71.6% for the same groups. Expressed as per cent of body weight, the liver protein contents of the female DEA injected rats and female control rats were 0.74 and 0.71 respectively, while the corresponding values for the male DEA injected rats and male controls were 0.80 and 0.81 respectively.

Although DEA was not a growth inhibitor on the levels at which it was administered, the differences in liver composition of DEA fed animals and controls are of interest, particularly in view of similar experiments with ethionine(5). The fact that feeding either DEA or ethionine can result in higher water and protein content of livers as compared with controls suggests that these effects are due to their properties as amino acid analogues *per se*, and not to interference with specific functions of the amino acids involved (*e.g.* transmethylation). Since the effect of DEA is to lower and the effect of ethionine is to raise the level of liver lipide(5), it appears likely that the mechanisms of action of these compounds on lipide metabolism are different. The scope of these experiments is not sufficient to suggest the mechanism by which the DEA diet lowers the liver lipide content.

Summary. The effect of the administration of β,β-diethylalanine (DEA) upon the composition of rat livers has been studied. Feeding DEA to male rats resulted, at the higher of 2 levels, in lower liver lipide and higher liver water and protein content than in controls. Injections of massive doses of DEA were without significant effect. The implications of these results with respect to similar studies with ethionine are discussed.

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Effect of Aldosterone and Desoxycorticosterone on Adrenalectomized Dogs.* (21034)

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Since 1950, data have rapidly accumulated concerning the presence in adrenal cortical extracts and in adrenal vein blood, of a highly active Na-retaining substance(1-6). This work has culminated in recent announcements from 3 laboratories of the isolation in crystalline form of a new mineralcorticoid(3,7,8), provisionally designated by Simpson *et al.*(7) as electrocortin. Chemical degradation of the new crystalline adrenal steroid by Simpson, Tait, Wettstein, Neher, Euw, Schindler and Reichstein(9) shows that the compound is 11β-21-dihydroxy-3,20-diketo-4-pregnene-18-al. According to these investigators, this reacts mainly as the 11-hemiacetal when in solution; they suggested aldosterone as a definitive name for the compound. Owing to scarcity of material, few detailed physiological studies on the pure substance have appeared (10-12).

Through the courtesy of Professor T. Reichstein, the writers secured enough crystalline material for detailed tests on 4 adrenalectomized dogs. The present study is a comparison of the life maintenance and Na-retaining activity of pure crystalline aldosterone with that of the free alcohol of desoxycorticosterone (DOC).

* Expenses of this investigation were defrayed by grants from Sharp and Dohme Division of Merck & Co., and the National Science Foundation. DOC was supplied by Ciba Pharmaceutical Products, and the aldosterone by Professor T. Reichstein of the University of Basle, Switzerland.

Material and methods. Earlier work(13), showed that 10% aqueous-alcohol is an excellent vehicle in which to administer the free alcohol of adrenal steroids when rapid absorption is necessary. This vehicle also appears to be superior to oil or water preparations or micro-crystalline suspensions for life maintenance studies. Therefore, the aldosterone and DOC crystals were dissolved in sufficient 95% ethanol which when diluted to 30% with distilled H_2O yielded a solution containing 25 μg/cc of aldosterone and 1.5 mg/cc of DOC. This constituted the stock solution which if kept in sterile bottles can be left at room temperature for long periods without deterioration. Each day the dosage to be used was removed from the stock solution, diluted to 10% alcohol and administered subcutaneously in divided doses, one-half at 9:30 a.m. and the remainder at 4:30 p.m. Four dogs were used for testing aldosterone, 3 for DOC, and 4 each for assay of cortisone and hydrocortisone. In this brief report, however, but 2 representative experiments (one each for aldosterone and DOC) will be discussed. The dog given DOC had been adrenalectomized 1 year and weighed 17 kg; the aldosterone animal weighed 20 kg and the adrenals had been removed 3 years previously. Both animals, prior to these experiments had been employed in bioassay work and studied through numerous cycles of adrenal insufficiency. During the intervals between the various experiments they were maintained in good health on 0.5 mg DCA in oil injected intramuscularly once daily. The diet used in these and earlier experiments has been described(14); however each dog's daily ration contained a total of 1.47 g of Na and 0.94 g of K. This is a low sodium diet for an adrenalectomized dog. The animals were kept at each dose level for 10 days and at the termination of this interval blood samples were taken, the arterial pressure determined and the dosage reduced by 50%. This procedure was followed until subminimum dose levels were reached and signs of adrenal insufficiency appeared.

Results. The essential data regarding the physiological activity of aldosterone and DOC are given in Fig. 1. It is obvious that both steroids, in very small doses, when administered in 10% alcohol are capable of maintaining a normal serum electrolyte pattern, arterial pressure and blood urea nitrogen. The minimum maintenance dose of aldosterone for the dog whose analyses are represented in Fig. 1, is 6.25 μg/dog/day, whereas it required 250 μg of DOC/dog/day to maintain the animal in equally good health. The dogs retained full activity, vigor and appetite at these dosages. Upon reducing the daily amount of aldosterone by one half, or to 3.125 μg/dog/day, the arterial pressure declined, serum K increased as did also the blood urea nitrogen. However, serum Na exhibited little, if any, change. The appearance, appetite and activity of the dogs seemed unimpaired throughout the 10-day interval of this low dose level. Further reduction of dosage to 1.56 μg/dog/day led to development of symptoms of adrenal insufficiency such as lassitude, weakness and spasticity of the hind limbs. Serum K generally increased to the level at which early signs of hyperpotassemia became evident. The arterial pressure slowly declined and blood urea nitrogen became elevated. None of the dogs receiving aldosterone completed the 10-day test period at this lowest dose level.

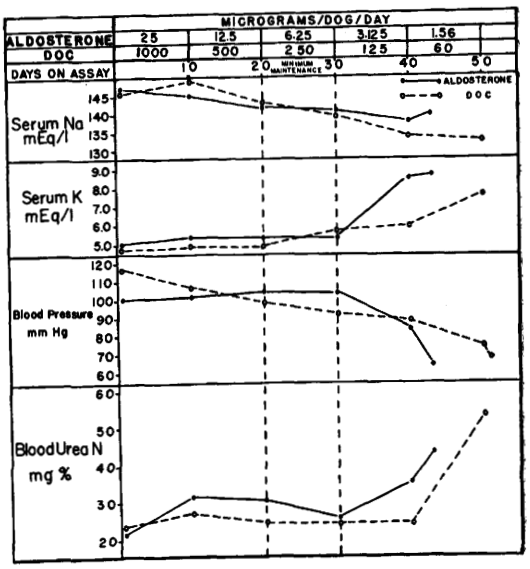

FIG. 1. Effect of aldosterone and DOC on arterial pressure, serum electrolytes and blood urea nitrogen of adrenalectomized dogs.

Thus, the minimum maintenance requirement for this aldosterone-treated dog is 6.25

μg/dog/day, the subminimum dose is 3.125 μg/dog/day, and the level at which substitution therapy is necessary is 1.56 μg/dog/day. However, when the data obtained from study of all 4 animals that received this steroid are analyzed, individual variations appear. In all cases the minimum dose found adequate to maintain normal serum electrolytes, arterial pressure, blood urea nitrogen and body weight, was approximately 10 μg/dog/day. The 3 animals receiving DOC exhibited some variation with respect to their maintenance requirement for the steroid, which was 250-125 μg/dog/day. Thus, aldosterone appears to be about 25 times more potent than DOC under these experimental conditions. These data are in agreement with those of others (7-10). However, our data show that a larger daily dose is required to prevent a slow decline in arterial pressure than is necessary to maintain a normal serum electrolyte pattern. Excluding this slow decline in blood pressure, the minimum daily dose of aldosterone is 6.25 μg/dog/day and for DOC, the dose required varied between 250-125 μg per day with 2 of 3 dogs requiring the larger dose.

Earlier observations(6) by the writers, employing a refined (maximum 60% purity), Na-retaining preparation of the amorphous fraction of adrenal extracts given to us for testing by Dr. E. C. Kendall of this University, had shown that it was not uncommon for adrenalectomized dogs receiving the Na-retaining material, to develop severe symptoms of adrenal insufficiency despite retention of normal levels of serum Na. It was also noted that hyperpotassemia accompanied by severe cardiac symptoms developed even though the serum Na remained normal. Similar changes may become manifest in the lower dose ranges of aldosterone, *e.g.*, 1.56 μg/day. It is clear from the data in Fig. 1 that the steroid in low dosage is not as effective in maintaining a normal level of serum K as it is in keeping the serum Na constant.

Discussion. Isolation of aldosterone and the observation, made by several groups of investigators that it is much more potent than desoxycorticosterone in its Na-retaining properties, raises the question whether the new steroid differs qualitatively as well as quantitatively from DOC compounds. A ready answer is not now forthcoming owing to the paucity of material available for physiological study; however, certain facts seem to be definitely established. Pure aldosterone is about 25-30 times more active than either the free alcohol of desoxycorticosterone in 10% alcohol or its acetate in oil, when tested for Na-retention and life maintenance on adrenalectomized dogs(7-10). According to some investigators(10), the steroid differs in several notable respects from DOC: 1) it is far superior in retaining Na; 2) its activity in inducing K excretion is only about 5 times greater, and finally, 3) aldosterone has no demonstrable effect on water excretion. Knauff *et al.*(5), state that their partially purified material had no effect on liver glycogen deposition and also gave negative results when tested for reduction of circulating blood eosinophils. On the other hand, Schuler, Desaulles and Meier(12), found aldosterone to be about 30 times as active as free desoxycorticosterone in increasing the liver glycogen of mice, but it was somewhat less active than cortisone and corticosterone in this respect. Gaunt *et al.*(15), found the steroid to have an activity equal to or even greater than cortisone when used on rats in the cold stress test and to be potent, though less so than cortisone in the eosinophil depletion test. These data strongly suggest that although aldosterone exhibits activity resembling that of desoxycorticosterone in certain important respects, it also appears to possess a number of other physiological properties which are qualitatively different from those of DOC compounds.

Summary. The minimum daily dose of aldosterone when administered in 10% alcohol to adrenalectomized dogs is approximately 10 μg/dog/day; the dose of the free alcohol of desoxycorticosterone required is 250-125 μg/day. Thus, aldosterone is about 25 times more active than DOC. The new steroid in low dosage is less efficient in regulating the serum K level than it is in maintaining a normal level of serum Na. Hyperpotassemia and symptoms of adrenal failure may be present in dogs presenting normal levels of serum Na. A larger dose of this mineralocorticoid is required to

maintain a normal level of arterial pressure than is necessary to maintain a normal serum electrolyte pattern. Aldosterone appears to possess certain physiological properties which distinguish it from desoxycorticosterone compounds.

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Relationship between Plasma Mucoproteins and Protein Sugar in Patients with Rheumatoid Arthritis Receiving Cortisone.* (21035)

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It has been shown by Winzler *et al.*(1) that serum mucoprotein concentrations are significantly increased in patients with rheumatoid arthritis. Shetlar and associates(2), and Kelley(3) have demonstrated that in the same disease there is a considerable rise in the carbohydrate content of the total serum protein. Serum mucoproteins are comparatively rich in polysaccharides since they contain approximately 14.5 mg % of carbohydrate(3), whereas in normal sera the total globulin fraction contains only 1.5 to 3.9 mg % of carbohydrate and the albumin fraction, about 1.2 mg %(4). Thus, although mucoproteins represent approximately 0.7% of the total serum protein, they contain about 10% of the total protein sugar.

The relationship between the concentrations of serum mucoprotein and total protein sugar has been studied in the present report in order to test the hypothesis that variations of mucoproteins and total protein sugar in inflammatory diseases may depend upon a common regulating factor.

Methods. Mucoproteins were estimated by the Winzler method and are expressed as mg of tyrosine per 100 ml of original serum (Tyrosine Index). Total protein sugar was determined by the tryptophan method of Shetlar as modified by Badin, Jackson, and Schubert(5). Error in duplicate analyses averaged ±0.3 mg for the Tyrosine Index.

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