigs

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SUMMARY

Plasma concentrations of free fatty acid (FFA) and blood glucose were measured in normal and 25-day scorbutic guinea pigs 15 minutes after injection of either saline or epinephrine. The normal group showed a large increase in FFA after epinephrine and a smaller increase after saline as compared to no injection at all. The scorbutic animals had no increase of FFA either after saline or epinephrine. The injection of 50 mg ascorbic acid intraperitoneally into 25-day scorbutic guinea pigs 5 hours before sacrifice restored to this group the normal response to epinephrine and saline. The mean plasma 17-OH corticosteroid concentration was 206.5 μg/100 ml for six 25-day scorbutic guinea pigs and was 71.5 μg/100 ml for six normal guinea pigs. Scorbutic animals had marked increases of glucose both after saline and epinephrine injections as compared to uninjected scorbutic animals.

In a recent paper, Mueller and Cardon (1) described the failure of fasting to increase plasma free fatty acid concentrations (FFA) in guinea pigs deprived of vitamin C. In 1956, Dole (2) and Gordon and Cherkes (3) noted that injections of epinephrine are followed by marked increases in FFA. It was decided to investigate the effect of scurvy upon this phenomenon. Shafrir and Steinberg (4) have noted that adrenalectomy abolishes FFA response to epinephrine in dogs; injections of cortisone permit these animals to respond again in a normal fashion.

There is conflicting data in the literature concerning adrenal cortical activity in scurvy. Bacchus and Heiffer (5) have noted decreased urinary corticoids in the later stages of scurvy in guinea pigs, and Daughaday *et al.* (6) described low formaldehydogenic steroids in three human patients with scurvy. However, Done *et al.* (7) noted a tenfold increase in plasma 17-OH corticosteroids in scorbutic guinea pigs, and Burstein *et al.* (8) described a 300% increase in urinary cortisol under similar conditions. Therefore, plasma 17-OH corticosteroid concentrations of scorbutic guinea pigs after 25 days on a vitamin-C-deficient diet have been compared with those of normal guinea pigs to investigate the possibility that the scorbutic state may cause adrenal insufficiency.

METHODS

Twelve male, NIH stock, mixed-color guinea pigs were fed a normal diet of greens and chow for one week and then injected intraperitoneally with 25 μg of epinephrine in saline at approximately 3:45 PM after a 5-hour fast. They were then sacrificed in groups of three at intervals of 5, 10, 15, and 20 minutes to determine the time of peak effect of epinephrine on blood glucose and FFA. Blood was collected from the severed necks for 30 seconds through silicone-coated funnels into silicone-coated test tubes, each containing three drops of heparin (10,000 U. S. P. units/ml). Blood samples were immediately placed in crushed ice and analyzed for blood glucose and FFA.

Additional guinea pigs, weighing from 245 to 310 g, were fed a commercial powdered ascorbic acid-free diet (Nutritional Biochemicals, Inc.) and tap water *ad libitum* as in the previous study (1). Every morning each animal was given a 0.10-ml intraperitoneal injection. Scorbutic animals received normal saline; normal animals received 50 mg of ascorbic acid in solution.

Twenty-five days after the beginning of the experiment, food was removed from all cages at 11 AM. The animals were injected intraperitoneally with either 25 μg of epinephrine in 0.25 ml saline or 0.25 ml normal saline at approximately 3:45 PM. Exactly 15 minutes later the animals were sacrificed and blood was collected as before. The order of injection and sacrifice was alternated between normal and scorbutic animals as well as between saline and epinephrine injections. One group of 25-day scorbutic animals (25-day scorbutic plus vitamin C) received injections of 50 mg of ascorbic acid

TABLE 1. PLASMA FREE FATTY ACID CONCENTRATION AND BLOOD GLUCOSE IN VARIOUS EXPERIMENTAL GROUPS

	15 Minutes After Saline						15 Minutes After Epinephrine					
	Glucose			FFA			Glucose			FFA		
	n	$\bar{X}$ ± S.D.	Range	n	$\bar{X}$ ± S.D.	Range	n	$\bar{X}$ ± S.D.	Range	n	$\bar{X}$ ± S.D.	Range
25-day normal	5	134 ±13	118–153	5	0.72 ±0.20	0.50–0.97	6	127 ±12	114–144	6	1.19 ±0.25	0.88–1.43
25-day scorbutic	6	163 ±9	146–171	6	0.58 ±0.14	0.41–0.76	6	183 ±30	147–227	6	0.58 ±0.16	0.40–0.84
25-day scorbutic + vitamin C	6	145 ±19	117–174	6	0.81 ±0.45	0.72–0.99	6	147 ±22	126–190	7	1.15 ±0.45	0.71–2.03

instead of the usual saline immediately after food was withdrawn—5 hours before sacrifice.

Twelve guinea pigs, six normal and six 25-day scorbutic, were fasted for 5 hours and then anesthetized with 15 mg of pentobarbital intraperitoneally. Their abdomens were opened and as much blood as possible was removed from the abdominal aorta with a heparinized syringe and needle. The plasma was removed after centrifugation and frozen immediately. At a later date, 17-OH corticosteroids were determined on these plasmas by the method of Silber and Porter (9).[1]

Plasma free fatty acid concentrations were determined by extraction of 1 ml of plasma for 24 hours in a mixture of 2,2,4-trimethyl-pentane (isooctane), acetic anhydride, and glacial acetic acid, followed by two washings and titration against approximately 0.02 N NaOH (10). Blood glucose was determined by the Glucostat ®[2] method (11).

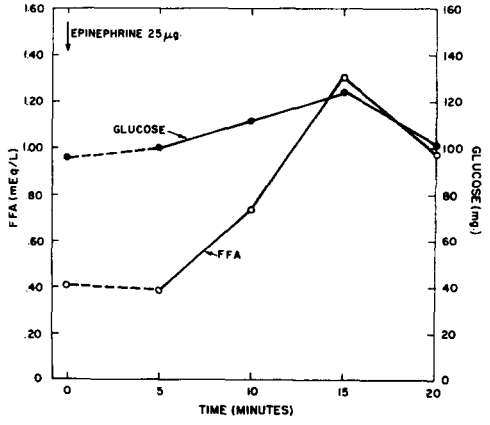

FIG. 1. The effect of 25 μg epinephrine injected intraperitoneally on blood glucose and plasma free fatty acid concentration. Each point on the graph represents the average values of at least three animals.

[1] The author is indebted to Mr. Silas Jackson, Endocrinology Service of the National Cancer Institute, for the 17-OH corticosteroid determinations.

[2] Worthington Biochemical Corporation.

Statistical methods employed included the standard "*t*" test and the standard correlation coefficient.

RESULTS AND DISCUSSION

Figure 1 shows the effect of 25 μg epinephrine intraperitoneally on FFA and glucose in relation to time. The zero determinations were made at a previous date. Large increases in FFA and smaller increases in glucose follow epinephrine with the peak reached at 15 minutes. Accordingly, the 15-minute period between injection and sacrifice was chosen for the subsequent studies.

The effect of epinephrine and saline injections upon FFA and glucose is shown in Table 1. The normal guinea pig FFA after a 5-hour fast without any injection has been determined to be 0.41 ± 0.12 mEq per liter (1). Apparently saline injections alone cause enough distress to increase FFA to 0.72 mEq per liter in 15 minutes. Epinephrine, however, increases FFA further to a mean 1.19 mEq per liter, which is significantly greater ($p < 0.01$) than the mean FFA after saline.

In a previous study (1), the mean FFA for 25-day scorbutic, 5-hour fasted guinea pigs without injection was 0.55 mEq per liter. In the present study, the mean FFA concentrations after saline and after epinephrine in the 25-day scorbutic guinea pigs were similar (0.58 mEq per liter) and significantly lower ($p < 0.001$) than the FFA after epinephrine in the normal group.

The injection of vitamin C five hours prior to sacrifice into the 25-day scorbutic guinea pigs apparently restores their ability to increase FFA both after saline and epinephrine. The FFA means of 0.81 mEq per liter after saline and 1.15 mEq per liter after epinephrine are very similar ($p > 0.1$) to those observed in the normal animals. Three of the seven plasmas obtained from the guinea pigs receiving epinephrine were lipemic.

The mean blood glucose in uninjected normal and

TABLE 2. PLASMA 17-OH CORTICOSTEROID CONCENTRATIONS IN NORMAL AND SCORBUTIC GUINEA PIGS (μg/100 ml)

	1	2	3	4	5	6	X ± S.D.
Normal	70	59	52	63	98	87	71.5 ± 18
25-day scorbutic	116	280	199	134	315	195	206.5 ± 78

scorbutic guinea pigs was 95.3 ± 13 mg/100 ml and 107.8 ± 11 mg/100 ml, respectively. The normal groups had similar moderate increases in blood glucose after both saline and epinephrine injections. The scorbutic animals, after saline and epinephrine, had marked increases in blood glucose significantly greater than uninjected normal ($p < 0.01$) and 25-day scorbutic guinea pigs ($p < 0.01$) but not significantly different ($p < 0.1$) from each other. The injection of 50 mg of ascorbic acid prior to sacrifice lowers the blood glucose toward normal in both saline- and epinephrine-injected animals. The mean glucose values after epinephrine and saline are not significantly different from each other or from the mean glucose of any of the other groups.

Plasma 17-OH corticosteroid concentrations are presented in Table 2. The mean for the six normal animals is 71.5 μg/100 ml and for the six scorbutic guinea pigs, 206.5 μg/100 ml. There is no overlap of the two groups, and the means are significantly different ($p < 0.01$).

These data show that vitamin-C deficiency of 25 days duration abolishes the normal increase in FFA but not the normal increase in glucose, which follows epinephrine injection. After 25 days of deprivation, a single 50-mg injection of ascorbic acid 5 hours prior to sacrifice restores FFA mobilization to normal. A possible explanation for these phenomena might be that the elevation of blood sugar in the scorbutic animal abolishes any response of FFA to epinephrine. Increasing blood glucose by oral ingestion or intravenous injection may inhibit FFA response to epinephrine (12) presumably by increasing the utilization of glucose. In situations of impaired glucose utilization (i.e., diabetes mellitus), however, the high blood sugar does not decrease FFA (13). The high blood sugar in scurvy does not result in increased glucose utilization (14, 15, 16) but, on the contrary, reflects decreased glucose utilization. Therefore, this explanation seems less likely for the presently observed phenomenon. In the 25-day scorbutic group receiving vitamin C five hours prior to sacrifice and epinephrine 15 minutes prior to sacrifice, there was a negative correlation between FFA and blood glucose ($r = -0.68$); in the similarly treated group receiving saline 15 minutes prior to sacrifice, there was a positive correlation ($r = +0.25$). Neither of these correlations was significant ($p > 0.1$).

The almost threefold increase of plasma 17-OH corticosteroids in the 25-day scorbutic guinea pigs agrees well with the findings of Burstein *et al.* (8) and Done *et al.* (7). The mean plasma 17-OH corticosteroid concentration noted by Done *et al.* in scurvy was 301.9 ± 43.03 μg/100 ml and 32.9 ± 4.60 μg/100 ml in controls. Therefore, the mechanism of abolition of increased FFA after epinephrine appears to be apart from adrenal insufficiency in scurvy. The possibility remains, however, that there may be an increase of a blood constituent in scurvy that reacts to the Porter-Silber test as 17-OH corticosteroid but that is not the same as the normal 17-OH corticosteroid, either in exact chemical structure or in its role of potentiating FFA release with epinephrine. The work of Burstein and associates (8) would make this possibility highly unlikely, as they identified in urine by chromatographic methods only normally appearing corticosteroids in high quantities in scorbutic guinea pigs.

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