terolemia (111 and 160 mg/100 ml). At autopsy there was no ascites; kidneys were pale.

Histologic findings. The following summarizes observations in the 8 rats which developed severe proteinuria and other aspects of nephrotic syndrome. With one exception, in which there was evident interstitial fibrosis and tubular atrophy (Fig. 2) kidneys appeared normal under low power, showing only a few hyalin or granular tubular casts. Under high power, the only significant changes were found in the glomeruli. These showed condensation and simplification of tuft patterns, coalescence of tufts and fraying and thickening of basement membranes (Fig. 4); glomeruli from animal with interstitial fibrosis showed in addition endothelial proliferation (Fig. 3). Some glomeruli contained deposits of "Schiff-positive" material (Fig. 5). These changes resemble those in clinical cases of nephrotic syndrome, occurring idiopathically or in association with renal vein thrombosis.

Summary. Among 41 albino rats subjected to partial occlusion of left renal vein with subsequent contralateral nephrectomy, 8 developed severe proteinuria and hypercholesterolemia, while 4 had also ascites and hypoalbuminemia. The kidneys were pale, with histological changes limited to glomeruli. These consisted of simplification of tufts, with coalescence, fraying and thickening of basement membranes. This technic requires further standardization, but seems to reproduce the clinical syndrome associated with renal venous thrombosis. To elicite the syndrome, venous occlusion must be incomplete and involve all functioning renal tissue.

ADDENDUM: Since completion of this manuscript, we found that G. Lagrue, B. N. Halpern, P. Milliez and A. Brannelec, *C. R. Soc. Biol.*, 1957, v151, 81, described a similar syndrome, although without noting edema or describing histologic appearance of kidneys, in rabbits subjected to bilateral sub-total occlusion of renal veins.

1. Pollak, V. E., Kark, A. M., Pirani, C. L., Shafter, H. A., Muehrcke, R. C., *Am. J. Med.*, 1956, v21, 496.
2. Harrison, C. V., Milne, M. D., Steiner, R. E., *Quart. J. Med.*, 1956, v25, 285.
3. Hasson, J., Berkman, J. I., Parker, J. G., Rifkin, H., *Ann. Int. Med.*, 1957, v47, 493.
4. Leger, L., George, L. A., *Presse Med.*, 1954, v62, 721.

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Physiologic Chloride Levels in Human Whole Saliva. (23891)

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Chloride concentration of human saliva has been shown to vary in certain systemic disease states(1,2). Dental research interest in salivary chlorides in relation to dental caries has stemmed primarily from the knowledge that chlorides enhance amylase activity and from reports that amylase activity of saliva from caries-active subjects is significantly higher than that from subjects free from carious lesions(3-6). There is diverse opinion as to significant differences in salivary amylase and chloride in subjects with caries activity(7-11) when compared to caries resistant subjects.

Our investigations involve multiple chemical analyses on saliva specimens collected from subjects presenting various oral pathologic states. Since there is considerable disagreement in the literature as to physiologic levels of some constituents under study, we instituted concurrent studies under identical experimental conditions to establish physiologic baselines.

The present study was designed to determine physiologic chloride levels under 2 different forms of stimulation. Also studied were effects of age and sex on chloride concentration and salivary flow rate and the effect of volume on chloride levels.

Procedure. All subjects had recently undergone thorough physical examination and each found to be fit. Times of arising and retiring, types of food and drink ingested, and environmental conditions were virtually identical in all instances. Oral health status was not a consideration on these otherwise healthy young adults. In the first portion of the experiment (3-RBS) whole saliva samples were collected at 9:00 a.m. from 323 males in 17-19-year age group and from 159 females between 18 and 21 years of age. Salivary flow was elicited with 3 large (size 32) crepe rubber bands, each subject chewing vigorously on the bands for 5 minutes. The advantages of this type eliciting agent have been previously discussed(12,13). In the second portion of the study (NES) 5 minute saliva samples were collected at 9:00 a.m. from 503 males between 17 and 24 years of age and from 179 females in the 18-26-year age group. No exogenous stimulation was employed with these participants. To preclude very small sample size, these subjects were instructed to produce by oral movements, as much saliva as possible during the collection interval. Analyses for variance included all subjects in the 3-RBS portion and 502 males and 164 females in the NES study. The inclusion of only these participants in the latter study was due to the extremely small number of subjects in certain age groups. Chloride concentration was determined by modification of the method of Sendroy(14). Salivary proteins were first precipitated with phosphoric-tungstic acid and the mixture then shaken with excess of solid silver iodate and centrifuged. After addition of sodium iodide to a portion of the supernate, the resultant free iodine was titrated with sodium thiosulfate.

Results. Three rubber band study. The mean volume for 323 male participants was 8.70 ml (S.D., 3.22), while for females was 6.24 ml (S.D., 2.58). No significant differences associated with age were found for either sex, but the difference between volume means for males and females was significant ($P<.01$).

The chloride mean for males was 31.78 meq/l (S.D., 6.96) and that for females was 27.90 meq/l (S.D., 6.22). Confidence limits for chlorides were estimated to be: males—17.9 to 45.7 meq/l; females—15.5 to 40.3 meq/l. No significant differences in chloride levels attributable to age were found for males or for females. However, a significant difference ($P<.01$) was evident when the sexes were compared.

TABLE I. Distribution of Volume and Chloride Means.

Exogenous stimulant	No. subjects	Sex	Volume Mean	Volume S.D.	Chloride Mean	Chloride S.D.
None	503	♂	6.32	2.21	23.28	4.10
3 rubber bands	323	♂	8.70	3.22	31.78	6.96
None	179	♀	4.67	1.30	23.67	3.97
3 rubber bands	159	♀	6.24	2.58	27.90	6.22

Correlation coefficients between volume and chlorides were: males—$r = .20$ ($P<.01$); females—$r = -.15$ ($P<.05$). Thus, for males, volume and chlorides were positively correlated and, for females, these variables were negatively correlated.

No exogenous stimulation study. The mean volume for 503 males was 6.32 ml (S.D., 2.21) and that for females 4.67 ml (S.D., 1.30). No significant volume differences attributable to age were found for males or females. The volume means for males and females, however, were significantly different ($P<.01$).

The chloride mean for males was 23.28 meq/l (S.D., 4.11) while that for females was 23.67 meq/l (S.D., 3.97). Confidence limits were estimated to be: males—15.1 to 31.5 meq/l; females 15.7 to 31.6 meq/l. For males, significant differences ($P<.05$) were found in chloride levels for the 6 age groups tested in the analysis of variance. For females, no significant differences were found in chloride levels for the age groups tested. No significant difference was found in chloride concentrations when the sexes were compared.

The correlation coefficients between volume and chlorides were: males—$r = -.23$ ($P<.01$); females—$r = -.16$ ($P<.05$). Thus, volume and chlorides were negatively correlated for both males and females.

Discussion. Chloride means. White, *et al.* (1) analyzed paraffin stimulated saliva samples from 73 control subjects and reported a mean value of 29.00 meq/l. Chauncey(15), Hubbell(9), and Brown and Klotz(16) also studied stimulated saliva and reported means of 20, 12.06, and 14.37 meq/l, respectively. Anders(17) studied "resting" saliva in 431 children and found a mean chloride value of 16.25 meq/l. Nonstimulated saliva has also been analyzed by Becks(18), Vladesco(19), and Roskin(11), who reported mean values of 21.04, 33.75, and 17.83 meq/l, respectively. The Russian investigator Popova-Milashevskaia(20) reported salivary chloride mean of 21.88 meq/l in 40 subjects, mode of stimulation not stated.

Sex effects. The significant difference between volume means for males and for females throughout our study has also been noted in earlier work(12,13). In the 3-rbs portion of the present study the female flow rate mean was 71.7% of the mean for males while, in an earlier study(13), in which an identical stimulation procedure was utilized, the female flow rate mean was only 53.4% of that for males. It was postulated that the relatively low flow rate for females might well be due in part to psychologic factors since female subjects did seem much more hesitant to expectorate in view of other participants than did the males. The only procedural variation between the 2 studies was that, in the present study, each group of subjects was given a thorough briefing as to the purpose of saliva sampling and the female subjects were sampled in situations of semi-privacy. The rate of flow for males increased by 0.65 ml/5 min. and that for females increased by 1.94 ml/5 min. under conditions of the present study.

That there was no significant difference in chloride levels attributable to sex in the NES study is in agreement with other reports(17, 20). The significant difference in chloride levels between the sexes in the 3-rbs portion can be explained only when the relationship between volume and chlorides is thoroughly understood.

Age effects. Within the narrow confines of age ranges, age was not found to exert a significant effect upon rate of salivary flow. No significant differences in chloride levels attributable to age were found except for males in the NES study. In this instance the means indicated neither a systematic increase nor decrease with age and are thus felt to be due to chance, which may occur in sampling. Anders(17) concluded that age exerted no influence on chloride levels. Becks(18) found that, under physiologic conditions, excretion of cations and anions in the saliva diminished (except for potassium) between 20-40 years of age but noticeably increased with advance in age. Dancis, *et al.*(21) found that salivary chloride was high at birth, that it dropped rapidly during the first week of life, continued to fall at a much slower rate during the first 2 years, and appeared to stabilize through 10 years of age.

Volume effects. McGrady and Bessman(2) found chloride concentrations to vary markedly with method of collection. Anders(17) presented findings on 9 subjects in whom chloride levels in "resting" and paraffin stimulated saliva were compared. In 5 subjects the chloride concentration was higher in nonstimulated saliva, while the opposite held true in the remaining 4 participants. Brown *et al.*(22) found that the chloride of saliva increased with rate of flow. Thaysen, *et al.* (23), reporting on analyses of parotid saliva from 47 subjects, found that chloride values increased with increases in secretory rate. White, *et al.*(1) could find no highly significant effect of rate of flow on chloride concentrations.

When the 2 portions of the present experiment are compared, volume and chloride levels for both males and females are significantly higher when exogenous stimulation is used. Correlation coefficients for volume and chlorides within each study, however, do not point out a positive conclusion. For females, a negative correlation was noted in both studies, there being no significant difference between correlation coefficients for females for the 2 experiments. For males, however, a highly significant difference between the 2 coefficients was noted. When no exogenous stimulation was used, volume and chlorides were negatively correlated but when 3 rubber band stimulation was employed, volume and chlorides were positively correlated. The indication is that, under conditions of the present experiment, if one attempted to predict volume from chloride levels or chloride levels from volume findings, for males or for females, the degree of success in prediction would be quite small.

Summary. When whole saliva samples were collected at 9:00 a.m. from 323 males and 159 females under stimulation of 3 large rubber bands and from 503 males and 179 females utilizing no exogenous stimulants, the following conclusions could be drawn: 1. No significant differences in volume attributable to age were noted. Rate of flow for males was significantly higher than that for females. Rate of flow for both males and females was significantly higher when exogenous stimulation was utilized. 2. No significant increase or decrease in chloride values attributable to age was noted. 3. For females, volume and chloride were negatively correlated while, for males, a positive correlation was found with exogenous stimulation and a negative correlation when no such stimulation was used. For both males and females, chloride levels were significantly higher when exogenous stimulants were used.

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1. White, A. G., Entmacher, P. S., Rubin, G., Leiter, L., *J. Clin. Invest.*, 1955, v34, 246.

2. McGrady, K., Bessman, S. P., *A.M.A. Am. J. Dis. Child.*, 1955, v90, 610.

3. Pickerill, H. P., *New Zealand M. J.*, 1924, v23, 485.

4. Turner, N. C., Crane, E. M., *J. Dent. Res.*, 1944, v23, 413.

5. Florestano, H. J., Faber, J. E., James, L. H., *J. Am. Dent. Assn.*, 1941, v28, 1799.

6. Turner, N. C., Crane, E. M., *Science*, 1944, v99, 262.

7. Anders, J. T., *J. Dent. Res.*, 1956, v35, 753.

8. Bergeim, O., Barnfield, W. F., *ibid.*, 1945, v24, 141.

9. Hubbell, R. B., *Am. J. Physiol.*, 1933, v105, 436.

10. Hess, W. P., Smith, B. T., *J. Dent. Res.*, 1948, v27, 593.

11. Roskin, M., PROC. SOC. EXP. BIOL. AND MED., 1927-28, v25, 465.

12. Shannon, I. L., School of Aviat. Med., USAF, Rep. No. 57-80, June, 1957.

13. ———, *ibid.*, No. 57-99, May, 1957.

14. Sendroy, J., *J. Biol. Chem.*, 1937, v120, 405.

15. Chauncey, H. H., The Chemical Composition of Human Saliva, Ph.D. Thesis, Boston Univ. Graduate School, 1955.

16. Brown, J. B., Klotz, N. J., *J. Dent. Res.*, 1937, v16, 19.

17. Anders, J. T., *J. Appl. Physiol.*, 1956, v8, 659.

18. Becks, H., PROC. SOC. EXP. BIOL. AND MED., 1928, v26, 93.

19. Vladesco, R., *Compt. Rend. Soc. Biol.*, 1938, v128, 317.

20. Popova-Milashevskaia, V. A., *Stomatologiia, Moskva*, 1950, No. 2, 14.

21. Dancis, J., Grobow, E., Boyer, A., *J. Pediatrics*, 1957, v50, 459.

22. Brown, J. B., Wright, H. D., Limbacher, H. P., *J. Dent. Res.*, 1938, v17, 191.

23. Thaysen, J. H., Thorn, N. A., Schwartz, I. L., *Am. J. Physiol.*, 1954, v178, 155.

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Synergistic Effect of Somatotropin with α-Corticotropin on the Adrenal of Hypophysectomized Rats.* (23892)

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Although an antagonism is often operative between adrenocorticotropic hormone (ACTH) and growth hormone (somatotropin) with respect to certain anabolic effects of the latter(1), it is becoming apparent that the 2 hormones may also act in synergism. Cater and Stack-Dunne(2) have studied the combined effect of somatotropin and ACTH in the hypophysectomized rat and demonstrated that the resulting increase in adrenal weight is greater than the sum of increases obtained with each alone. More re-

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