damage of deeper tissues has not been observed in these mice. It might be expected that these mice would show a more marked adrenal hypertrophy, but this cannot be evaluated with present data.

*Summary.* Amount of scarring and adrenal weight were compared for 280 male albino mice from 50 populations of 4, 5, or 6 mice each. No relationship was found between adrenal weight and absence or presence of scarring or between adrenal weight and amount of scarring. There was a significant decline in absolute adrenal weight with increased scarring, but this decline disappeared when adrenal weights were corrected for body weight. It was concluded that adrenal hypertrophy after one week of grouping did not reflect amount of injury received.

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## Effects of Adrenalectomy and Aldosterone on Sodium Concentration in Renal Medulla of Hydropenic Rats.* (24870)

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Relying upon the hairpin countercurrent theory proposed by Hargitay and Kuhn(1). Wirz suggested that concentrating ability of mammalian kidney depends on water-free active solute reabsorption taking place in the ascending limb of the loop of Henle(2). This theory has been supported by the demonstration in hydropenic animals of increasing osmotic pressure in the medulla, from its cortical margin to the extremity of the papilla (3,4) and by recent work of Gottschalk and Mylle showing high osmolality of tubular fluid obtained from the tip of the loop of Henle(5). Removal of solutes from the fluid flowing up the ascending limb of the Henle loop is suggested by the hypotonicity of urine obtained from the first portion of the distal tubule(2,6,7). Quantitatively, the major ion involved in active transport process upon which the concentrating mechanism depends, is likely to be sodium(6). In man, aldosterone causes, within 2 to 4 hours, a reabsorption of sodium by kidney greater than could be accounted for by exchange for potassium and hydrogen ions (Crabbé, J., unpublished observations), thought to occur in the distal convoluted tubule(8). This suggested that aldosterone might stimulate sodium transport system in the loop of Henle. Assuming that sodium is the solute actively reabsorbed at that site and relying upon the observation that its proportional contribution to total solute content of the papilla is constant(4), it should therefore be possible to examine this hypothesis by measuring concentration of sodium in the papilla of concentrating kidney.

*Methods.* Twenty-four Holtzman female rats, 4 months old, were deprived of water 40 hours, and fasted 28 hours, before sacrifice. Two groups of 6 rats (groups B and D) had been adrenalectomized under ether anesthesia 80 hours before start of experiment, *i.e.,* 120 hours before sacrifice, and had been maintained on food and 1% sodium chloride solution *ad lib.* during the interval. Twelve intact rats were placed on same regimen (Groups A and C). The animals were transferred to metabolic cages 28 hours before sacrifice.

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TABLE I. Sodium, Potassium and Water Content of Renal Tissue/100 g Dry Weight in Rats.

| | | Papilla | | | Cortex | | |
|---|---|---|---|---|---|---|---|
| | | Na | K | $H_2O$, | Na | K | $H_2O$, |
| | | meq | | ml | meq | | ml |
| Intact (Group A) | Mean | 98.7 | 34.7 | 520 | 25.7 | 32.1 | 247 |
| | S.D. | 10.2 | 3.3 | 64 | 2.5 | 1.4 | 28 |
| Adrenalectomized (Group B) | Mean | 102.3 | 41.0 | 693 | 26.1 | 36.3 | 306 |
| | S.D. | 22.4 | 7.5 | 150 | 4.4 | 3.7 | 35 |
| Intact + aldosterone (Group C) | Mean | 95.8 | 32.5 | 481 | 23.8 | 32.0 | 243 |
| | S.D. | 11.6 | 3.8 | 39 | 1.3 | 1.1 | 12 |
| Adrenalectomized + aldosterone (Group D) | Mean | 104.1 | 41.0 | 705 | 27.8 | 36.9 | 307 |
| | S.D. | 29.7 | 15.2 | 181 | 6.3 | 5.6 | 62 |

TABLE II. Concentrations of Sodium and Potassium in Tissue Water of Rats.

| | | Papilla | | Cortex | |
|---|---|---|---|---|---|
| | | Na | K | Na | K |
| | | meq/l | | | |
| Intact (Group A) | Mean | 193.7 | 66.7 | 106.0 | 130.1 |
| | S.D. | 38.5 | | 10.7 | |
| Adrenalectomized (Group B) | Mean | 149.6 | 59.1 | 84.3 | 118.5 |
| | S.D. | 27.8 | | 17.3 | |
| Intact + aldosterone (Group C) | Mean | 200.2 | 67.4 | 98.1 | 131.9 |
| | S.D. | 29.4 | | 6.0 | |
| Adrenalectomized + aldosterone (Group D) | Mean | 147.3 | 58.1 | 90.6 | 120.5 |
| | S.D. | 11.0 | | 9.5 | |

Each rat of Groups C and D was given subcutaneous injections of 20 $\mu$g of d-l aldosterone monoacetate§ in 0.02 ml of ethanol diluted to 0.1 ml with isotonic saline, 28 and 16 hours before death, and twice that dose 4 hours before sacrifice. Those of Groups A and B received only ethanol diluted in saline. All animals were exsanguinated from abdominal aorta under intraperitoneal Nembutal anesthesia. Kidneys were quickly excised and dissected with scissors; the inner part of medulla, consisting largely of papilla, could readily be identified and isolated due to its white appearance. Both papillae of each rat were weighed in stoppered tared tubes, dried 24 hours at 105°C, reweighed, ground, extracted with 0.75 N nitric acid for 48 hours at room temperature, and the tubes centrifuged before removal of supernatant for sodium and potassium measurement by internal standard flame photometry. Pairs of slices of renal cortex were handled similarly.

*Results.* Results of tissue analyses, expressed as meq of sodium and potassium and as ml of water/100 g of dry tissue, are summarized in Table I. Neither adrenalectomy nor aldosterone had any significant effect on sodium content/100 g of dry solids of either renal papilla or cortex. Adrenalectomy resulted in increase in potassium content of both papilla and cortex, of 18% and 13% respectively. This was not affected by administration of aldosterone. The most striking change following adrenalectomy was in degree of hydration of renal tissue. The water content of the papilla increased 33%, while the cortex increased 24%. Once again aldosterone was without influence on these values.

In contrast to uniformity of sodium concentration/unit dry tissue weight, the concentration of sodium in tissue water (Table II) showed considerable change in both parts of the kidney, as a result of changes in tissue water content. Adrenalectomy caused a fall of 23% in papillary sodium concentration ($p = 0.05$) and of 20% in sodium concentration of the cortex ($p < 0.02$). Aldosterone again was without effect. Potassium concentration in the papilla and cortex tissue water was

§ Kindly supplied by Dr. R. Gaunt, Ciba Pharmaceutical Products, Summit, N. J.

likewise lowered by adrenalectomy whether aldosterone was given or not.

*Discussion.* The operation of the countercurrent multiplier concentrating mechanism depends upon simultaneous presence of 3 factors: 1) antidiuretic hormone, to render renal tubule permeable to water, 2) active, water-free reabsorption of sodium in the ascending limb of Henle's loop, and 3) adequate flow of blood in capillary loops of medulla. An attempt was made to influence the sodium transport mechanism, while maintaining amount of antidiuretic hormone maximal by prolonged dehydration and assuming that blood flow in medullary capillaries remained comparable in all animals. Under these conditions any change in concentration of sodium in renal papilla of animals could be ascribed to a change in rate of active sodium transport. The papillae of adrenalectomized rats contained lower concentrations of sodium in tissue water than those from normal animals. This confirms the results of Guinnebault and Morel(9) and supports the view that adrenal secretions are required for adequate function of countercurrent system.

Change in sodium concentration in papilla following adrenalectomy was entirely the result of changes in water content. Dry tissue solids represent a far better base of reference for such analyses, since it can be safely assumed that they remain constant. In consequence, one can determine whether changes in sodium concentration in wet tissue are due to changes in hydration or in sodium accumulation.

In light of countercurrent multiplying theory, in presence of antidiuretic hormone even a small change in rate of sodium transport is bound to result in much larger variations in tissue hydration. This dominance of water change over sodium content has also been observed when antidiuretic secretion alone is changed(10).

Changes in water content of renal cortex which followed adrenalectomy were similar but less pronounced than those seen in the papillae. The significance of this is not clear.

Reasons why administration of aldosterone in relatively large amounts to adrenalectomized animals, failed to return the composition of renal papilla to normal, may be manifold. Duration of aldosterone treatment was probably sufficient since this hormone produces sodium retention within 2 to 4 hours, and changes observed in electrolyte content of serum and muscle of these rats (Borle, A. B.), *et al.*, unpublished observations). Perhaps aldosterone exerts its sodium-retaining effect elsewhere in the renal tubule. A second possibility is that other adrenal steroids may be required for aldosterone to increase sodium concentration in the medulla. This latter hypothesis is supported by clinical observation that glucocorticoids are required to enable a patient with Addison's disease to handle a water load in normal fashion(11,12).

The failure of aldosterone to produce noteworthy changes in renal tissue composition of normal rats is not so surprising. The conditions of these experiments were such that a maximal retention of sodium due to endogenous secretion of hormone might be expected (13,14) and thus addition of further exogenous hormone would have little effect.

*Summary.* 1. Water, sodium, and potassium contents of renal cortex and papilla have been measured in normal and adrenalectomized hydropenic rats with and without administration of exogenous aldosterone. 2. Sodium content of renal papilla and cortex expressed in terms of dry tissue solid were little changed by adrenalectomy of aldosterone. However, water content was increased by adrenalectomy, the increase being 33% in papilla and 24% in the cortex. Aldosterone had no significant effect on this phenomenon. 3. It is suggested that the adrenal secretion exerts an influence on active transport of sodium across the ascending limb of loop of Henle. 4. Under our conditions, aldosterone is not sufficient to restore renal tissue concentration of sodium to normal in adrenalectomized animals.

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## Hyperlipemia and Hemolysis. II. Interaction of Sodium Oleate with Human Erythrocytes in Homologous Plasma. (24871)

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Previous studies(1) on the interaction of sodium oleate with washed human erythrocytes suspended in buffered isotonic saline indicate that erythrocyte-oleate binding precedes hemolysis; once lytic quantities are bound, repeated iced saline washings fail to dissociate oleate or check hemolysis, and following lysis, hemolytic substances are released. The present studies consider the interaction of sodium oleate with washed human erythrocytes suspended in homologous plasma. This investigation is a continuation of studies concerned with intermediate pathways for *in vivo* hemolysis reported during conditions associated with rapid increases in serum unesterified fatty acid(2-5).

*Methods and materials.* Sodium oleate (Baker) was employed as the fatty acid; all solutions were prepared in sodium phosphate buffered saline, ionic strength 0.15. The pH of buffered oleate solutions, measured with glass electrode, was 7.10 at 37°C and 7.20 at 25°C. All oleate solutions remained at room temperature 24 hours prior to use since hemolytic potency declined upon standing, rapidly at first, then more slowly. Mechanical fragility of human erythrocytes suspended in fresh heparinized plasma or in buffered saline was determined at 25°C with pipette shaker generating 2 inch horizontal strokes at 220/minute; all suspensions were tested in 1 x 12 cm test tubes. On completion of shaking, the tubes were spun at 2500 rpm, 0°C, 15 minutes and supernatant hemolysis determined by a Klett-Summerson photoelectric colorimeter (550 mu. filter).

*Results. Binding of oleate by plasma.* Ability of mammalian plasma and plasma proteins to bind fatty acid and neutralize hemolytic activity has been documented extensively (6-9). The present data are consistent with these findings. The assumption is made that human plasma inhibition of oleate hemolysis is dependent on plasma oleate binding. Table I indicates capacity of bovine serum albumin (Armour, Fraction V) to render exogenous oleate hemolytically inert; 1 mole of bovine serum albumin binds the hemolytic equivalent of 5 to 6 moles exogenous sodium oleate at pH 7.1, 37°C. These results do not reflect *total* oleate binding capacity of bovine serum albumin since initial oleate albumin levels were not determined. Lysis inhibition by bo-

TABLE I. Capacity of Bovine Serum Albumin to Bind Exogenous Sodium Oleate.

| | Exogenous sodium oleate ($\times 10^{-7}$ moles) | Bovine serum albumin* ($\times 10^{-7}$ moles) |
|---|---|---|
| | 24 | 4.6 |
| | 12 | 2.3 |
| | 6 | 1.1 |
| | 3 | .5 |
| Mean | 11 | 2.1 |

* Minimum quantity required to prevent oleate lysis of washed human erythrocytes at 37°C, pH 7.1. Each value is mean of 3 determinations.