ination against strontium with time. It is inferred that the observed discrimination occurs in the kidney and that endogenous excretion *via* the gut is negligible in this case. The point to which attention is directed is the differential observed between parts of long bones in the rat under the experimental conditions used. The more rapid turnover of the radioisotopes in processes of accretion, exchange, and resorption in the epiphyseal ends of the femur as contrasted with the less actively growing shaft leads to a preferential loss of strontium in the ends of the femur as compared with the shaft. It is conceivable that vascular arrangements in the epiphyses as compared with the shaft play a role in the discrimination observed.

Summary. After intraperitoneal injection of a mixture of Ca^{45} and Sr^{89} in the rat, the Sr/Ca Observed Ratio in parts of a long bone, the femur, was obtained 15 minutes to 48 hours after injection. A maximal Sr content appeared in bone within 30 minutes, with the shaft showing discrimination against Ca; during the next 48 hours the Observed Ratios fell but remained consistently higher in shaft portions of the bone. Data on blood Sr/Ca ratios are also presented.

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Effect of Phenethylbiguanide on Adrenal Function and Responsiveness as Measured by ACTH Test.* (24128)

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The quest for an oral hypoglycemic agent has received considerable impetus since reintroduction of the aryl-sulfonylureas by Franke and Fuchs(1), extending the work that Janbon(2) and Loubatieres(3) had begun 13 years earlier. Unger, Freedman and Shapiro (4), reviewing a group of potentially hypoglycemic drugs, discovered N B′ Phenethylformadinylimourea (DBI)†, a biguanide. DBI has been used by Pomeranze(5,6), Williams, *et al.*(7), and in our own clinic (unpublished) in treatment of hyperglycemia in man. It lowers blood sugar concentration in patients with diabetes mellitus but the moderately severe side effects of nausea and anorexia have precluded its continued use in many. The mechanism by which biguanides act is unknown. Since adrenal steroids can affect blood glucose level by increasing gluconeogenesis and decreasing glucose utilization, it was believed that one action of DBI might involve the blockade of adrenal steroid production. Our studies were designed to measure the effect of DBI on adrenocortical secretion of hydrocortisone and demonstrate either a decrease in or a blockade of adrenal cortical activity by comparing baseline plasma hydrocortisone levels with levels after DBI, and by comparing baseline responses of adrenal gland to pituitary stimulation (ACTH test) to responses to ACTH following DBI therapy.

Methods. The 6 subjects, all on wards of St. Luke's Hospital had recognized diabetes mellitus for 1 month to 20 years and were well controlled on 20 to 80 units of insulin/day. These patients received DBI for 8 to

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TABLE I. Adrenal Responsiveness to ACTH in 6 Patients with Diabetes Mellitus before and after Treatment with DBI.

Patient (yr & sex)	Duration of diabetes mellitus	Insulin dosage (units)	Duration DBI treatment (days)	Daily dosage DBI (total mg)	Pre-DBI ACTH test			Post-DBI ACTH test		
					Baseline*	2 hr	4 hr	Baseline	2 hr	4 hr
					γ %					
R.C. 21 ♂	1 mo	40 (NPH)	15	250 (no insulin)	13	33	41.1	24	30	37
P.M. 17 ♂	9 yr	60 (lente)	12	150 (40 u lente)	13	40.1	49.3	6	29	41
I.W. 85 ♀	17 "	35 (PZI)	15	100 (no insulin)	30	49	50	18.5	32	101
A.B. 21 ♂	10 mo	40 (NPH)	8	250 (no insulin)	24.3	27	44	42	55	61
I.Q. 36 ♀	3 yr	80 (NPH)	8	275 (30 u regular)	15	29	42	23	26.3	26.3
E.S. 67 ♀	12 "	DBI initial therapy	15	150 (no insulin)	25		59	7.3		28.6

* Plasma 17,21-dihydroxy-20-ketosteroid.

15 days. Two patients required insulin in reduced amounts for control of hyperglycemia during treatment with DBI, while 4 were maintained on drug alone. All patients had an ACTH test as outlined by Christy, Wallace and Jailer(8) before and after treatment with biguanide. The test consists of baseline, 2 hour and 4 hour plasma hydrocortisone levels measured during infusion of 25 units of aqueous ACTH in 500 cc of isotonic saline. 17, 21-dihydroxy-20-ketosteroid concentrations in plasma were determined by the method of Silber and Porter(9). By this method 80% of the chromogenic reacting material is hydrocortisone. DBI added to pooled plasma in concentrations varying 0.05 mg to 0.3 mg % did not alter values for hydrocortisone as determined by Silber-Porter technic.

Results. In pre-treatment ACTH tests, the average baseline plasma hydrocortisone level was 20 γ/100 ml (range 13-30 γ/100 ml), and after ACTH administration the average level was 47.6 γ/100 ml (range 41.1-59 γ/100 ml). After DBI therapy the average baseline value was 20.1 γ 100 ml) (range 6-42 γ/100 ml) and after ACTH administration the average level was 49.1 γ/100 ml (range 26.3-101 γ/100 ml). (Table I). Patients (P.M., I.W., E.S.) showed a depression of post-treatment baseline hydrocortisone levels while 3 (R.C., A.B., I.Q.) showed elevated baseline levels. Patients (R.C., I.Q.) had a diminished response to ACTH following biguanide therapy, 2 (P.M., A.B.) showed essentially no change, I.W. had an increased response while patient E.S. had an increased response but depressed baseline level. All these values are within normal limits for this test with the exception of I.W. who showed a high 4 hour level.

Discussion. Gluconeogenesis and glucose release by liver are of major importance in maintaining normoglycemia(10). Although Tyberghein and Williams(11,12) demonstrated marked reduction in liver glycogen content in the guinea pig following DBI administration, further work(12) indicates another effect on hyperglycemia aside from that of hepatic carbohydrate metabolism as shown by the success of Nielsen, *et al.*(13) in inducing hypoglycemia with DBI in hepatectomized guinea pigs maintained with glucose.

A decrease in adrenal steroidogenesis and concomitant decrease in gluconeogenesis might explain why Williams, *et al.*(12), found a decrease in both liver glycogen and urinary nitrogen in animals fed alanine and DBI. While increased peripheral utilization by insulin enhancement or some other mechanism could account for Nielsen's findings. Our results in human subjects failed to show any consistent pattern of alteration in plasma hydrocortisone baseline concentration or ability

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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‡ Established Investigator of Am. Heart Assn.