lationship was established between height of serum bilirubin concentration and degree of Bromsulphalein retention and elevation of angiotensinase activity, thus suggesting a more specific association with cholestasis. Angiotensinases have been isolated from both liver tissue and bile(11), and liberation of these enzymes from damaged hepatic cells, a decrease in their biliary excretion associated with cholestasis, or an increase in production of the enzymes by the liver are all consistent with our results. Klaus and associates(11) found very high values in patients with hepatitis, metastatic disease of the liver, or biliary obstruction, as well as in those with active cirrhosis; in some instances, the observed fluctuations coincided with the degree of jaundice.

The present findings might account for the decreased pressor responsiveness of patients with cirrhosis to infusions of angiotensin(8), but they do not readily explain the paradoxic effect of the drug on excretion of sodium. The more rapid utilization of angiotensin resulting from plasma angiotensinase hyperactivity could contribute to the hypotension of hepatic failure(17) and, in a less extreme form, might explain the relative freedom from arterial hypertension reported by some authors (6,7) in association with cirrhosis.

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Effect of Acetoacetate on Adrenocortical Activity. (29456)

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Administration of acetoacetate in gradually increasing amounts for 21 days, to guinea pigs fed a diet completely lacking in vitamin C, was found to cause hyperglycemia and a rapid depletion of liver and muscle glycogen(1). Earlier studies to evaluate the role of the adrenals in mediating the deleterious effects of acetoacetate showed that acetoacetate administration for 21 days had no appreciable effect on adrenal weights and adrenal cholesterol and ascorbic acid contents of normal and scorbutic guinea pigs(2). However, acetoacetate increased greatly the adrenaline content of the adrenals of normal and scorbutic guinea pigs(3). As adrenaline enhances ACTH secretion, it was thought necessary to

TABLE I. Adrenal Weights and Adrenal Ascorbic Acid. Average values with standard deviation.

Group	Adrenal wt, mg/100 g body wt		Adrenal ascorbic acid, mg/100 g adrenal	
	35 days	70 days	35 days	70 days
I Normal	80.1 ± 1.8 (10)*	82.1 ± 2.0 (8)	100.0 ± 8.8 (10)	98.6 ± 9.2 (8)
II Normal injected acetoacetate	83.0 ± 3.2 (8)	98.7 ± 1.4 (6)	96.4 ± 6.8 (8)	50.2 ± 3.4 (6)
III Scorbutic	94.6 ± .9 (8)	112.3 ± .7 (8)	65.3 ± 5.1 (8)	15.1 ± 1.1 (8)
IV Scorbutic injected acetoacetate	96.8 ± 1.1 (7)	128.3 ± 1.6 (7)	56.8 ± 4.6 (7)	5.5 ± .9 (7)

* Figures in parentheses indicate number of animals.

study the effect of acetoacetate injections for a longer period on adrenocortical activity of normal and scorbutic guinea pigs. This paper describes the effect of prolonged acetoacetate treatment on adrenal weights, adrenal cholesterol and ascorbic acid and urinary 17-ketosteroid excretion of normal and subacute scorbutic guinea pigs.

Methods. Healthy guinea pigs were divided into 4 groups as follows: I, Normal; II, Normal injected acetoacetate; III, Scorbutic; and IV, Scorbutic injected acetoacetate. Chronic scurvy was induced in guinea pigs of Groups III and IV by feeding 0.25 mg ascorbic acid daily to each animal along with a scorbutic diet(4). Animals of Groups I and II were fed 5 mg of ascorbic acid daily, along with the scorbutic diet fed to Groups III and IV. Animals of all groups were fed 2 drops of Adexoline (vitamin A and D) orally twice a week. The amount of diet consumed every day by the I and III Group animals was noted and the same amount was fed to animals of corresponding weights in the II and IV Groups respectively, on the subsequent days, to obviate results due to inanition. Half the animals of every group were killed after 35 days, and the other half after 70 days on the experimental regimen, and the following estimations were carried out.

Adrenal ascorbic acid: The adrenals were removed and freed from connective tissue immediately after killing the animals. The right adrenal was homogenized in 10% trichloracetic acid and the ascorbic acid content was estimated by the colorimetric method of Bessey(5). The results are presented in Table I.

Adrenal cholesterol: The left adrenal was homogenized in alcohol:acetone (1:1) mixture. Free and total cholesterol were determined by the method of Sobel and Mayer(6) as adapted to tissues by Mookerjea and Sadhu(7). The results are shown in Table II.

Urinary 17-ketosteroids: Fifty female guinea pigs were divided into 4 groups as above and animals of each group were housed in individual metabolic cages during the 5th, 7th and 9th weeks of the experimental period. Urine was collected over concentrated hydrochloric acid and the 17-ketosteroids from 24 hour samples were estimated by the method of Robinson *et al* as described by King(8). Table III gives the average 17-ketosteroid excretion values per guinea pig per day during the 5th, 7th and 9th weeks.

TABLE II. Adrenal Cholesterol in mg/g Adrenal. Average values with standard deviation.

Group	35 days			70 days		
	Esterified	Free	E/F	Esterified	Free	E/F
I Normal	(10)*49.3 ± 1.4	5.6 ± .5	8.8	(8)* 48.7 ± 2.1	5.9 ± .4	8.1
II Normal injected acetoacetate	(8) 48.0 ± 4.8	5.7 ± .8	8.4	(6) 42.8 ± 5.2	6.0 ± .4	7.0
III Scorbutic	(8) 35.0 ± 1.1	4.5 ± .4	7.7	(8) 22.8 ± .8	4.2 ± .7	5.9
IV Scorbutic injected acetoacetate	(7) 33.1 ± 3.2	4.1 ± .7	7.4	(7) 17.5 ± 4.3	3.5 ± .5	5.0

* Figures in parentheses indicate number of animals. E/F, Esterified/Free ratio.

TABLE III. Urinary 17-Ketosteroid Excretion of Female Guinea Pigs in mg/Animal/Day. Average values with standard deviation.

Group	5th wk	7th wk	9th wk
I Normal control	.32 ± .06 (10)*	.41 ± .04 (10)	.48 ± .10 (8)
II Normal injected acetoacetate	.38 ± .03 (10)	.46 ± .08 (9)	.70 ± .04 (7)
III Scorbutic	.65 ± .05 (9)	.76 ± .08 (8)	.84 ± .05 (7)
IV Scorbutic injected acetoacetate	.68 ± .02 (8)	.81 ± .06 (7)	.98 ± .07 (7)

Dose of acetoacetate injected as indicated in text.

* Figures in parentheses indicate number of animals.

Results and discussion. The results presented in Tables I and II show that there is progressive depletion of adrenal ascorbic acid and adrenal cholesterol in the scorbutic guinea pigs. The weights of adrenals of scorbutic guinea pigs show a similar progressive increase. These results are in agreement with our results on guinea pigs totally denied vit. C in their diet(2).

Acetoacetate administration for 35 days does not have an appreciable effect on adrenal weights, cholesterol and ascorbic acid of normal as well as scorbutic guinea pigs (Tables I and II, Groups II and IV). However, if acetoacetate injections are continued for 70 days, there are marked changes. Adrenal weights increase, ascorbic acid decreases and cholesterol also decreases. The fall in adrenal cholesterol is small, perhaps because of some synthesis from acetoacetate. There is a drop in the ratio esterified/free cholesterol in the scorbutic guinea pigs. Acetoacetate administration for 70 days causes a further drop in the ratio.

Urinary 17-ketosteroid excretion increases progressively in the scorbutic female guinea pigs. These results are in agreement with those of Clayton and Prunty(9,10). Hein and Oertel(11,12) also found a greatly enhanced elimination of 17-ketosteroids in scorbutic guinea pigs, whereas Banerjee and Deb(13) and others(14,15) found a decreased excretion in scurvy. Banerjee and associate(16) later found an enhanced urinary excretion of 17-ketosteroids in 7 out of 10 scorbutic guinea pigs and in all 3 scorbutic monkeys.

17-ketosteroid excretion of normal and scorbutic guinea pigs injected with acetoacetate for 35 days is not significantly altered. Acetoacetate injections for 70 days cause an appreciable increase in urinary 17-ketosteroids.

An increase in adrenal weight and a decrease in adrenal cholesterol concentration are indicative of hyperactivity of the adrenal cortex(17). As judged by these indices, our results show that acetoacetate administration for 35 days does not enhance adrenocortical activity but after administration for 70 days, there is a significant hyperfunction of the adrenal cortex. This indication is further supported by the decrease in adrenal ascorbic acid and increase in 17-ketosteroid excretion.

Summary. Adrenocortical activity of normal and scorbutic guinea pigs, administered acetoacetate for a prolonged period, was evaluated by determining adrenal weights, adrenal ascorbic acid and cholesterol and urinary 17-ketosteroids. There was a progressive decrease in adrenal cholesterol and ascorbic acid of scorbutic guinea pigs and an increase in adrenal weights and urinary 17-ketosteroids. Acetoacetate injections for 35 days to normal as well as to scorbutic guinea pigs did not cause significant changes in adrenal weights, adrenal cholesterol, ascorbic acid and urinary 17-ketosteroids. Acetoacetate administration for 70 days caused an increase in adrenal weights and urinary 17-ketosteroid and a decrease in adrenal ascorbic acid and cholesterol. The results indicate hyperactivity of the adrenals in scorbutic guinea pigs. Normal and scorbutic guinea pigs subjected to prolonged acetoacetate administration also exhibited increased adrenocortical activity.

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Acetaldehyde, A Product of Deoxynucleoside Metabolism in Human Erythrocyte Ghosts.* (29457)

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Acetaldehyde has been reported in human red blood cells(1,2) as well as in the blood of several mammalian species(3). The significance of its presence is unknown. Its reactivity and low concentration (40-100 μg/ml) render it difficult to detect in extracts of whole blood made with common protein precipitants such as tricholoracetic acid, tungstic acid, or zinc hydroxide. However, it may be distilled quantitatively from blood due to its high vapor pressure in aqueous solutions at 37°C(4,2).

Human erythrocyte ghosts contain no acetaldehyde as the small endogenous concentration of intact cells is removed during hemolysis and washing. However, when ghosts are incubated with deoxynucleosides or deoxyribosephosphate a catabolic pathway may be described in which acetaldehyde is produced along with several phosphate esters.

Experimental. Ghost suspensions were made and equilibrated as described previously(5). They were reconstituted with physiological buffer to give cell counts of 5×10^6 cells per mm^3. Thirty-five ml of ghost suspension were incubated with deoxyinosine or deoxyadenosine at 37°C in incubation flasks kept under an atmosphere of 5% CO_2-95% O_2 for 5 hours at shaking rates of 50-60 oscillations per minute. Phosphate buffer (pH 7.4) was added to a final concentration of 4.0 mM. In several experiments hemolysates were made by freezing and thawing twice washed, packed erythrocytes and incubated (5.0 ml total volume) with deoxyribose-5-P.

Samples were withdrawn at intervals and precipitated with cold 30% metaphosphoric acid, centrifuged, the precipitate washed with 2% metaphosphoric acid, the combined supernatants adjusted to a pH 6.8 and diluted (final concentration metaphosphoric acid 4%). Acetaldehyde was determined with yeast alcohol dehydrogenase(6) and confirmed colorimetrically with semicarbazide. The extracts from the incubations were gassed at 37°C with nitrogen in a small train and the vaporized acetaldehyde trapped in a buffered solution of semicarbazide (pH 7.0). The acetaldehyde semicarbazone chromophore was then estimated from its absorbancy at 224 mμ(2).

The magnitude of deoxynucleoside metabolized was measured by comparing the hypoxanthine produced(7) with the diminution

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