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Diet and Temperature Effects on Excretion of Fat-Mobilizing Substances in Rat Urine.* (29843)

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Chalmers *et al*(1) reported the isolation of a fat-mobilizing substance (FMS) from the urine of fasting humans. Subsequently (2) we reported the extraction of a similar material from the urine of fasting rats (rat FMS). More recently, the separation of rat FMS into 2 chemically and biologically distinct fractions was accomplished in this laboratory and some of the characteristics of these fractions were determined(3,4). One fraction, FMS IA induced hypophagia but not fat mobilization when injected into rats; the second fraction, FMS IB, caused fat mobilization without an effect upon food intake. Both substances exhibited lipolytic activity *in vitro,* the activity of IB exceeding that of IA. Both substances in their present state of purity contain 16-17 amino acids, carbohydrate, phosphorus, hexosamine and a cholesterol-like material but are chemically distinct as shown by thin layer chromatography and electrophoresis(3).

The present communication reports our observations on the effects of previous diet and of environmental temperature on excretion of FMS, FMS IA and FMS IB in the urine of fasting rats.

Methods. Male rats of the Wistar strain were fed one of the 4 experimental diets shown in Table I. FMS, FMS IA and FMS IB were extracted from urine (collected under toluene) and fractionated as previously described(3). Omission of solution in water to separate FMS IA and IB results in extraction of FMS. Lipolytic activity *in vitro* was determined by measuring increase of free fatty acid release when the substance (100 μg) was added to about 250 mg adipose tissue (rat epididymal fat pad) in an incubation medium of Krebs-Ringer bicarbonate buffer (pH 7.3-7.4) with 4% bovine serum albumen and the whole incubated for 3 hours with shaking at 37°C. Free fatty acids in the incubation medium were measured by the procedure of Dole(5).

I. *Effect of previous diet.* Four groups of 8 rats each, weighing 400-450 g, were housed in stainless steel metabolism cages at 23 ± 1°C and each group was fed one of the 4 diets for a period of 6 days. The animals were then fasted for 24 hours during which urine was collected and pooled for each group. FMS IA and FMS IB were separately extracted and assayed for lipolytic activity *in vitro*. The same animals were then refed their experimental diets for a period of 6 days but with a caloric restriction of approximately 50%. Urine was collected during fasting, pooled for each group and FMS was extracted and assayed *in vitro*.

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TABLE I. Constituents in % by Weight of Experimental Diets.

Constituent*	High carbohydrate (HCD)	High protein (HPD)	High protein-high fat (HP-HFD)	High fat (HFD)
casein	20	50	50	20.8
sucrose	64	34	24	10.4
corn oil	10	10	20	4.6
lard	—	—	—	26.6
salts	4	4	4	4
alphacel	2	2	2	—
celluflour	—	—	—	33.6
cals/g	4.26	4.26	4.76	4.06

* Plus adequate amounts of B vitamins, vit A and D, choline and inositol.

II. *Effect of environmental temperature.* This experiment was carried out on 2 separate occasions with comparable results. The details of one experiment are reported here. Twenty-five male rats, weighing 260-280 g, were housed in each of 2 large metabolism cages and were provided with the high-fat diet *ad libitum*. After 6-day feeding periods at 24°C, 25 rats were fasted for 24 hours at each of the following environmental temperatures in random manner: 30, 24, 19, 15 and 5°C. Urine was collected during the fast, pooled for each group and FMS IA and FMS IB were extracted and assayed *in vitro*.

Results. I. *Effect of previous diet.* From the data of Table II, it is apparent that FMS is excreted by fasting rats previously fed any of the 4 diets whether *ad libitum* or in restricted amounts. With *ad libitum* feeding, FMS excretion was considerably greater than previously observed with a high-fat diet (2-4) possibly due to the greater age, and hence body weight of these animals. It would appear that increased amounts of dietary protein and fat (HPD or HP-HFD) are conducive to increased excretion of FMS; the greatest excretion observed in this investigation was with animals previously fed the HP-HFD either *ad libitum* or in restricted amounts. After a high carbohydrate diet (HCD) fed *ad libitum*, fasting excretion of FMS was considerably less than that with other diets; with previous restricted feeding, fasting excretion was relatively small in animals fed either the HCD or the HFD. All urine extracts possessed appreciable lipolytic activity as measured *in vitro*. Although there was no consistent correlation between previous diet and lipolytic potency of the extracts *in vitro*, there was some suggestion of a positive correlation between total activity excreted per 24 hours of FMS IA and proportion of protein calories in the diet, and between that of FMS IB and proportion of non-carbohydrate calories in the diet.

II. *Effect of environmental temperature.*

TABLE II. Effect of Quantity and Quality of Diet on Excretion of FMS, FMS IA and FMS IB (Results obtained from pooled urine from 8 rats/group).

Diet§	Caloric intake, cals/rat/day	FMS IA			FMS IB			FMS	
		mg/rat	Activity specific*	Total†	mg/rat	Activity specific*	Total†	mg/rat	Activity*
HCD	112	1.3	1.18	15.3	12.5	2.16	270	13.88‡	—
HPD	98	6.6	1.57	103.6	24.1	1.77	427	30.7‡	—
HP-HFD	110	7.5	1.77	132.8	39.3	2.35	924	46.8‡	—
HFD	104	2.0	1.96	39.2	32.6	3.14	1024	34.6‡	—
HCD	50							10.6	1.77
HPD	50							21.5	1.96
HP-HFD	50							22.1	1.60
HFD	50							10.1	1.18

*μM free fatty acids released by 100 μg material acting on approximately 250 mg rat epididymal fat pad *in vitro*.

† Calculated as specific activity × mg material excreted.

‡ Calculated as the sum of FMS IA and FMS IB.

§ See Table I for description of diets.

TABLE III. Effect of Environmental Temperature on Excretion of FMS IA and FMS IB in Rat Urine. Results were obtained from pooled urine of 25 male rats during 24-hours fasting.

Environmental temperature °C	FMS IA		FMS IB		Total FMS IA + IB	Activity* *in vitro*	
	mg/rat	% total	mg/rat	% total	mg/rat	IA	IB
30	1.77	26	4.99	74	6.76	1.39	1.39
24	3.48	34	7.06	66	10.54	.79	1.39
19	2.47	21	9.12	79	11.59	.79	2.18
15	4.90	13	33.50	87	38.40	.92	1.48
5	6.68	32	13.40	68	20.08	1.12	1.48

* μmoles free fatty acids released by 100 μg material acting on approximately 250 mg rat epididymal fat pad *in vitro*.

As shown in Table III, decreasing the environmental temperature from 30 to 5°C resulted in a progressively increasing excretion of FMS IA. Similarly, excretion of FMS IB was increased although to a slightly greater degree and with a very marked increase at about 15°C. This dramatic increase at 15°C has been observed in 2 separate experiments. All extracts had appreciable lipolytic activity *in vitro*, the potency of FMS IB exceeding that of FMS IA as previously reported(3) except when collected at 30°C, at which temperature the potencies were equal. There was no apparent correlation between lipolytic potency *in vitro* and the temperature at which fasting occurred.

Discussion. It is apparent from the results of these experiments that the excretion of both FMS IA (anorexigenic) and FMS IB (fat-mobilizing) is affected in quantity at least by the nature and amount of food fed prior to fasting and by the environmental temperature to which the animals are exposed during the 24-hour fasting period. Male rats, while in the fed state, do not excrete an active material at 24°C but will do so when exposed to a cold environment (5°C)(4). Similarly, excretion of a fat-mobilizing material in urine of cold-exposed rabbits has been reported(6). It is obvious that, in investigations of fat-mobilizing substances in urine, diet and environmental temperature must be considered at least with regard to the quantities excreted. Lipolytic potency *in vitro*, on the other hand, does not appear to be related to either previous diet or environmental temperature. The sudden and very marked increase in excretion of FMS IB at about 15°C is of considerable interest but the cause and significance of this increase are not known. An earlier study in this laboratory has demonstrated a peak of spontaneous activity at about 10-15°C in rats exposed to environmental temperatures in the range 5-33°C(7). It may well be that increased spontaneous activity (exercise) or shivering is the causative factor in increased excretion of FMS IB at about 15°C. Exercise has been reported to increase serum concentration of free fatty acids, an index of fat mobilization(8).

Summary. The effects have been investigated of 4 diets (high carbohydrate, high protein, high fat and high protein-high fat) and of environmental temperature (5-30°C) on urinary excretion of FMS IA (anorexigenic) and FMS IB (fat-mobilizing) by the fasting rat. The greatest excretion of FMS IA and FMS IB was observed when the HP-HFD was fed prior to fasting; for FMS IB, the greatest total activity excreted per 24 hours was obtained after the diet highest in non-carbohydrate calories (HFD). Excretion of these materials increased with decreasing environmental temperature. A very marked increase in FMS IB excretion was observed between 10 and 15°C.

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Adrenal Gland Dehydrogenases and Corticosteroid Production in Normal and Arteriosclerotic Female Rats.*† (29844)

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Female rats develop arteriosclerosis when repeatedly bred. The incidence and severity of the arteriosclerosis increases with the number and frequency of the breedings(1). The arterial lesions consist of subintimal accumulations of mucopolysaccharides which become converted into fibrotic plaques. With advancing arteriosclerosis, there is medial pooling of mucopolysaccharides, elastosis, ground substance degeneration and eventual calcification. Droplets of lipid are found at the periphery of intimal plaques, greater quantities of lipid at sites of medial elastic tissue degeneration(1). Cortical hyperplasia and necrosis, lipid depletion, and other indications of adrenal gland exhaustion are also commonly observed in breeder females(2). Also, when maximally stimulated *in vitro* by ACTH, the adrenal glands of the breeder female rats produce significantly less corticosteroid than adrenals from virgin females (3). The evidence that a causal or permissive relationship exists between abnormalities in adrenal function and the pathogenesis of the arteriosclerosis in these animals has been previously discussed(2).

The experiments described here were carried out in an attempt to find the biochemical basis for the reduced cortical steroid production observed in the adrenal glands of breeder female rats. McKerns has published data which indicate that in corticosteroid biosynthesis there is a specific requirement for reduction of TPN by the dehydrogenases of the pentose phosphate cycle. Also, the production of TPNH appears to be a limiting factor in the rate of corticoid synthesis(4). Therefore, it occurred to us that the decreased steroid production by the adrenal glands of breeder rats might be due to a relative deficiency of these dehydrogenases. The data reported here are consistent with this hypothesis.

Materials and methods. The rats used were obtained from the Sprague-Dawley Farms, Madison, Wis. All were females, either 250 ± 10 g virgins or 320 ± 15 g discarded breeders. The animals were killed by decapitation and their adrenal glands were removed and trimmed free of extraneous tissue. The aortas of the breeder animals were examined for evidence of gross arteriosclerosis. The adrenal tissue was then classified according to whether it was derived from virgin animals or from breeder animals with or without gross arteriosclerosis of the aorta. Virgins were the same age as breeder female rats and were uniformly free of arteriosclerosis. Enzyme assays were carried out on supernatants derived from 5% homogenates of the adrenal tissue in distilled water. Particulate matter was removed by centrifugation at 8000 × *g* for 15 minutes. The initial composition of the assay system is indicated in Table I. Measurements were made of the rate of change in optical density of TPNH at 340 μ at one minute intervals using

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