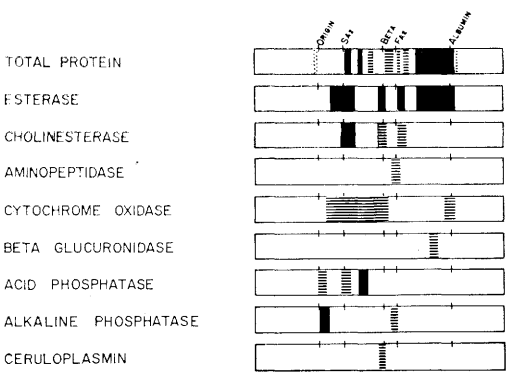

FIG. 8. Enzyme distribution in the mouse.

fractions with low mobility in all species except the dog. Cytochrome oxidase exhibited the greatest divergence with respect to number of bands, varying from one in the guinea pig to 5 in the rabbit. Beta-glucuronidase activity was found in only one band in all species. It, along with aminopeptidase and ceruloplasmin, had the most uniform migration. Although there was some variability in intensity of the various zones after both protein and enzyme stains among various members of the same species, the overall pattern for that species was constant.

Summary. The occurrence and distribution of serum enzymes and proteins in 7 mammalian species have been studied by means of starch gel electrophoresis and appropriate staining technics. Species differences were observed with respect to the presence, number, mobility, and concentration of components exhibiting various enzymatic activities. Similar observations were also made with respect to bands staining for protein.

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Comparison of "Meal Eating" and "Nibbling" on Severity of Alloxan Diabetes.* (26181)

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That rate of ingestion of diet plays a significant role in regulation of over-all body metabolism is suggested by the following data: animals fed full-spaced meals ("meal eaters") demonstrate (a) increased quantities of body fat, (b) decreased body protein, (c) altered thyroid activity and (d) changed tissue enzymatic activity, when compared to animals eating the same diet in frequent small feedings ("nibblers")(1). To determine whether manner of eating plays a role in severity of diabetes, experiments were undertaken dealing with relationship of eating habits to glycosuria and nitrogen excretion in alloxanized rats. Results indicate that rate of ingestion of diet does influence excretion of both substances in presence of experimental alloxan diabetes.

Methods. Male Holtzman rats, received when approximately 100 g in weight, were placed on a 50% carbohydrate diet *ad libitum,* and when about 130 g were given 50

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TABLE I. Effect of Rate of Ingestion of Diet on Urinary Glucose and NPN Excretion in Alloxan Diabetic Rats.

Rat No.	Diet and days of observation	Cumulative excretion* Glucose *Ad libitum*	Glucose Force fed	Glucose % increase†	NPN *Ad libitum*	NPN Force fed	NPN % increase†
Series 1							
1	50% CHO 8 days	12224	18238	49	1824	2312	27
2	*idem*	1605	4613	187	1448	1776	23
3	"	287	548	91	1928	1968	2
5	"	667	2144	221	1784	2072	16
6	"	451	1523	238	2120	2496	18
10	"	171	1082	536	1608	2424	51
	Mean increase in excretion, ± S.E.			220 ± 70			23 ± 6.6
Series 2							
10	50% CHO 7 days	373	871	134	1610	2198	37
5	*idem*	471	1322	181	2002	2219	11
11	"	852	1417	66	1603	2198	37
15	"	18273	20645	13	1960	2149	10
	Mean increase in excretion, ± S.E.			99 ± 37			24 ± 7.6
Series 3							
10	71% CHO 6 days	275	1139	314			
5	*idem*	141	1075	662			
11	"	258	974	278			
15	"	11297	15348	36			
	Mean increase in excretion, ± S.E.			323 ± 129			

* The excretions are given in mg for total No. of days; food ingested the same for both periods.

† Excretion during *ad libitum* period = 100%.

mg/100 g body weight of alloxan ("sub-diabetogenic" dose) at pH 4.0 subcutaneously (2). One week later, animals were started and continued, for duration of experimental periods of observation, on subcutaneous injections of 2 U protamine Zn insulin‡ daily. Experiments started 4-8 weeks after alloxanization.

At start of periods of observation, rats were put into individual metabolism cages, their *ad libitum* food intake measured daily and their 24 hour urines collected in citric acid under toluene for 6, 7 or 8 days. Animals were then adapted to a forced feeding regimen with the same diet twice daily; by the end of 2 weeks, it was possible to force feed each day the same quantity of food each animal had previously consumed so that every rat was his own control ("pair-fed" against itself). Urines were collected daily throughout. Four rats were put through the *ad libitum*-force feeding cycle once, 4 rats were cycled twice and 2 rats cycled 3 times. Two of the cycles were accomplished with a moderate (50%) carbohydrate diet and one with a high (71%) carbohydrate diet. Preparation and composition of diets were described previously(3,4). Urinary glucose determinations were accomplished by the method of Nelson(5), urinary NPN evaluated according to Conway(6). The results are given as total urinary excretions during number of days of study.

Results and discussion. When animals were force fed twice daily the same quantities of diet they had eaten previously *ad libitum,* excretions of glucose and NPN were significantly greater than excretions measured under nibbling conditions. These findings were true not only for the total period but for each comparative day. The increase in glycosuria varied between 13% and 662%, averaging 215%. NPN excretions increased by an average of 23% (Table I). A greater loss of glucose and nitrogen occurred during the force-feeding period, irrespective of whether diabetes was "poorly" or "well" controlled during nibbling period.

‡ Generously supplied by Dr. W. R. Kirtley, Eli Lilly Co.

The increased glycosuria observed with force feeding could have resulted from either greater postprandial hyperglycemia attendant on this manner of feeding or from a relatively more marked insulin deficiency. It is conceivable that, with the decreased rate of protein synthesis that accompanies the meal eating regimen, synthesis of the protein hormone insulin might be affected likewise. The effects of forced feeding on nitrogen metabolism might be attributed to (a) a rate-limited ability of the body to handle ingested protein or (b) increased gluconeogenesis. It has been observed(1) that "pair-feeding" rats in a limited time period (giving the amount of diet consumed by other animals over a 24 hour period) results in a decreased ability to store body protein. The data of Wu and Wu (7) in the human suggest that this finding results from a limitation in quantity of protein the organism can handle in a given time period. Also, a possible cause for the increased NPN excretion seen with forced feeding is accelerated gluconeogenesis, secondary to the increased glycosuria. However, both our data and those of Wu and Wu favor rate-limitation as the cause for the greater nitrogen excretion that accompanies forced feeding.

Animals were recycled through the two feeding patterns to exclude the exacerbations and remissions that characterize alloxan diabetes(8) as being responsible for our findings. Constant insulin dosage was suboptimal in order to compare glycosuria under the conditions of both *ad libitum* and forced feeding.

The implications of our findings with respect to man appear obvious. Increased frequency of feedings is now used in therapy of complications of diabetes. During treatment of diabetic acidosis, glucose when given is administered intravenously in small constant dosage. When "brittle" diabetics are in need of control or a diabetic patient is faced with complications making regulation difficult, feedings every 6 hours have been given(9,10). Our results suggest that in therapy of human diabetics, the use of more frequent meals might lead to better control and to reduction in insulin dosage needed.

Summary. The food intake and urinary excretion of glucose and NPN were measured daily in alloxan diabetic rats under the conditions of free access to food. The animals were then adapted to force feeding and fed in this manner twice daily the same quantity of diet they had consumed *ad libitum*. Insulin dosage was kept constant throughout. Under conditions of forced feeding, glycosuria increased by 215% and NPN excretion by 23%. These findings were reproducible when animals were recycled 2 or 3 times, demonstrating that eating patterns, not ebb and flow of severity of diabetes, were responsible for the findings.

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