pattern of enzyme availability(8).

Summary. Acid deoxyribonuclease activity was measured in rat kidney tissue divided into the various renal segments: outer cortex (O.C.), inner cortex (I.C.), outer medulla (O.M.) and inner medulla (I.M., papilla). In terms of total activity, the O.C. had the highest enzyme content. The activity of the I.C. was considerably less and the medullary segments had the lowest values. If freeze-thaw procedures were not utilized, all segments except the papilla markedly decreased in enzyme activity. The enzyme values of the cortical segments were increased by lowering the sucrose content of the homogenate media. Those findings suggest that in addition to regional variation of activity, the relationship of acid deoxyribonuclease to the cell is different among the renal segments.

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An *in vitro* Preparation of Dog Parotid Gland.* (31884)

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An *in vitro* preparation of an exocrine gland might serve as a convenient model for the study of exocrine gland electrolyte transport. Burg and Orloff have recently produced isolated renal tubuli by the treatment of kidney tissue with collagenase(1). With some modifications their procedure has been applied to the production of seemingly intact acinar and tubular elements from the parotid gland of the dog. This paper presents the method of preparation and an investigation of the viability of this system.

Methods. Parotid glands excised from young pentobarbital-anesthetized dogs were diced and washed in Krebs Ringer bicarbonate solution containing 200 mg% glucose (KRBG) at pH 7.4. The tissue was placed in an Erlenmeyer flask containing fresh KRBG and 400 mg% collagenase.‡ It was shaken in an Eberbach reciprocating shaker at 227 cycles/min with oscillations of approximately 4 cm at 25-27°C for 2 hours. At the end of this time a high percentage of tissue had been reduced to minute fragments. Unreduced tissue was discarded. The yield was washed with 4 volumes of fresh KRBG and centrifuged at 50 × *g* and 0°C for 5 minutes. The washing procedure was repeated 3 times.

As an index of viability of the preparation, its capacity to concentrate iodide was investigated. This parameter was chosen since

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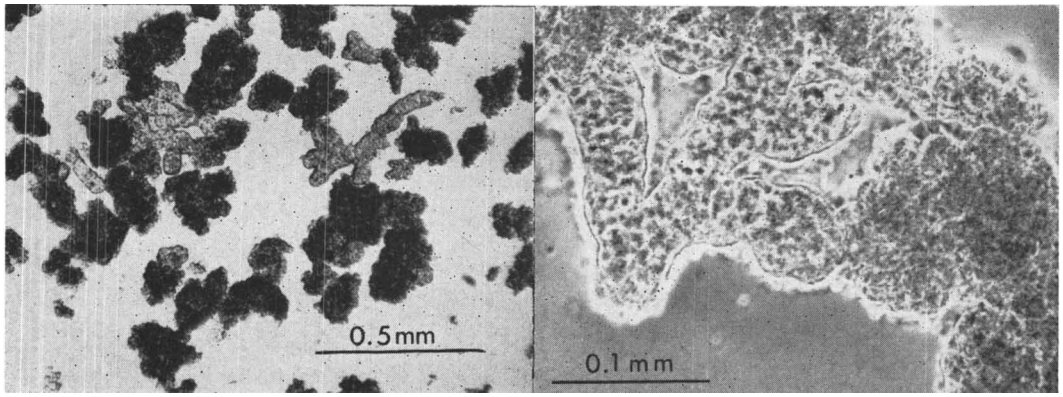

Fig. 1. Bright field view of tissue yield. Photograph shows population of different glandular elements.

Fig. 2. Phase contrast view of tissue yield. Photograph shows tubular system.

it is felt to require metabolically active cells. The distribution of inulin was studied in order to assess the ability of cell membranes to restrict permeation of large molecules. Approximately 0.3 g aliquots of washed tissue yield were incubated in 10 ml KRBG for 1 hour at 37°C in a Dubnoff metabolic shaker. The medium contained 0.9 μc/10 ml carrier-free Na I^{131}§ and 500 mg% inulin.‖ Atmospheres of 95% O_2/5% CO_2 or 95% N_2/5% CO_2 were maintained. Some incubations were carried out under a 95% O_2/5% CO_2 atmosphere but with 10^{-3} M NaCN in the medium. At the end of incubation flask contents were centrifuged at 500 $\times$ *g* at room temperature for 5 minutes. Centrifuge tubes were used which minimize tissue-medium exchange and allow collection of the tissue plug *via* the tube bottom(1).

Duplicate 0.1 ml aliquots of supernatant and accurately weighed aliquots of approximately 0.1 g tissue plug were analysed for Na I^{131} with a well scintillation detector. Before counting 2.0 ml of water were added to all tubes. Exposure to water for 2 hours at room temperature effectively leached I^{131} from the tissue. This was judged from the fact that samples counted as alkaline digest showed no significant counting difference from samples counted in water.

Each water-leached aliquot was analyzed for the presence of inulin by the alkali-stable method of Walser, Davidson and Orloff(2). Recovery of inulin was determined to be 101.3 $\pm$ 2.7% from tissue and 99.5 $\pm$ 2.4% from medium.

Radioisotope accumulation was expressed as the ratio of

$$\frac{\text{CPM/g wet tissue}}{\text{CPM/ml medium}}$$

Inulin space was calculated as the ratio of

$$\frac{\text{wet tissue inulin concentration} \times 100.}{\text{medium inulin concentration}}$$

Correcting for inulin space the I^{131} uptake ratio was expressed as

$$\frac{\text{CPM/g cells}}{\text{CPM/ml medium.}}$$

Results. Fig. 1, a low power view of the tissue yield, illustrates the population of different glandular elements. One can observe small groups of acini, single acini and tubuli. Acinar elements appear more numerous than tubuli.

Fig. 2, a phase contrast micrograph, illustrates the apparent integrity of cellular detail in a tubular complex. There is relative absence of tissue debris, adherent collagenase and blood cells.

Fig. 3, presents the results of an experiment testing the viability of the preparation. Under aerobic conditions a wet tissue/medium I^{131} ratio of 1.39 $\pm$ 0.15 was observed, thus showing accumulation of isotope. The inulin space averaged 49.3 $\pm$ 6.4%. On correction for the radioactivity emanating from the inulin space the cell/medium I^{131} ratio increased to a mean of 1.75 $\pm$ 0.24. In con-

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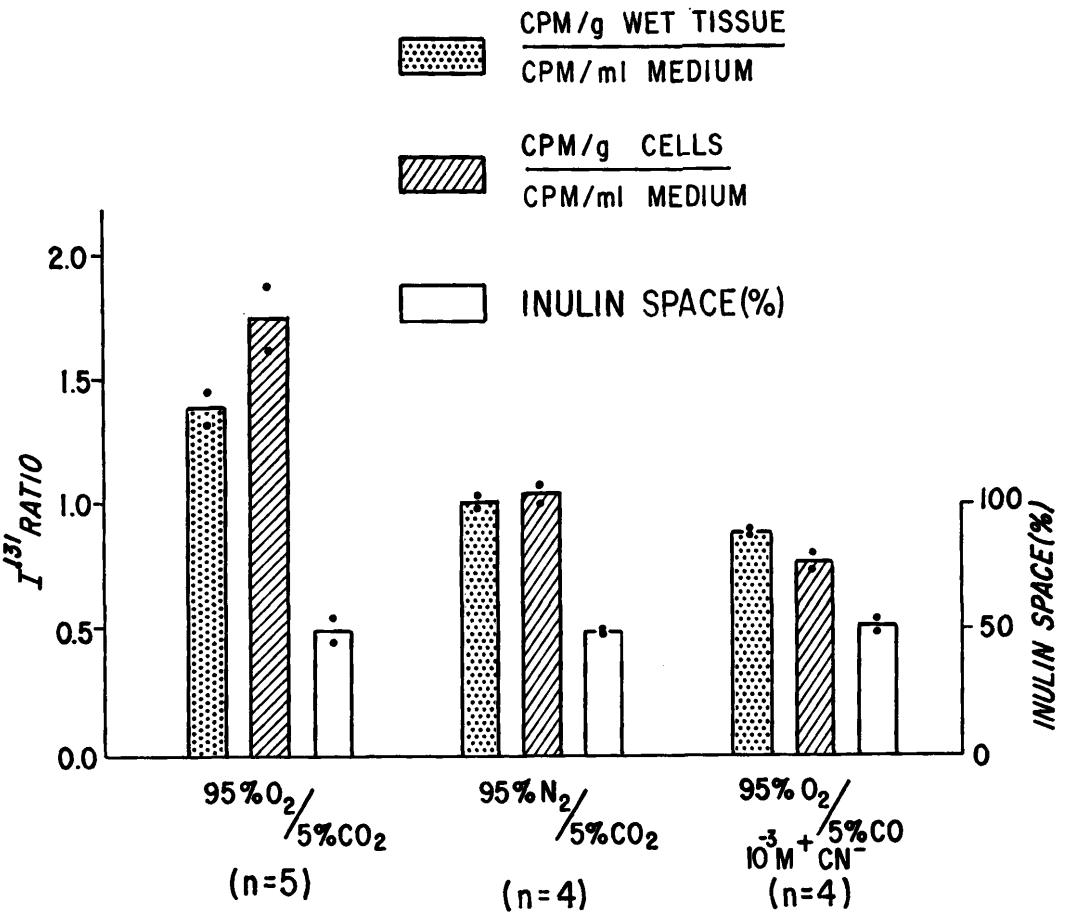

Fig. 3. Effect of anaerobiosis and cyanide on I^{131} uptake and inulin space of collagenase treated dog parotid gland. Bars represent mean ± SD.

trast, anaerobic conditions reduced the wet tissue/medium I^{131} ratio to 1.04 ± 0.05 after correction for an inulin space of 49.3 ± 1.2%. Cyanide further inhibited the I^{131} uptake, producing a wet tissue/medium I^{131} ratio of 0.89 ± 0.02, inulin space of 51.8 ± 4.5%, and cell/medium I^{131} ratio of 0.77 ± 0.05. I^{131} ratios for the 3 groups are different at a $p<0.001$. The inulin space values are not significantly different. Thus it is clear that accumulation of I^{131} by the preparation is depressed by anaerobiosis and obliterated by CN^-.

Discussion. Cohen and Myant observed that dog parotid gland *in vivo* is capable of accumulating I^{131} in excess of serum by a ratio of 1.21 ± 0.14, without correction for extracellular fluid space(3). The collagenase treated preparation described here is seen to accumulate I^{131} to a similar or greater extent. The depression of I^{131} uptake by anaerobiosis and cyanide indicates that this process is metabolically dependent. Hence the iodide accumulating capability is suggestive of the viability of the tissue.

The inulin space observations under the conditions of the experiment indicate that the cell membranes of the collagenase preparation are not freely permeable. An inulin space of approximately 50% is not surprising in comparison with 20% values of Burg and Orloff with kidney cortex preparation. The anatomy of parotid glandular and renal tubular fragments is quite different. Furthermore, the method of Burg and Orloff utilizes collection of the tissue plug by centrifugation at 15000 × *g* while the present study uses only 500 × *g*.

Two lines of inquiry concerning the function of exocrine glands, especially the salivary glands, have recently come into focus. The first deals with the problem of how these glands produce hypotonic secretions whose electrolyte and osmolal composition is flow dependent. By indirect methods, several workers have attempted to dissect the role of acinar from tubular components to understand this phenomenon. Their conclusions, however, are contradictory(4,5). By direct methods of micropuncture and microcatheterization other workers have clarified some aspects of this problem, at least in the rat submaxillary gland(6,7). The tubular system buried deep in the gland, however, remains invulnerable to micropuncture. Until this system can be made accessible, an understanding of water and solute movements between cell, interstitium and lumen will not likely be achieved.

The second area of inquiry relates to the questions of the metabolic substrates for secretion and the controls of secretory events. This area is relatively unexplored in these glands(8), owing in part to the complexity of their blood supply and the difficulty of critically manipulating their milieu *in vivo*.

An *in vitro* preparation of these tissues could allow exposure of the tubular system, critical adjustment of the cellular environment, and convenient detection of cellular functional changes. It is felt that the collagenase preparation described here provides the opportunity for fulfilling these requirements.

Conclusions. This paper has presented a method of preparation of acini and tubuli from the parotid gland of the dog. The viability of the system is evidenced by its capacity to accumulate I^{131} by a metabolically dependent process. Free inulin distribution is restricted. It is suggested that this *in vitro* preparation might prove useful in the solution of two of the major issues in exocrine gland physiology.

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Low Carcinogenicity of the K-Region Epoxides of 7-Methylbenz(a)-anthracene and Benz(a)anthracene in the Mouse and Rat.* (31885)

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The induction of tumors at sites of application of sub-milligram quantities of the carcinogenic polycyclic aromatic hydrocarbons and the very low carcinogenic activity of the phenolic or quinone metabolites which have been tested(1) have led to the idea that the hydrocarbons may be carcinogenic without metabolic conversion. On the other hand, correlations have been obtained between the covalent binding of various hydrocarbons to proteins and nucleic acids in mouse skin *in vivo* and the carcinogenic activities of the

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