mined. The total sulfur as determined (0.63% of the dry weight) is much lower than previously reported values. Adrenal glands were incubated briefly with radioactive cysteine and the tissue was then analyzed for total uptake of the isotope and its distribution within the fractions. The effect of added adrenocorticotropin upon this uptake and distribution was observed. The hormone preparation causes a marked reduction in uptake by the soluble fraction, and a rise in the uptake by the lipid and protein fractions. The concentration of the isotope in the lipid sulfur is very striking.

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Secretion of Aldosterone by the Zona Glomerulosa of Rat Adrenal Glands Incubated *in Vitro.** (22416)

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(Introduced by J. S. L. Browne.)

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Previous studies(1) have shown that rat adrenal glands incubated *in vitro* release at least seven compounds including a substance with marked sodium retaining activity. The latter compound was identical with aldosterone as far as blue tetrazolium and triphenyl tetrazolium reactions, soda fluorescence, chromatographic mobility, mixed chromatograms and biological activity were concerned. Since the classical work of Deane, Shaw and Greep(2) much indirect evidence has pointed to the adrenal zona glomerulosa as the site of production of mineralocorticoids. The present work provides evidence for the *in vitro* secretion of aldosterone by the zona glomerulosa following the separation of this zone from the rest of the gland by adrenal decapsulation.

Methods. Adrenal glands were obtained from male rats weighing between 170 and 180 g. These animals were subsequently used for bioassay purposes. The glands were carefully dissected free from fat. A small incision was made in the adrenal capsule and the gland was extruded by gentle pressure. In each experiment a total of 50 to 70 glands and their capsules were weighed separately and placed in 50 ml Erlenmeyer flasks containing 1.5 ml per 25 mg of wet tissue of Krebs Ringer bicarbonate solution with added glucose (200 mg %)(3). The incubation of the tissue was carried out in a water bath at 38°C for 2½ hours. After the first hour (pre-incubation period) the medium was replaced by the same volume of fresh Krebs Ringer bicarbonate solution, and the

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TABLE I. Comparison of Aldosterone Production of Decapsulated with That of Whole Rat Adrenal Glands Incubated *In Vitro*.

Decapsulated glands			Whole glands		
	Aldosterone			Aldosterone	
Tissue wt incubated, mg	μg/total tissue wt /hr	μg/100 mg tissue/hr	Tissue wt incubated, mg	μg/total tissue wt /hr	μg/100 mg tissue/hr
			870	3.22	.37
			868	4.34	.50
1186	.76	.06	875	5.60	.64
1186	.68	.05	733	2.64	.36
527	1.21	.23	750	5.93	.79
1913	.57	.03	798	6.15	.77
528	.66	.13	1006	6.24	.62
1913	.80	.04	730	8.76	1.20
		.09 ± .03			.66 ± .09

incubation was allowed to continue for 90 minutes. Oxygen, containing 5% CO_2, was bubbled through the medium continuously. Incubation media from the glands and from the capsules were each extracted, first with twice and then with 1.5 times their volume of redistilled chloroform. Chloroform extracts were centrifuged to break down the emulsion and evaporated to dryness *in vacuo* at 50°C. Dry chloroform extracts (crude fraction) were chromatographed in the benzene-aqueous methanol system of Bush(4) run at room temperature for four and a half hours. The detection of the steroid material after chromatography was done by direct visualization under ultra violet light, by scanning of the paper strip in a Beckman DU spectrophotometer at 2400 Å; by the soda fluorescence, the blue tetrazolium and the triphenyltetrazolium reactions on the paper chromatogram. The aldosterone band was cut from the paper in the limits of its ultraviolet absorption as defined by the scanning pattern and eluted with redistilled methanol. This fraction was evaporated to dryness, redissolved in ethanol and bioassayed on adrenalectomized rats according to a modification of the Singer-Venning assay(5).

Results. Table I compares the aldosterone production of decapsulated and whole rat adrenal glands incubated *in vitro*. In each series of experiments the aldosterone production is expressed per total weight and per 100 mg of tissue per hour. The whole glands have a mean of aldosterone secretion of 0.66 ± .09 μg/100 mg of tissue per hour, which is in the range of results previously obtained(1), whereas the decapsulated glands only produce 0.09 ± .03 μg of aldosterone per 100 mg of tissue per hour. This difference is statistically significant ($P<.001$).

A typical scanning pattern of two paper chromatograms on which have been developed respectively the extracts of decapsulated gland and of capsule incubates is presented in Fig. 1. In the chromatogram from the capsule there is an obvious aldosterone peak and very little other Δ^4-3-ketosteroid material, whereas in the chromatogram from the decapsulated glands there is no aldosterone peak

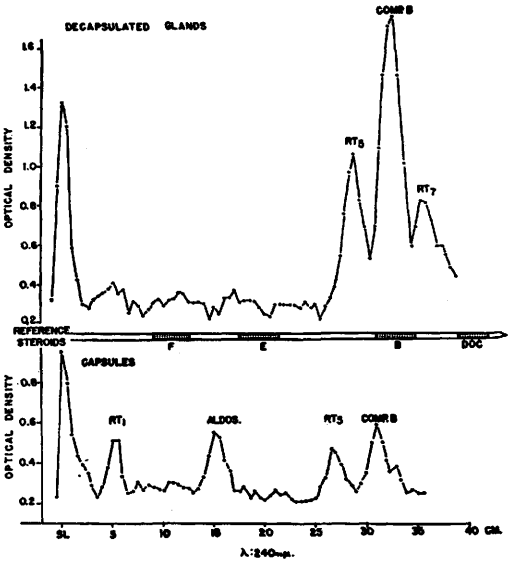

FIG. 1. Location of Δ^4-3-ketosteroids as determined on chromatograms in a calibrated scanner fitted to the Beckman spectrometer. (Wave length: 240 mμ.)

TABLE II. Comparison of Aldosterone Production of Rat Adrenal Capsules with That of the Corresponding Decapsulated Glands Incubated *In Vitro*.

Total wt, mg		Aldosterone				
		Capsules		Decapsulated glands		Whole glands
Capsules	Decapsulated glands	μg/total tissue wt /hr	μg/100 mg tissue/hr	μg/total tissue wt /hr	μg/100 mg tissue/hr	μg/100 mg tissue/hr (calculated)
168	650	4.16	2.46	.48	.11	.71
180	650	4.80	2.66	1.23	.19	.57
244	506	3.54	1.44	1.60	.32	.70
197	542	2.04	.77	1.17	.17	.33
165	547	3.02	1.83	.00	.00	.43
175	569	2.78	1.59	.30	.05	.42
			1.79 ± .28		.14 ± .05	.53 ± .07

and most of the material detected is in the corticosterone area.

In 6 experiments the production of aldosterone, by the decapsulated glands on one hand and by their own capsules on the other, was compared (Table II). The results of the biological assay show that a mean of 1.79 ± .28 μg of aldosterone per 100 mg of tissue per hour is produced by the "capsules," and a mean of 0.14 ± .05 μg/100 mg of tissue/hour by the decapsulated glands ($P < .001$). Although values have been given in Table II for the production of aldosterone by the decapsulated gland, in only 2 cases a statistically significant sodium retaining activity was obtained in the bioassay. Thus the average production of aldosterone by the decapsulated gland is probably much lower than that expressed and the difference between the amount produced by the decapsulated gland and by the capsule is even more marked. It should also be noted that the amount of al-

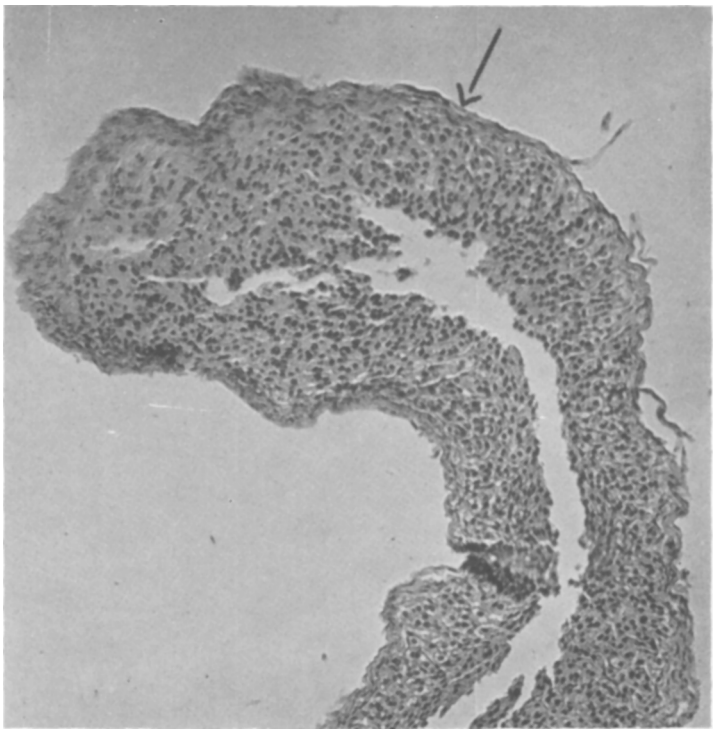

FIG. 2. A) Part of a capsule after enucleation of adrenal gland. Zona glomerulosa and outermost part of fasciculata remained attached to the capsule. (× 100, Masson trichrome.)

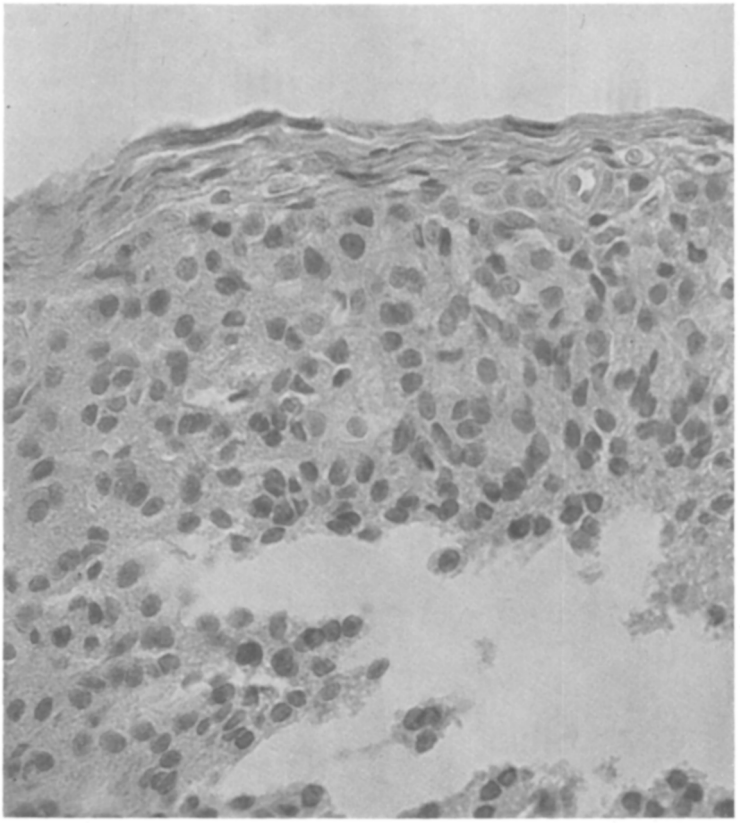

FIG. 2. B) Higher magnification of the area indicated by the arrow on A (× 425).

dosterone produced by the total tissue mass of the capsule is much greater absolutely than that produced by the total mass of the decapsulated gland ($P<.001$).

The last column of Table II gives the value of aldosterone production per 100 mg of tissue per hour calculated on the basis of the total gland weight (capsule + gland). These values agree well with the amount actually produced by incubated whole glands as shown in the last column of Table I. The difference is statistically not significant ($P>.3$).

Serial histological sections of the adrenal capsules and of the decapsulated glands show that following decapsulation the whole zona glomerulosa and about one-fifth of the zona fasciculata (Fig. 2 A and B) are removed with the connective tissue of the capsule whereas the decapsulated glands contain the rest of the fasciculata, the reticularis and the medulla. Evidence that such a clear cut picture is not always obtained is afforded by the finding of occasional clusters of glomerulosa cells present in the decapsulated portion of the gland. The histological studies have been limited, up to now, to a small number of samples as it would be next to impossible to carry out systematic histological studies on each of the hundreds of the capsules and glands which have been incubated in these experiments. There is no serious reason to believe, however, that a standardised procedure of handling the tissue would lead to a striking difference in the distribution of glandular tissue between the capsular and glandular portion of the adrenal after decapsulation.

Only in 2 instances out of 6 experiments did the decapsulated glands produce enough aldosterone to give a significant sodium retention in the bioassay. Even in these 2 cases the aldosterone production of the "capsules" was more than four times greater than that of the glands when expressed per 100 mg of

tissue per hour of incubation.

Clearly the convention of expressing aldosterone output in terms of tissue weight minimizes the secretory capacity of the decapsulated portion since the non-productive medulla is included in the weight of this part of the gland. It is believed that this consideration does not invalidate the conclusion that aldosterone is produced primarily by the capsular portion and by the zona glomerulosa. This conclusion is based on the following reasons: an arbitrary correction factor which assumes the weight of the medulla to be 50% of the decapsulated gland still yields a significant difference when the corrected output of the decapsulated glands is compared to that of the capsules ($P<.001$). Calculation of aldosterone output in terms of total weight of tissue incubated shows a significantly greater output by the capsules ($P<.001$). Finally as pointed out previously, the aldosterone production of the intact glands (Table I) is strictly comparable to that of the sum of the two portions of the glands incubated separately ($P>.3$), emphasizing the difference in output in favor of the capsule containing the zona glomerulosa. It is therefore concluded that the present findings strongly suggest that aldosterone is produced principally by the cells of the zona glomerulosa.

Conclusion. Rat adrenal glands incubated *in vitro* release aldosterone at the rate of 0.66 μg/100 mg of tissue per hour. Decapsulated rat adrenal glands secrete only negligible amounts of the hormone. Comparison of aldosterone production between decapsulated rat adrenals and their capsules shows that most of the hormone is secreted by the capsular portion which contains the cells of the zona glomerulosa. These findings are strong evidence that the principal site of aldosterone secretion in the rat adrenal is the zona glomerulosa.

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Evidence for Occurrence of 10-Formyltetrahydrofolic Acid in Human Urine. (22417)

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The term "citrovorum factor activity" is commonly used to denote the growth-promoting effect of certain substances for *Pediococcus cerevisiae,* formerly known as *Leuconostoc citrovorum*(1,2). Citrovorum factor (CF) activity is shown by 5-formyl-5, 6, 7, 8-tetrahydropteroylglutamic acid (5CHOTHFA) (3,4), by crude preparations of tetrahydropteroylglutamic acid (THFA)(5), and by massive concentrations of pteroylglutamic acid (FA)(1).

The urinary excretion of CF activity in adult males was shown to increase markedly following the oral administration of FA(6,7), thus indicating that FA was converted *in vivo* to CF. However, these studies were based upon microbiological assays in which the samples were autoclaved and hence gave no indication as to whether heat-labile substances with CF activity were present in the urine. Recent studies of the mechanism of CF formation in certain enzyme systems have demonstrated the existence of such heat-labile substances(8,9) thus prompting the con-