statistically significant peak and low hours in both the 5039 spontaneous and the 5507 low-forceps deliveries, just as was seen in the total for each hospital and in the grand total.

It is interesting that Charles (*2*) and also Guthmann (*1*) noted a daily disproportion in the frequency of the onset of labor. Charles found that 62 percent of labors began between 9 P.M. and 9 A.M., the midpoint of this period being 4 hours before the midpoint of the peak period of birth given here.

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9 November 1955

Adrenal Cortex and the Parotid Secretion of Sodium-Depleted Sheep

The isolation of aldosterone (*1*) has stimulated inquiry into the role of the adrenal gland in the changes occurring in the activity of electrolyte-secreting tissues as a result of sodium ion (Na^+) depletion (*2*). The necessity of adequate techniques of bioassay has been emphasized accordingly (*3*).

It has been reported elsewhere (*4*) that sheep with a modified Pavlov-Glinski fistula of the parotid duct lost 2 to 4 lit of hypertonic alkaline saliva each day (composition: Na^+, 170 to 180 mequiv/lit; K^+, 5 to 15 mequiv/lit; Cl^-, 8 to 15 mequiv/lit; HCO_3^-, 120 to 150 mequiv/lit; HPO_4^{--}, 15 to 40 mequiv/lit; $Na^+/K^+ = 18$ to 25). If the fistula loss of Na^+ was not replaced, these sheep became rapidly depleted of large quantities of Na^+, and the Na^+/K^+ ratio of the saliva decreased commensurately

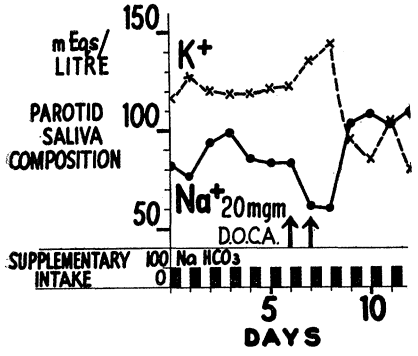

Fig. 1. Parotid fistula No. 7. Effect of DOCA on electrolyte composition of parotid saliva of a sheep moderately depleted of Na^+.

from 18 to 0.1. Since the $Na^+ + K^+$ total remained virtually constant, and the anion pattern was little changed, the saliva retained its hypertonicity and alkalinity.

At the outset, a tentative hypothesis was that the change in Na^+/K^+ ratio was mediated by the activity of the adrenal cortex, responding in some way to Na^+ depletion by enhanced production, presumably of aldosterone, which in turn affected the electrolyte output of the sheep's parotid gland. The following experiments were carried out (*5*).

Desoxycorticosterone (DOCA) was administered to a normal sheep with a parotid fistula. The animal, which had been moderately depleted of Na^+ during the previous 15 days, was secreting saliva of altered pattern (Na^+, 76 to 100 mequiv/lit; K^+, 115 to 128 mequiv/lit). On 2 successive days 20 mg of DOCA was injected intramuscularly, and a definite alteration of salivary Na^+/K^+ ratio resulted (Fig. 1). If, however, the same dosage of DOCA was given when the animal was grossly depleted of Na^+ (salivary pattern $Na^+/K^+ = 30/170 = 0.18$), there was less than 5 mequiv/lit effect on the concentrations. Thus DOCA was shown to produce an effect on the salivary electrolyte pattern. But was the adrenal cortex the regulator of the change seen during Na^+ depletion?

Sheep with unilateral parotid fistulas were bilaterally adrenalectomized.

1) If adequate Na^+ replacement was given, the sheep could be maintained in good condition indefinitely on a daily dose of DOCA (5 to 10 mg) and cortisone (25 mg). DOCA was the more critical component. The volume and electrolyte pattern of parotid saliva were normal.

2) If adrenal-hormone supplement alone was withdrawn, the usually observed fall in plasma Na^+/K^+ ratio and large Na^+ loss in the urine occurred (Fig. 2). However, despite the negative Na^+ balance (136 mequiv), there was no characteristic fall in the salivary Na^+/K^+ ratio. In fact this ratio rose (Fig. 2). The animal's condition rapidly deteriorated. When the adrenal hormones were replaced, the salivary Na^+/K^+ ratio fell to a level consistent with the existing degree of Na^+ depletion.

3) If the DOCA supplement was increased (40 mg/day), the salivary Na^+/K^+ ratio fell from 17 to 2.0 in 5 days.

4) If Na^+ replacement was withheld, and the usual maintenance-hormone supplement continued, a state of adrenal insufficiency developed within 2 to 4 days. Despite the Na^+ depletion, there was little or no change in the salivary Na^+/K^+ ratio.

5) If, however, Na^+ was withdrawn during a period of constant but increased

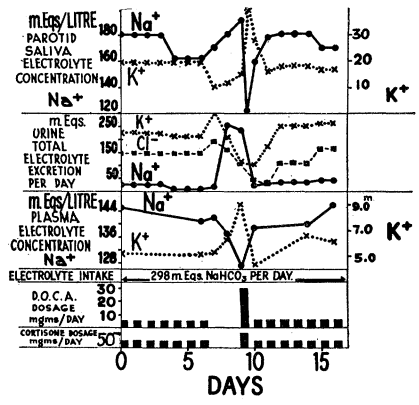

Fig. 2. Adrenalectomy No. 1. Effect on parotid saliva, urine, and plasma of withholding DOCA and cortisone from a bilaterally adrenalectomized sheep with a parotid fistula. The daily Na^+ supplement was given throughout the experiment.

DOCA dosage (20 mg/day), the salivary Na^+/K^+ ratio fell to that seen during Na^+ depletion in a nonadrenalectomized sheep. Figure 3 shows that the increased DOCA dosage lowered the salivary Na^+/K^+ ratio, and that an equilibrium state was reached before the Na^+ withdrawal. The pattern returned to this equilibrium ratio upon restoration of Na^+ balance. Hence, adrenal steroids do alter the sheep's salivary electrolyte pattern, and it is a necessary condition that they be provided in excess of basal-maintenance dose if this electrolyte pattern is to vary commensurately with Na^+ depletion as in a normal animal.

However, the finding in item 5 of an unequivocal response to Na^+ depletion on a constant DOCA dosage suggests that the "cause" of this parotid behavior in the normal animal is the simultaneous interaction of at least two factors. Probably the adrenal secretion is one contributory condition in a set of jointly

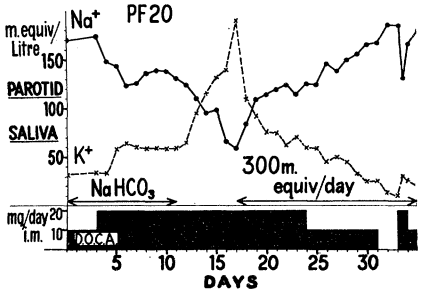

Fig. 3. Parotid fistula No. 20. Na^+ depletion (515 mequiv) in a bilaterally adrenalectomized sheep with a parotid fistula. Na^+ depletion caused a clear-cut fall in the Na^+/K^+ ratio of parotid saliva, in addition to that caused by increased DOCA dosage. After the recovery period, DOCA alone was withheld for 2 days (days 31 and 32) and, in this circumstance, the Na^+/K^+ ratio of saliva rose, despite a negative Na^+ balance of 300 mequiv.

sufficient and severally necessary conditions, and it would seem more correct, in this situation, to describe its role as such, than as permissive of the action of other factors. Lorenz (*6*), in analyzing innate behavior patterns, has used Erhlich's term *amboceptoric* in like circumstances.

The value of this preparation is that the electrolyte activity of adrenal steroids can be examined in a conscious animal in normal correspondence with its environment at strictly controlled levels of Na^+ depletion.

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Cholinesterase Activity of Human Leucocytes

The erythrocytes and the serum of blood are known to possess cholinesterase activity, but the experiments of Fegler, Kowarzyk, and Szpunar (*1*) have been generally accepted (*2*) as proof of the absence of cholinesterase in the white blood cells of man. Hines (*3*) also reports that he was unable to find cholinesterase activity in the white layer of centrifuged human blood, but he gives no details. We wish to report here experimental evidence that human leucocytes contain appreciable amounts of an acetyl-β-methylcholine chloride (Mecholyl) (*4*) splitting enzyme.

White-cell preparations were obtained from the blood of seventeen young, apparently healthy males. The method of separation selected was the flotation technique of Vallee, Hughes, and Gibson (*5*), with the exception that the flotation solution was made up with dextran instead of the albumin used by these authors. Cholinesterase activity determinations were performed according to a modification of the electrometric method of Michel, (*6*), in which the cleavage of Mecholyl identified the white-cell enzyme as being similar to the cholinesterase of erythrocytes and brain tissue (*7*). The use of this substrate also obviated the necessity for complete removal of residual plasma from the preparations.

Concomitant determinations, using Mecholyl, of the erythrocyte cholinesterase activity were performed on each sample of whole blood for purposes of comparison. Hematocrit determinations permitted the activity values to be expressed in terms of a unit volume of packed cells, thus compensating for variations in the total packed red-cell volume.

The flotation solution was made up by first dissolving 214.2 g of sodium chloride-free clinical dextran (*8*) in 900 ml water. Then 0.6092 g of solid sodium chloride was added to 100 ml of the dextran solution to give a freezing-point depression of 0.57C° and a specific gravity of 1.079. This solution is isoosmotic with plasma and has a density that is intermediate between the densities of the normal erythrocyte and leucocyte.

For each determination, 15 ml of freshly drawn heparinized blood was carefully layered onto 10 ml of dextran solution in a 40-ml test tube having an inside diameter of 25 mm. The tube was centrifuged for 10 minutes at 42 *g* and then for 20 minutes at 1500 *g*. The plasma was drawn off and the white cells that had collected at the dextran-plasma interface were pipetted into a graduated 15-ml centrifuge tube. The volume was increased to 5 ml by the addition of isotonic saline, and the cells were homogeneously suspended by inversion.

A Van Allen microhematocrit tube of known total volume and with a calibrated stem was filled to the new calibration mark above the bulb with an aliquot of this suspension and was centrifuged at 1400 *g* for 10 minutes. Some cells usually adhered to the sloping sides of the upper part of the tube; these were freed from the wall with the aid of a thin piece of wire. The tube was then returned to the centrifuge and was spun for an additional 20 minutes. In this way, values for white-cell concentrations were obtained that were expressed in terms of volume of packed white cells per unit volume of suspension. Microscopic examination of stained smears of the packed cells showed that contamination by erythrocytes was negligible.

The remaining portion of the white-cell suspension, of known volume and leucocyte concentration, was packed in the centrifuge tube by spinning for 5 minutes at 360 *g*. The cells were resuspended in 0.5 ml of saline and transferred undiluted to a 5-ml beaker for the electrometric activity determination. To the leucocyte suspension (or to 0.02 ml of whole blood in 0.5 ml of water) was added 0.5 ml of Michel's buffer No. 2 (*9*), followed by 0.1 ml of 0.22 F Mecholyl (*10*). The initial *p*H reading was made immediately, using a model G Beckman *p*H meter equipped with glass and calomel electrodes. The beaker was then placed in a water bath at 25 ± 1°C for 1 hour, after which, the *p*H was again determined. All experimental *p*H differences were corrected for nonenzymatic hydrolysis by means of accompanying blank determinations. Hematocrits of the whole blood were determined in Wintrobe tubes by spinning at 1500 *g* for 30 minutes.

Triplicate determinations were performed on 3 successive days on four subjects in order to permit an evaluation of the analytic precision. Day-to-day variations were thus included in the measure of precision. There is a wide variation among the subjects in the values for white-cell cholinesterase, but the red-cell values fall within a narrow range, in agreement with previous results. The mean leucocyte-cholinesterase activity is 0.028 Δ *p*H/hr mm^3 packed cells, with 95-percent confidence limits of ± 0.016. The standard deviation of the triplicate analyses is ± 0.014 (coefficient of variation = 49 percent), which indicates the relatively low order of precision of the individual results. Application of the F test yielded a value exceeding the 1-percent point, from which it may be concluded that the method is sufficiently precise to detect differences from one individual to another. The mean value for erythrocytes is 0.069 Δ *p*H/hr mm^3 packed cells (standard deviation = ± 0.007); the ratio of the mean activity of packed erythrocytes to the mean activity of packed leucocytes is then 2.5. Graphic comparison of the activities of the two cell types showed the absence of any systematic relationship between them.

This investigation adds another item to the long list of tissues that are known to contain cholinesterase. Further studies will be required to elucidate the function of this enzyme in the leucocytes.

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9. This is 0.006 formal in sodium barbital, 0.001 formal in KH_2PO_4, and 0.30 formal in NaCl. The *p*H is adjusted to 8.00 by the addition of 0.1 F HCl.