of adrenal to respond to ACTH. This would seem to eliminate suppression of adrenal cortical activity as a mechanism whereby DBI lowers blood glucose.

Summary. Adrenal function was studied in 6 diabetic patients with hyperglycemia controlled by DBI (N B′ phenethylformadinylimourea) or by DBI and insulin. DBI administration did not significantly affect baseline plasma hydrocortisone levels or response to ACTH. These results appear to eliminate adrenal steroid suppression as a possible mode of action of this biguanide.

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Metabolism of dl-Adrenaline-2-C^{14} in the Human*† (24129)

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Little information is available on metabolic products of adrenaline in the human. After intravenous infusion of adrenaline only 1 to 3% of the amount infused can be recovered in the urine as adrenaline or a conjugate of adrenaline(1,2,3). Following intravenous infusion of adrenaline-2-C^{14}, Resnick and Elmadjian(4) recovered 90% of radioactivity in urine within 30 hours; when adrenaline-methyl-C^{14} was infused, about 34% of the radioactivity was recovered. Schayer(5) previously found similar results in the rat and was able to distinguish 6 radioactive peaks by paper chromatography. Armstrong and McMillan(6), and Armstrong, McMillan and Shaw (7) identified 3-methoxy-4-hydroxymandelic acid (MOMA) as a product of noradrenaline metabolism in humans. In addition, Axelrod (8), and Axelrod *et al.*(9) found 3 methyl-O-adrenaline (metadrenaline) and 3 methyl-O-noradrenaline (normetadrenaline) and their corresponding glucuronides in urine of the rat.

This report describes a procedure which utilizes a combination of filter paper and ion exchange chromatography, to quantitatively separate the bulk of radioactive compounds which appear in urine after intravenous infusion of dl-adrenaline-2-C^{14}.

Materials. The sources of materials were as follows: dl-adrenaline-2-C^{14}, specific activity 1.09 mc/m mole from Tracerlab, Boston, Mass.; Amberlite IRC-50, XE-64 from Rohm and Haas Co., Philadelphia, Pa.; Dowex 1-2X, 200-400 mesh, from Dow Chemical Co., Midland, Mich. *Methods.* Infusion of dl-Adrenaline-2-C^{14} and Collection of Urine

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Specimens: Healthy males aged 20 to 35 years were given an intravenous infusion of 5 μcuries (4.5 μmoles) of the labeled adrenaline in 200 ml of physiological saline over a one-hour period. Urine was collected at end of infusion period, at one hour intervals for the next 5 hours, and at 6 hour intervals for subsequent 18 hours. An aliquot of each specimen was pooled to give a 24-hour urine sample. Radioactivity in each specimen was determined with a thin window Geiger tube (16 c.p.m. background) and corrected to infinite thinness by reference to a previously prepared absorption curve. *Separation of urinary metabolic products.* A modification of the method previously reported by Kirshner, Klingman, and Goodall(10) was used. An aliquot of urine (5 to 10 ml) containing 15,000 to 30,000 counts/minute is passed through a 3 X 1 cm column of Amberlite IRC-50(11). The column is then washed with 15 ml of water. The effluent and wash are combined and saved. The IRC-50 column is then eluted with 20 ml of 0.5 N acetic acid. The eluate is evaporated to dryness and the residue dissolved in 0.5 ml of water. This solution is then chromatographed for 15-18 hours on Whatman No. 1 filter paper using n-butanol saturated with N HCl as the solvent. After drying, the paper is cut into 1 cm strips; each strip is eluted with water and an aliquot plated out for assay of radioactivity. Three radioactive peaks are found. One of the peaks corresponds to adrenaline; another corresponds to metadrenaline and the third peak has not yet been identified. The adrenaline and metadrenaline peaks account for 80 to 90% of the radioactivity found on the paper. The combined effluent and wash from the IRC-50 column is then passed through a 10 X 1 cm column of Dowex-1 acetate. The column is washed with 15 ml of water and the effluent and wash are combined and assayed for radioactivity. About 3% of total activity of the 24 hour urine specimen is obtained in this effluent and wash. The Dowex-1 column is then attached to an automatic fraction collector and eluted with 0.3 M ammonium acetate at pH 4.8. Three ml fractions are collected. The course of the fractionation is followed by assaying an aliquot of each third sample for radioactivity. When radioactivity in the eluate returns to background, usually after 80 to 90 ml of the buffer have passed through the columns, the column is eluted with 1 M ammonium acetate at pH 4.8. The fractionation is again followed by assaying each third fraction for radioactivity and again a sharp radioactive peak is obtained. When the radioactivity in the eluates again returns to background, the column is eluted with 3.0 M ammonium acetate pH 4.8. The fractionation is again followed similarly and a third peak is obtained. *Identification of Dowex-1 Eluates. 0.3 M Eluate.* This fraction is largely an acid labile conjugate of metadrenaline. After heating for 15 minutes with 1 N hydrochloric acid at 100°, 60% of the radioactivity originally present can be recovered as metadrenaline. The metadrenaline was isolated from the hydrolysate by absorption on IRC-50 and identified by paper chromatography using butanol-1 N HCl and isopropanol-ammonia-water as solvents. The conjugate is not a glucuronide since incubation of either whole urine or the Dowex eluate with either bacterial (Sigma) or animal (Warner-Chilcott) β-glucuronidase for 24 hours caused no increase in the metadrenaline fraction. However, the conjugate was hydrolyzed by incubation with Mylase P (Nutritional Biochemicals). In a parallel experiment in which rats were given an intraperitoneal injection of adrenaline-2-C^{14}, a radioactive fraction was obtained from the 24 hour urine specimen which was hydrolyzable by β-glucuronidase. This radioactive fraction was identified as metadrenaline glucuronide by isolation of radioactive metadrenaline after treating the fraction with β-glucuronidase. The metadrenaline glucuronide obtained from rat urine can be differentiated from the acid labile conjugate of metadrenaline obtained from human urine by ion exchange and filter paper chromatography. *1 M eluate.* Radioactive fractions were combined and concentrated. An aliquot was chromatographed on Whatman No. 1 paper in n-butanol saturated with N HCl and in isopropanol-ammonia-water (8:1:1). Indicator amounts of 3-methoxy-4-hydroxy mandelic acid were added in each instance. After de-

TABLE I. Distribution of Radioactivity in 24 Hr Urine following Infusion of Dl-adrenaline-2-C^{14}. Figures represent % of total radioactivity which appeared in urine. The .3 M, 1.0 M and 3.0 M eluates contain respectively, conjugated metadrenaline, 3-methoxy-4-hydroxymandelic acid and 3,4-dihydroxymandelic acid.

	% recovery of infused dose	IRC-50 fractions				Dowex-1 fractions				
		Total	Adr.	Metadr.	Unknown	.3 M eluate	1.0 M eluate	3.0 M eluate	Dowex-1 effluent	% recovery of radioactivity in urine
	70	11	4	5	2	43	27	16	2	99
	65	12	4	5	1	52	23	9	3	99
	75	14	5	6	2	39	29	8	3	93
	72	10	4	5	1	46	24	8	4	92
	78	10	3	5	1	35	31	14	3	93
	76	12	4	4	1	35	29	18	2	96
Avg	73 ± 5	12 ± 1	4 ± 0	5 ± 0	1 ± 0	42 ± 7	27 ± 3	12 ± 4	3 ± 0	95 ± 3

veloping for 15 to 18 hours, the paper was dried and sprayed with diazotized p-nitroaniline followed by 10% Na_2CO_3. After drying, the paper was cut into 1 cm strips, eluted with water, and an aliquot of each eluate assayed for radioactivity. In each case radioactivity was found only in the area coincident with the added 3-methoxy-4-hydroxymandelic acid. *3 M eluate.* The radioactive fractions were treated similarly to those of the 1.0 M eluate. A sharp radioactive peak corresponding to the position of 3,4-dihydroxymandelic acid (DOMA) was obtained in the butanol-N HCl solvent; in the isopropanol-ammonia-water solvent, 2 radioactive peaks were obtained. However, destruction of the DOMA which had been added as a marker was observed.

Results. Table I presents the data from experiments in which aliquots of 24 hour urine specimens obtained from 6 different subjects were assayed. The average recovery of $73 \pm 5\%$ of the infused dose is lower than that obtained by Resnick and Elmadjian(4) in humans and by Schayer(5) in rats. Of the total radioactivity in urine, 4% is due to adrenaline. This amount corresponds to 3% of the infused dose which is in accord with previous reports(1,2,3). Radioactive adrenaline could be detected only in the urine samples collecting during the infusion period and during the first and second hours after the infusion.

No previous data on excretion of metadrenaline or its conjugate in human urine are available. The results reported here for excretion of metadrenaline in human urine are approximately the same as those for the rat (9). In addition, Axelrod *et al.*(9) reported that, in the rat, 20% of an intraperitoneal injection of adrenaline is excreted in the urine as the glucuronide of metadrenaline. We have been able to confirm this finding in the rat. In the human, the acid labile conjugate of metadrenaline accounts for about 30% of an infused dose of adrenaline. This acid labile conjugate does not appear to be a glucuronide since it is not hydrolyzed by β-glucuronidase and differs chromatographically from the metadrenaline glucuronide obtained from the rat.

Recently Armstrong and McMillan(6) identified 3-methoxy-4-hydroxymandelic acid as a metabolic product of noradrenaline and estimated that 30% of an infusion of noradrenaline was converted to this acid. In the 6 experiments reported here, 27% of the radioactivity which appeared in the urine after an infusion of dl-adrenaline-2-C^{14} was recovered as 3-methoxy-4-hydroxymandelic acid.

Dihydroxymandelic acid has been previously found in human urine(12), however, no estimates of the amounts of this acid derived from adrenaline metabolism were made. The figures presented for this compound are tentative pending more rigorous identification of the material eluted from the Dowex-1 column.

The radioactive material of human urine which appears in the Dowex-1 effluent has not yet been characterized. It is interesting to note that in rat urine, the glucuronide conjugate of metadrenaline is found in the Dowex-1 effluent.

Summary. A procedure is described which utilizes a combination of filter paper and ion exchange chromatography to separate the urinary metabolic products of adrenaline. After intravenous infusion of dl-adrenaline-2-C^{14} in 6 human subjects, 73 ± 5% of infused radioactivity was recovered in 24 hour urine. Radioactivity which appeared in urine was distributed among various metabolic products as follows: adrenaline, 4%, 3-methyl-O-adrenaline, 5%, 3-methyl-O-adrenaline conjugate, 42 ± 7%; 3-methoxy-4-hydroxymandelic acid, 27 ± 3%; 3, 4-dihydroxymandelic acid, 12 ± 4%; 2 unidentified fractions, 4%.

ADDENDUM: Recent work on the separation procedure has resolved the 0.3M eluate and the 3.0M eluates each into two fractions. Preliminary work indicates that the metadrenaline conjugate accounts for about 80% of the radioactivity in the 0.3M eluate and one unidentified compound accounts for the remainder. In the 3.0M eluate, 3,4-dihydroxymandelic acid accounts for 30 to 50% of the radioactivity. This work is still in progress and will be reported at a future date.

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Plasma Cholesterol in Growing Chicken as Influenced by Dietary Protein and Fat.*† (24130)

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In the human, Ahrens *et al.*(1), Beveridge *et al.*(2), and others have indicated a relationship between degree of saturation of ingested fat and resulting serum cholesterol level. Studies with "normal" experimental animals (those not receiving dietary cholesterol or other cholesterolemic agents) have shown essentially no differential response to dietary fat(3). Hegsted and coworkers(4) presented evidence of a significant negative correlation between the product of the essential fatty acids and total saturated fatty acids (EFA x TSFA) of dietary fats and serum cholesterol level. Serum cholesterol level can be experimentally elevated not only through dietary cholesterol but also by feeding low protein diets(5,6,7). Our objective was to evaluate the effect of level and type of dietary fat on plasma cholesterol in growing chickens receiving either 1) low protein diet plus 0.3% cholesterol or 2) high protein diet with 2% cholesterol. Since differences in serum cholesterol as a result of varying the dietary fat (as observed by Hegsted *et al.*) might be related to differences in absorption of dietary cholesterol it was of particular interest to find out whether the low protein induced hypercholesterolemia would respond differen-

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