TABLE I. Oxidation of 0.4 mg Each of Phloroglucinol and Orcinol. Pre-incubation period was 90 min., pH 7.8, 37°. Figures are mm³ oxygen uptake. Autorespiration has been subtracted.

Compound	Pre-incubated with	Min. 30	60	90	120	150	180
Phloroglucinol	—	−4	10	23	53	77	98
"	50 γ phloroglucinol	10	24	38	72	99	129
"	50 γ orcinol	−4	12	25	56	81	108
Orcinol	—	−9	−9	−6	−5	15	34
"	50 γ phloroglucinol	−1	14	39	72	108	143

however, oxidized orcinol after a long latent period which could be shortened by pre-incubation with phloroglucinol. This is shown in Table I in comparison with the oxidation of phloroglucinol. Pre-incubation with orcinol had only a slight effect on the latent period for phloroglucinol. For the formation of the enzyme, therefore, 3 hydroxy groups meta to one another on the ring are necessary. If one was substituted by a methyl group as in orcinol, the enzyme was formed much more slowly, but the enzyme once formed was able to oxidize orcinol fairly rapidly. The substitution of a carboxyl group as in 3, 5, dihydroxybenzoic acid caused complete loss of activity. It did not cause formation of the enzyme, was not oxidized by it, nor did it inhibit formation or oxidation. This was also true of phloridzin which is a glucoside of phloroglucinol. Enzyme formation occurred at the normal rate in the absence of added phosphate.

The oxygen uptake varied between 4 and 6 atoms per molecule of phloroglucinol and 2 molecules of carbon dioxide per molecule were produced. The ring was therefore split and some assimilation of the end products, if it occurred, could account for the variability of the oxygen uptake. It is probable that more than one enzyme is involved in the overall reaction.

Summary. The rate of formation of the enzyme which oxidizes phloroglucinol is not affected by factors which alter the rate of formation of the enzymes which oxidize benzoic acid and inositol. Some of the characteristics of the phloroglucinol oxidation are described.

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Effect of Adrenocorticotropin on Uptake of S^{35}-Labelled Cysteine by Rat Adrenal Glands.* (22415)

CLARKE DAVISON† AND FREDERICK G. HOFMANN.‡ (Introduced by A. B. Hastings.)

Department of Biological Chemistry, Harvard Medical School, and Biological Research Laboratory, Harvard School of Dental Medicine.

Reports of the effects of the adrenocorticotropic hormone (ACTH) upon sulfur distribution in the animal body have been largely confined to extra-adrenal tissues and fluids. Kinsell *et al.*(1) demonstrated a 50% rise in urinary cystine and cysteine after the administration of the hormone. Hess *et al.* (2) found a marked fall in the concentration of reduced glutathione in blood but no change in total glutathione levels after administration of ACTH to human patients. Ingbar *et al.* (3) found that the hormone produced no changes in the blood glutathione of rats although it did cause a reduced non-protein

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† Present address: Department of Pharmacology, George Washington University Medical School, Washington, D.C.

‡ National Research Council Fellow in Medical Sciences. Present address: Department Pharmacology, College of Physicians and Surgeons, Columbia University, New York.

sulfhydryl level in the kidney and an increased level in the liver. No changes in the amount of protein sulfhydryl were observed. Goldzieher *et al.*(4) observed a 50% rise in rat adrenal non-protein sulfhydryl concentration 3 hours after injection of 0.1 mg of Armour ACTH, but a reduced sulfhydryl level after larger doses or longer treatment. Liver and diaphragm exhibited no such changes at the lower dose, but non-protein sulfhydryl content was reduced with the larger amounts of hormone. In distribution studies by Lee and Williams(5) ACTH was demonstrated to increase the incorporation of S^{35}-labelled DL-cystine in a number of tissues of the rat. No studies were made with the adrenal itself. The present investigation was undertaken to study the *in vitro* effect of this hormone upon uptake and distribution of cysteine in the rat adrenal gland.

Materials and methods. The animals used were 150 to 200 g female Sprague-Dawley rats. Adrenal glands were removed, halved and incubated for 20 minutes at 37°C in an artificial medium consisting of 0.123 M NaCl, 0.005 M KH_2PO_4, 0.015 M K_2HPO_4 and 0.011 M glucose. The pH was 7.28. An oxygen atmosphere was present within the flasks. After incubation the adrenals were filtered onto glass wool and thoroughly rinsed with isotonic sodium chloride solution. About one-third of the tissue was set aside for oxidation of the total sulfur content to sulfate. The remainder was fractionated into lipid, protein and water-soluble fractions according to the methods of Folch(6). The sulfur of these fractions was also oxidized to sulfate. In experiments with labelled cysteine carrier sulfate was added and the sulfate was precipitated with benzidine by conventional methods, and counted. In early experiments, oxidation was carried out in open tubes by successive treatment with hydrogen peroxide-glacial acetic acid, aqua regia and finally perchloric and nitric acids. Since considerable variation and poor recoveries were obtained by this procedure, oxidation with aqua regia in a sealed Carius tube as described by Lees (7) was adopted and proved more satisfactory. The samples of whole adrenal gland and fractions were dried and weighed in 125 mm x 14 mm combustion tubes. Small test tubes (30 mm x 6 mm) containing one-half ml of freshly prepared aqua regia (three parts concentrated nitric acid to one part of concentrated hydrochloric acid) were introduced into the large tubes. This amount was sufficient to oxidize twenty mg of organic material. Combustion tubes were drawn out, sealed and inverted to permit contact of the sample and oxidizing agent. The tubes were wrapped in asbestos paper and placed in iron bombs (capped one inch pipes) in an oven at 250°C for 8 hours. The tubes were opened in the hood and the contents carefully washed out and evaporated to dryness for precipitation. When benzidine sulfate was to be determined, rather than counted, the colorimetric method of Letonoff and Reinhold was used(8). L-cysteine hydrochloride was a sulfur-labelled preparation by Abbott Laboratories. The ACTH used was an Astwood preparation, assaying 80 International Units per milligram.

Results. Values for total and fractional solids and total and fractional sulfur contents of the rat adrenal were determined initially so that calculations of uptakes could be made on weight basis. The sulfur content of the rat adrenal has been reported to be 1.5% of the dry weight(9). This would far exceed that of any other tissue and repetition of this determination seemed desirable. Accordingly sulfur analyses were performed upon the normal adrenal by the closed Carius method. A few independent checks by oxidation of samples in the Parr bomb under thirty atmospheres of oxygen agreed very well, and these data were pooled with the Carius data. The adrenals of 2 rats were used for each total sulfur determination; those of 5 rats for the fractionation studies. The results are seen in Tables I and II. The data show much less sulfur than had been previously reported(9).

Experiments were then carried out to determine whether or not the adrenal gland took up cysteine *in vitro,* how it was distributed among the protein, lipid and water-soluble fractions and the effect of added ACTH on the uptake and distribution. In preliminary

TABLE I. Solid Content of Rat Adrenal Gland.

Component of gland	No. of determinations	Avg % of wet wt	Stand. error of mean	% of total solid
Whole	20	29.0	.51	
Protein	16	14.8	.28	51.0
Lipid	15	12.4	.34	42.8
Water-soluble	15	2.8	.31	9.5
			Recovery	103.3

TABLE II. Sulfur Content of Rat Adrenal Gland.

Component of gland	No. of determinations	Avg % of dry wt	Stand. error of mean	% of adrenal sulfur
Whole	13	.633	.032	
Protein	8	.467	.026	73.9
Lipid	9	.025	.006	4.0
Water-soluble	9	.177	.024	28.0
			Recovery	105.9

experiments using both inactive and radioactive cysteine, the disappearance of the substrate from the medium was too small to measure with accuracy. Therefore, the actual amount of radioactive cysteine taken up was measured by oxidizing the glands themselves.

The adrenal glands of 6 rats (290 mg) were incubated in each of 4 flasks for 20 minutes in 3 ml of the buffered medium. S^{35}-labelled cysteine was present in each flask at a concentration of 10 mg%. Duplicate incubations were run for each value reported. Two of the flasks contained ACTH at a concentration of 5 International Units per 100 mg of tissue. The total uptake of radioactivity averaged 4.62% in the flasks with cysteine alone, 5.20% with cysteine and the hormone. The distribution of radioactivity found is shown in Table III.

The initial activity of the medium was 3.2 x 10^5 counts per minute per ml, or 1.02 x 10^6 counts per minute per mg S as cysteine. Computations of the radioactivity which could be present in the glands if all the water (71% of the adrenal weight) was in equilibrium with the medium gives a value of 66,000 counts per minute. The actual values found were 44,300 and 50,000 counts per minute for the cysteine and cysteine plus ACTH samples respectively.

Discussion. On the basis of these experiments it appears that ACTH causes a marked change in the uptake of labelled cysteine from the water-soluble fraction of the gland to the lipid and protein fractions. Total uptake was also increased slightly, in agreement with the S^{35} cystine data on other tissues(5).

The increase in total uptake might be expected from adrenal stimulation under the influence of ACTH, but the significance of the rise in radioactivity of lipid sulfur and indeed of the very striking amounts of activity in the lipid sulfur as compared to the protein is interesting. It might indicate a very rapid turnover of some lipid, possibly proteolipid. However, the possibility of cysteine or some other sulfhydryl compound reacting with steroids or other lipid components must not be overlooked. The formation of thiazolidines has been reported by the interaction of cysteine with many steroids under very mild conditions in the test tube(10). Whether this might have a physiological importance is impossible to state at this time.

Summary. Values for total sulfur content of the rat adrenal gland and its protein, lipid and water-soluble moieties have been deter-

TABLE III. Effect of ACTH Preparation upon Uptake of Labelled Cysteine by Rat Adrenals.

Component of adrenal	Cysteine alone (I) Counts/min. /mg dry adrenal	Cysteine alone (I) % of total	Cysteine alone (I) Counts/min. /mg S of fraction × 10^4	Cysteine plus ACTH (II) Counts/min. /mg of adrenal	Cysteine plus ACTH (II) % of total	Cysteine plus ACTH (II) Counts/min. /mg S of fraction × 10^4	Difference II − I %
Whole	527 ± 85.0*		8.33	595 ± 45.0*		9.40	+13.0
Protein	118 ± 4.1	22.4 ± .8*	2.52	194 ± 24.0	32.6 ± 4.0*	4.16	+64.5
Lipid	36.2 ± 8.5	6.9 ± 2.4	14.5	48.9 ± 2.7	8.2 ± .5	19.5	+35.1
Water-soluble	267 ± 26.2	50.7 ± 5.0	15.6	185 ± 25.7	31.1 ± 4.3	10.4	−30.6
Recovery		80.0			71.9		

* ± Stand. error of mean.

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)

C. J. P. GIROUD,† J. STACHENKO, AND E. H. VENNING.
(Introduced by J. S. L. Browne.)

Department of Investigative Medicine, McGill University and McGill University Clinic, Royal Victoria Hospital, Montreal.

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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† Fellow of the National Research Council of Canada.