the corticosteroids—has been achieved by Hogg *et al.* (*4*). Byrnes and associates have found two of these steroids to be more potent than their nonmethylated analogs both in glycogen-depositing activity and in sodium-retaining activity as tested in the rat (*5*).

The present report (*6*) represents the initial use of these steroids in two additional species—man and the dog. We have studied in normal human subjects, in patients with Addison's disease, and in adrenalectomized dogs the comparative pharmacology of hydrocortisone (F), 2-methylhydrocortisone (methyl F), 9-α-fluorohydrocortisone (FF), and 2-methyl, 9-α-fluorohydrocortisone (methyl FF). In the dogs, additional observations were made on the effects of desoxycorticosterone (DOC) and aldosterone.

In human subjects, the oral administration of single doses of methyl FF (0.025 to 1.0 mg), FF (0.2 to 1.0 mg), methyl F (10 to 400 mg) and F (100 mg) induced retention of sodium and loss of potassium. More precise assays of these properties, performed in adrenalectomized dogs by a method previously reported (*7*), are summarized in Table 1. In brief, the 2-methyl compounds are many times more potent than their nonmethylated analogs. Methyl FF is seen to be more potent than aldosterone, and thus to be the most active sodium-retaining and potassium-losing steroid known at the present time.

The mechanism whereby the methylated steroids affect cation excretion was investigated in dogs. Methyl FF and, to a lesser degree, methyl F were capable of producing decreases in sodium excretion despite concomitant increases in glomerular filtration rate, indicating that these steroids increase the reabsorption of sodium by the renal tubules.

The decrease in circulating eosinophils that follows the administration of steroids may be used as one index of their "glucocorticoid" activity. Observations in both man and the dog indicated that the methylated steroids were only slightly more potent than the nonmethylated compounds during the first 4 hours following treatment. Eosinopenia persisted much longer, however, with use of the methylated steroids.

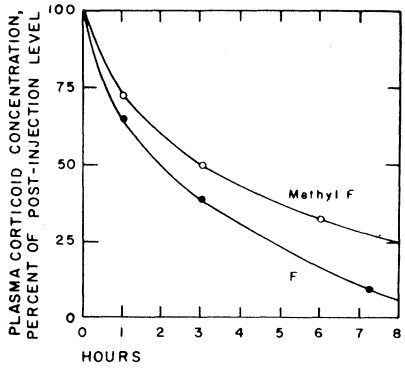

Fig. 1. Plasma 17,21-dihydroxy-20-ketosteroids (unconjugated) levels following intravenous injection of F and methyl F. The steroid concentration 10 minutes after injection is represented at "100-percent" concentration and "zero" hour. Curves are averages of three steroid tolerance tests in each of three subjects.

Whereas the direct biologic effects of a single oral dose of hydrocortisone ordinarily disappear in less than 24 hours, the effects of the 2-methyl derivatives were found to persist for approximately 48 hours in our studies in human subjects. Studies were accordingly designed to determine whether this prolonged action of the methylated steroids could be related to a slower rate of metabolic inactivation by the body.

Following administration of F and methyl F to human subjects, blood levels were determined at various intervals using a modification of the method of Silber and Porter (*8*) for measuring dichloromethane-soluble 17,21-dihydroxy-20-ketosteroids. It was consistently found that methyl F was removed from the circulation at a slower rate than F (Fig. 1). This was true whether the steroids were administered by vein or by mouth.

Following the administration of F to human subjects, one can account for approximately 30 percent of the administered dose by the determination of 17,21-dihydroxy-20-ketosteroids in the urine, using the method of Silber and Porter (*8*). The principal product thus measured is tetrahydrocortisone glucuronide. By way of contrast, following the administration of methyl F one can account for no more than approximately 5 percent of the administered dose using the same chemical methods.

It is suggested that the presence of the 2-methyl group alters the susceptibility of the steroid to enzymatic attack so that the processes by which F is metabolized operate less efficiently, and other processes assume greater prominence. As a result, removal of the methylated steroid from the circulation proceeds slowly, and during the metabolism of the steroid the 17,21-dihydroxy-20-keto configuration is lost. To some degree, the enhanced potency as well as the prolonged action of the 2-methyl steroids might be explained by the slower rate at which the body metabolizes them to inactive forms.

GRANT W. LIDDLE
JUNE E. RICHARD
Clinic of General Medicine and Experimental Therapeutics, National Heart Institute, Bethesda, Maryland

Table 1. Relative effectiveness of various steroids on excretion of Na^+ and K^+ in the adrenalectomized dog, using DOC as a standard. The figures in parentheses represent 95-percent confidence limits. Ratios of potency are adjusted for molecular weight, and dosages are compared on an equimolar basis.

Steroid	Sodium		Potassium	
	Effect	Potency	Effect	Potency
DOC	Retention	1	Loss	1
Aldosterone	Retention	39 (26–61)	Loss	29 (20–41)
Methyl FF	Retention	49 (19–100)	Loss	155 (75–353)
FF	Variable		Loss	5.5 (3.1–10.7)
Methyl F	Variable		Loss	1.0 (0.5–2.1)
F	Loss		Loss	0.04 (0.026–0.063)

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2 December 1955

Oral Phosphatase Levels and Caries Activity

Oral phosphatases in human saliva are thought to be principally of bacterial cell origin (*1–4*) and in part of glandular origin (acid phosphatase from the parotid glands, *3*). The significance of these enzymes in saliva is not clear, yet correlations have been suggested between their titers and certain oral debilities (*5, 6, 7*).

Lactobacilli show little phosphatase activity (*2, 4*); however Bray and King (*8*) demonstrated high degrees of phosphatase activity in 14 groups of organisms that are commonly found in the oral environment. Fitzgerald (*4*) has suggested a possible caries-activity test based on the titer of phosphatases in saliva.

Our experiment (*9*) was conducted to determine a possible relationship between

Table 1. Statistical analysis of data obtained from phosphatase activity in the whole salivas of 26 caries-free and 26 caries-active persons.

Activity	Caries-free	Caries-active
*Alkaline**		
Range	0.06– 1.98	0.06– 1.98
Mean ($\bar{x}$)	0.58	0.68
S. D. (σ)	0.66	0.50
	$\frac{\text{Dev.}}{\sigma} = \frac{\bar{x}_1 - \bar{x}_2 - O}{\sigma x_1 - x_2} = 0.61$	
*Acid**		
Range	0.90–12.60	2.40–13.80
Mean ($\bar{x}$)	5.65	7.61
S. D. (σ)	4.11	3.30
	$\frac{\text{Dev.}}{\sigma} = \frac{\bar{x}_1 - \bar{x}_2 - C}{\sigma \bar{x}_1 - \bar{x}_2} = 1.9$	

* Units of phosphatase per 100 ml of whole saliva.

caries activity and phosphatases found in whole stimulated saliva. Analyses for both acid and alkaline phosphatase were performed on a randomly selected group and on caries-free and caries-active individuals.

Fifteen milliliters of paraffin-stimulated saliva were collected from each of 100 naval recruits ranging in age from 17 to 20 years. Acute cases of gingival infection were eliminated from this otherwise randomly selected group. Similar saliva samples were collected from 26 caries-free and 26 caries-active individuals who had had no dental restorations. The caries-free group was selected by clinical and radiographic examination. The caries-active individuals were selected clinically on the basis of the existence of ten or more carious lesions.

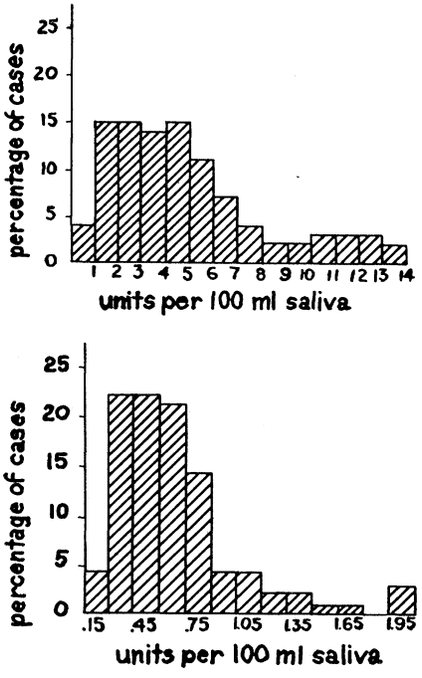

Fig. 1. Phosphatase activity in whole salivas of 100 randomly selected naval personnel of age ranging from 17 to 20 years. (Top) Acid pohsphatase: mean, 4.72; range, 0.60 to 13.8; standard deviation, ± 3. (Bottom) Alkaline phosphatase: mean, 0.57; range, 0.12 to 1.92; standard deviation, ± 0.405.

The colorimetric method of Seligman *et al.* (*10*), as adapted for saliva tests by Chauncey (*3*), was used for phosphatase determinations. The Coleman junior spectrophotometer at an optimum wavelength of 525 mμ and 10 by 75-mm cuvettes were used.

The mean acid phosphatase activity in whole salivas of 100 randomly selected persons was 4.72 units/100 ml, while that for alkaline phosphatase was 0.57 units/100 ml (Fig. 1). The mean acid and alkaline phosphatase activities in whole salivas of 26 caries-free individuals were 5.65 units/100 ml and 0.58 units/100 ml, respectively. The caries-active group showed mean phosphatase activities of 7.61 units/100 ml (acid) and 0.68 units/100 ml (alkaline).

Statistical analysis of the differences in the mean acid and alkaline phosphatase levels for caries-free and caries-active groups did not reveal significance. The probability of obtaining the observed differences in the means for acid phosphatase was 7 percent or less; for alkaline phosphatase, it was 54 percent or less (Table 1).

That oral phosphatases are predominately of bacterial cell origin seems well established (*1–4*). Rosebury (*6*) and others (*4, 7*) have ascribed possible roles to phosphatases in saliva that are based solely on known activity characteristics of these enzymes isolated from other tissues (bone, liver, kidney, serum, and so forth). The mere existence of a phosphatase associated with the bacterial cell does not, however, preclude the action of this enzyme according to previously accepted theory. Thus it becomes important to look on the oral phosphatases as entities and to characterize them according to the specific oral debility in which they are suspected to be taking part. For instance, recent tests in this laboratory showed that acid phosphatase of the parotid secretion was inhibited by tartrate, which has been shown to inhibit prostatic phosphatase (*11*). This would immediately bring to mind specific correlations regarding tests for cancer of prostate gland. However, characterizations of both parotid and prostatic enzymes become essential before conclusions may be drawn.

The failure to correlate the presence of phosphatases in saliva with certain oral debilities (dental caries, calculus formation, periodontal disease, and so forth) probably results from the fact that these enzymes are present in so many of the organisms normally present in the mouth that any association is obscured.

WILLIAM J. CARTER
School of Dentistry, University of Kansas City, Kansas City, Missouri
KIRK C. HOERMAN
HAROLD R. ENGLANDER
IRVING L. SHKLAIR
Dental Research Facility, U.S. Naval Training Center, Great Lakes, Illinois

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25 August 1955

Possible Function of Serum Proteins in Tissue Culture

In a recent article by Eagle (*1*) it was found that, with the exception of the necessity of serum proteins, completely defined chemical media are possible that support both growth and multiplication in certain lines of mammalian cells. The possibility that the serum proteins contribute as yet undetected trace elements or vitamins is being investigated by him (*1*, p. 503).

Although the presence of such trace elements or vitamins is certainly a possibility, there is another alternative that I feel should be considered. Both from the work described and what is known about protein chemistry and function, it seems that the essential substances could be proteins, perhaps certain of the serum proteins themselves.

From the results given in the paper (*1*, p. 503) it appears that the activities of the protein fractions were determined by the extent to which denaturation could not occur in the fractionation procedure. Exhaustive dialysis would remove most of the salts and other impurities, and this is known to render many proteins unstable and susceptible to structural changes (*2*, pp. 211–213; *3*). Alcohol is a denaturing agent for many proteins (*2*, p. 207; *4*, p. 173), and