with hexosamine, while ⅓ of total dermal amino sugar is found intimately associated with the insoluble residue, probably as a mucopolysaccharide. This residue is highly purified dermal collagen. Finally, fresh skin contains 55% water, 6% fat, 24% collagen and 15% non-collagen protein and glycoprotein.

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Metabolism of Cortisol by the Liver of Sodium Deficient Rats.‡ (26099)

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A major step in the metabolism of corticosteroids is reduction of the Δ^4-3-ketone group(1). It has been shown that rat liver contains steroid Ring A hydrogenases capable of catalyzing this reduction(2). Urquhart *et al.*(3) and Yates *et al.*(4) have proposed that rate of hepatic reduction of adrenal cortical steroids is a controlling factor in rate of secretion of those steroids by the adrenal through a negative-feedback mechanism by which the anterior pituitary (ACTH) regulates plasma corticosteroid concentration. It is likely then that conditions which produce changes in adrenocortical secretion rates might also alter the ability of the liver to metabolize active corticosteroids.

Eisenstein and Hartroft(5) have shown that total steroid secretion by the isolated adrenal of sodium deficient rats was significantly less than that of controls. The purpose of this study was to evaluate the effect of sodium deprivation on capacity of rat liver to reduce the Δ^4-3-ketone group of cortisol.*

Methods and materials. Female white rats of the Carworth strain (maintained on Purina pellet chow), weighing 140-200 g, were divided into two groups. One group (21 rats) was fed a commercially† prepared synthetic low-sodium diet which included all the nutrients essential for normal growth except sodium and contained 14.6 mg Na^+/100 g of diet and 910 mg K^+/100 g of diet. The other group (20 rats) was fed a diet† similar in all respects to the low-sodium diet except that

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* Δ^4-pregnene-11β,17α,21-triol-3,20-dione.

† Nutritional Biochemicals Corp.

TABLE I. Effect of Sodium Deprivation on Rat Liver Steroid Hydrogenase Activity.

Group*	No. rats	Days on diet, avg	μg/cortisol reduced/g liver/min.
Control	20	35	40.7 ± 7.4†
Low Na	21	60	30.6 ± 6.3
			P <.01

* Avg intake of Na for control group 1.60 meq/day; low-sodium group 0.16 meq/day.
† Stand. dev.

it included normal amounts of electrolytes. It contained 148 mg Na^+/100 g of diet and 500 mg K^+/100 g of diet by analysis and will be referred to as the normal synthetic diet. Information pertaining to length of the regimen and average sodium intake is indicated in Table I. The animals were killed by decapitation, and liver, blood and muscle samples obtained. The liver was rinsed in iced saline solution, blotted and weighed. It was then homogenized in a solution of 0.25 M sucrose with 0.04 M nicotinamide (4 ml/g liver). The liver homogenate was centrifuged at 7000 × g for 15 minutes and the supernatant fraction was used for assay of the steroid hydrogenase. Two ml of this supernatant fraction were added to 3.5 ml of a solution(6) containing the necessary co-factors for TPNH generation. One tenth ml of a 0.01 M solution of hydrocortisone in ethanol was used as the substrate and added to each flask. The samples were incubated in a Dubnoff shaker for 15 minutes at 38°C in an atmosphere of nitrogen and then 20 ml of chloroform were added. To measure the difference in enzymatic reduction of the hydrocortisone, comparable control tubes were set up in which the substrate was added after addition of 20 ml of chloroform. The samples were extracted by shaking for 10 minutes and the chloroform layer was washed with 0.1 N NaOH and 0.1 N HCl. Aqueous layers were removed by aspiration. Five ml aliquots were taken to dryness under reduced pressure and the residue dissolved in 10 ml of methanol.

The ultraviolet absorption was determined in a Beckman DU spectrophotometer at 242 mμ. The difference between the optical density of the controls and the samples was used to calculate the amount of Ring A reduction which had taken place.

Results. The results presented in Table I show that the capacity of the livers of sodium deficient rats to reduce Ring A of cortisol is greatly reduced when compared to animals maintained on a normal synthetic diet. The livers of sodium deficient animals had about 25% less steroid hydrogenase activity than control animals. This difference was found to be highly significant ($P<0.01$). Table II shows electrolyte content of plasma and muscle of both groups. The sodium deficient group had significantly less sodium and significantly more potassium in their skeletal muscle than the control group. There were no significant differences in plasma sodium and potassium levels, which is to be expected since plasma sodium and potassium levels tend to remain within normal limits except in extremely severe chronic sodium deficiency. Muscle electrolyte changes are therefore a better index of sodium deficiency.

Discussion. The deceased capacity of the liver of sodium deficient rats to reduce the Δ^4-3-ketone group of cortisol could have been the result of: (a) a decreased requirement for Δ^4-hydrogenases due to a diminished steroid output from the adrenal; or (b) a reduced enzyme concentration or activity within the liver cells elicited by the stress of salt deprivation. If the latter possibility is correct, a decreased secretion of adrenal steroids by salt deprived rats(5) can be explained, at least in part, by the feedback stabilization of plasma corticosteroids by the hepatic-hypo-

TABLE II. Electrolyte Content of Skeletal Muscle and Plasma.

Group	Skeletal muscle, meq/100 g (wet wt)		Plasma, meq/l	
	Na	K	Na	K
Control	3.80 ± .71*(12)†	9.58 ± .73 (12)	142 ± 5 (20)	7.0 ± .7 (20)
Low sodium	2.92 ± .37 (19)	10.3 ± .5 (19)	145 ± 4 (19)	7.5 ± 1.1 (12)
	P <.01	P <.01		

* Stand. dev. † No. in parentheses represents No. of values obtained.

thalamic-pituitary-adrenal axis described by Urquhart *et al.*(3) and Yates *et al.*(4). It is also possible that both mechanisms occur simultaneously. However, the results of this study do confirm previous evidence that the liver plays an important role in regulation of plasma corticosteroid levels.

It is not likely that the observed decrease in hepatic metabolism of cortisol by these animals would precipitate an adrenal crisis during stress because there still remains a considerable capacity for steroid reduction in the salt deficient rats(5). Further studies under more severe conditions of sodium restriction and for longer periods might be valuable in assessing this effect in chronic sodium deficiency.

Summary. A decreased capacity of liver homogenate to metabolize cortisol in sodium deficient rats has been demonstrated. The rate of Ring A reduction was diminished by about 25% below that of control animals. The possibility that this effect may result in a decreased adrenal corticosteroid secretion *via* the negative-feedback mechanism by which plasma corticoid levels are stabilized is discussed.

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Vi Antigens of the Enterobacteriaceae. IV. Purification and Properties of Vi Antigen of *S. paratyphi C*.* (26100)

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Isolation and purification of relatively undegraded Vi antigen by mild methods from *Salmonella typhosa, Escherichia coli* and *Paracolobactrum ballerup* has been reported by Baker *et al.*(1). Production of Vi antigen by some strains of *Salmonella paratyphi C* was reported by Rouchdi(2). However, neither the technic of purification mentioned above(1) nor that devised by Webster *et al.* (3,4) proved useable for purification of the Vi antigen of *S. paratyphi C*. In the present communication a method for the purification of the Vi antigen of this organism is outlined together with chemical and biological properties of this antigen.

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Methods. The culture of *S. paratyphi C* (C-32) used was obtained from Dr. F. Kauffmann. The organisms were grown, as previously described(1), on a casamino acid medium containing only dialysable components. After about 18 hours growth the cultures were killed by addition of 1.5 to 2 volumes of acetone (technical grade), dehydrated by washing with acetone, and dried in air.

Nitrogen was determined by the usual micro-kjeldahl method(5), total acetyl as described by Kabat and Mayer(5), O acetyl by a modification of the method of Pippin, McCready and Owens(6), rhamnose by the method of Dische and Shettles(7), hexose by the method of Dische, Shettles and Osnos (8), and phosphorus by the method of Allen (9). Viscosities were determined by means of an Oswald pipette. The Ouchterlony technic was used for the serologic tests which