factors too, that can greatly affect the tumorgenic response of a gland to a carcinogen.

It is becoming increasingly important that when working with the prostatic gland of the rat that the designation of the prostatic lobes be carefully indicated despite having a common origin arising as buds of the urogenital sinus. The observation that the prostatic lobes in an animal is not a homogenous structure as judged by its response to estrogen, enzyme concentration, chemical constituents (7) and zinc[65] uptake(8,9) has been late in discovery notwithstanding the fact that histological procedures do not reveal any differences among the lobes of the prostate gland. It is therefore reasonable to assume that lobes of the rat prostate gland can react differently to methylcholanthrene or to other carcinogens. Preliminary studies using different carcinogens to various prostatic lobes seem to support this concept.

Summary. (1) Intraprostatic deposits of 20-methylcholanthrene crystals induced three types of prostatic neoplasms in the ventral lobes of the Wistar rat's prostate gland: adenocarcinoma (2%), leiomyosarcomas (3%) and squamous cell carcinomas (30.3%). The squamous cell carcinomas and adenocarcinomas metastasized. (2) A detailed account of the distribution of invasion and/or metastases of these prostatic tumors to various organs are presented as well as an account on the growth characteristics of transplantable squamous cell carcinomas.

The authors wish to express their thanks to Miss Helen Fox, Edward Dywinski, and Andre Bulba for technical assistance, Dr. L. Simpson for her assistance in diagnoses of histological slides.

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Received August 30, 1956. P.S.E.B.M., 1956, v93.

Nitrogen Sparing Action of Neutral Fat and Fatty Acids in the Phlorhizinized Rat.* (22788)

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In a recent review devoted to the relationship of protein metabolism to that of fat and carbohydrate, Munro summarized the extensive data indicating that carbohydrate is a protein sparer, regardless of whether the animal is starving or is receiving a protein free or protein containing diet(1). By contrast, Munro noted that the results of studies dealing with the ability of fat to prevent excessive nitrogen loss are in conflict. Thus, some reports indicate that when fat is the sole source of calories, the nitrogen output of an animal is as great as during total starvation(2,3,4,5); on the other hand, data has been presented which demonstrates that fat is a protein sparer, even in the absence of protein in the diet, if minimal caloric requirements are satisfied(6,7). In view of the divergent results, and because it seemed to us that fat, under

* This investigation was supported by research grant from National Institute of Arthritis and Metabolic Diseases, Public Health Service.

† This department is supported in part by the Michael Reese Research Foundation.

TABLE I. Composition of Diets.

Vitamins and salt			
Salt (Wesson)	20 g	Vit. B syrup	30 ml
Cod liver oil	5 ml	2-methyl naphtho-quinone	50 mg
Wheat germ oil	5 "		

To the above were added, for the respective diets:

	High carbohydrate	Moderate carbohydrate	High fat
	g		
Casein hydrolysate (enzymatic)	95	95	95
Lactalbumin hydrolysate (enzymatic)	10	10	10
Methionine	2	2	2
Liver powder	10	10	10
Corn starch	185	110	
Dextrin	170	105	
Sucrose	170	110	
Mazola oil	10	100	245
Gum ghatti			10

Solid diet: To moderate carbohydrate diet were added 300 ml of 5% agar agar and 300 ml of water.

Liquid diets: To appropriate diet were added 30 g of cellu flour and water to make 1000 ml.

Protein-free diets: Made up similar to liquid diets with exception that casein and lactalbumin hydrolysates, methionine and liver powder were omitted.

Oleic acid diet: To vitamins and salt, 30 g of cellu flour, 10 g of gum ghatti, 240 g of oleic acid and water to make 1000 ml were added.

Glycerol diet: To vitamins and salt, 30 g of cellu flour, 10 g of gum ghatti, 25 g of glycerol and water to make 1000 ml were added.

appropriate conditions, should spare protein in the phlorhizinized rat, the experiments described below were undertaken. Protein sparing effects were obtained in phlorhizinized rats tube fed protein free diets, irrespective of whether the calories were supplied by neutral fat, fatty acids or carbohydrate.

Methods. Male Holtzman rats, weighing approximately 120 g at the start of the experiment, were employed and fed diets modified from those described by Ingle(8). The food was available to the animals eating *ad libitum* in solid form at all times but was administered by stomach tube to the force fed groups in liquid state twice a day, before 9 a.m. and after 4 p.m. The compositions of the diets are given in Table I. Four series of rats were employed: (1) Group 1 was *ad libitum* fed the solid moderate carbohydrate diet; (2) Group 2 was force fed the moderate carbohydrate diet; (3) Group 3 was force fed the high carbohydrate diet; (4) Group 4 was force fed the high fat diet. The rats were placed on their respective diets for 3-4 weeks, until they weighed between 210 and 220 g, the amount of diet administered to the rats in the force fed series being adjusted so that the weight gains of these animals approximated that of the *ad libitum* fed ones. At the completion of the feeding period, the rats were injected intramuscularly on each of 2 successive days with 50 mg of phlorhizin in 1 ml of sesame oil. The dietary management of the animals during the 2 days is shown in Table II. All animals were placed in individual metabolism cages during the second day of phlorhizinization and their urines collected under toluene with a gram of citric acid as an acidifying agent. These 24 hour specimens

TABLE II. Dietary Management of the Rats during the Two Days of Phlorhizinization.

Rat group	Period of starvation		Protein-free diets fed*				
	Day 1	Day 2	Moderate carbohydrate	High carbohydrate	High fat	Oleic acid	Glycerol
1†	+	+	0	0	0	0	0
2‡ (a)	0	0	+	0	0	0	0
(b)	+	0	+	0	0	0	0
3§	0	0	0	+	0	0	0
4‖ (a)	0	0	0	0	+	0	0
(b)	+	0	0	0	+	0	0
(c)	+	0	0	0	0	+	0
(d)	+	0	0	0	0	0	+

* Diet force fed the day(s) the animals were not starved. † These rats previously were on *ad lib* diet. ‡ Had been previously force fed the moderate carbohydrate diet. § High carbohydrate diet. ‖ High fat diet.

+ = Treatment indicated; 0 = no treatment given.

TABLE III. Excretion of Nitrogen and Glucose after Injections of Phlorhizin.

Rat group†		N excreted*	Glucose excreted*
		⌐mg/100 g body wt¬	
1‡	(7)	125 109–156	331 233– 429
2§	(a) (6)	42 39– 54	946 743–1220
3	(7)	35 28– 41	1053 790–1305
4¶	(a) (5)	67 54– 88	445 413– 500

* Values given are mean excretions and ranges for the group.

† No. in parentheses refers to No. of animals in group.

‡ These rats had previously been fed *ad lib*; they were starved for 2 days of phlorhizinization.

§ These rats were force fed the moderate carbohydrate protein free diet for 2 days of phlorhizinization.

Force fed high carbohydrate protein free diet for 2 days of phlorhizinization.

¶ Force fed high fat protein free diet for 2 days of phlorhizinization.

TABLE IV. Excretion of Nitrogen and Glucose after Injections of Phlorhizin.

Rat group†		N excreted*	Glucose excreted*
		⌐mg/100 g body wt¬	
1‡	(7)	125 109–156	331 233– 429
2§	(b) (5)	40 37– 43	1126 803–1394
4	(b) (6)	59 43– 75	463 176– 947
	(c) (5)	62 55– 81	368 211– 424
	(d) (6)	47 38– 57	482 404– 634

* Values given are mean excretions and ranges for the group.

† No. in parentheses refers to No. of animals in group.

‡ These rats had previously been fed *ad lib*; they were starved for 2 days of phlorhizinization.

§ These rats were starved for the first and force fed the moderate carbohydrate protein free diet for second day of phlorhizinization.

These rats were starved for first day of phlorhizinization; on second day, rats of groups (b), (c) and (d) were force fed protein free high fat, oleic acid and glycerol diets respectively.

were analyzed for N.P.N. by a modified Conway technic(9) and for reducing substances by the method of Nelson(10).

Results. Table III shows the urinary outputs of N.P.N. and glucose for the second day in rats force fed protein free diets during the 2 days of phlorhizinization, as well as the urinary losses of the starving animals. It can be seen that the N.P.N. output dropped from 125 mg/100 g in the animals who did not eat to approximately 40 mg/100 g in the animals force fed the carbohydrate diets. Little difference can be noted between the nitrogen excretions of the animals receiving the high or moderate carbohydrate intakes. When fat in the form of mazola oil was the source of calories, the nitrogen loss fell to approximately 67 mg/100 g of rat.

Starvation during the first day of phlorhizinization, followed by the feeding of the respective protein free diets on the second day, resulted in outputs similar to those noted above (Table IV). Fat feeding, in the absence of protein or carbohydrate, appeared to decrease the urinary nitrogen excretions.

The D:N ratios obtained in the above experiments suggested that gluconeogenesis from fat was occurring. However, since the dietary source of fat (mazola oil) is at least 90% trioleate‡ and therefore contains 10% available glycerol when hydrolyzed, it became apparent that both the "gluconeogenesis" and protein sparing we were observing might be attributable to the glycerol moiety of the fat. Accordingly, two groups of rats who had been force fed the high fat diet for 3-4 weeks were phlorhizinized for two days, starved on the first day but on the second day were force fed oleic acid or glycerol in the amounts calculated to be present in the high fat (mazola oil) diet. From the results in Table IV, it may be seen that the oleic acid, in the absence of glycerol or any other form of carbohydrate, prevented the excessive protein catabolism that results from phlorhizinization.

Discussion. The results obtained in our phlorhizinized rats, fed isocaloric amounts of protein free diets containing large or moderate amounts of carbohydrate or large amounts of neutral fat or fatty acids indicate that fat can inhibit endogenous protein catabolism. The

‡ We are indebted to Corn Products Refining Co. for this information.

degree to which fat prevents protein breakdown was not so great as that observed with carbohydrate; with the latter diets, N.P.N. excretions were decreased by 68%, with mazola oil by 53% and with oleic acid by 50%. Glycerol, in amounts of only 500 mg per rat per day decreased N.P.N. outputs by 63%.

The fats usually employed in laboratory diets are essentially triglycerides and contain 10% glycerol in ester linkage. In a high fat diet, as much as 500 mg/day of glycerol may come from this source. Failure to appreciate that 10% of dietary fat becomes available as carbohydrate when metabolized may be a factor in explaining the conflicting results obtained in previous studies.

Our first experiments were performed on animals force fed their respective protein free diets during the 2 days of phlorhizinization. To rule out the possibility that the apparent sparing of protein by fat may have been associated with the force feeding on the first day of phlorhizinization, thereby conserving body carbohydrate and protein stores, observations were made on animals starved for the first day of phlorhizin injections. On the second day, the animals were given their respective protein free diets or the oleic acid or glycerol diets (Table II). As is evident from Table IV, carbohydrate, neutral fat or fatty acids still prevented the usual protein loss seen with phlorhizinization.

While our findings are in accord with previously published data on the nitrogen sparing action resulting from feeding carbohydrate to phlorhizinized animals, they do not agree in respect to fat feeding. It has been reported that when fat, in the absence of protein, is administered to phlorhizinized animals, the accelerated nitrogen loss continued unabated (11,12,13). In contrast to previous work, we find a marked decrease in N.P.N. excretion when a protein free diet containing neutral fat or fatty acids is administered. The difference between our results and those of others may possibly be attributed to the pre-experimental dietary history of the animals; our rats were adapted and maintained on a high fat diet for 3-4 weeks before they were phlorhizinized whereas previous workers did not so adapt their animals. Munro is of the opinion that for the nitrogen sparing action of fat to be seen, studies should be performed several days after the animals have been placed on the high fat regimen(14).

It is not surprising that fat, like carbohydrate, should be found to spare nitrogen. Protein, carbohydrate and fat are considered to share a common final metabolic pathway with respect to carbon dioxide production (and energy yielding reactions). When there is a demand for energy, carbohydrate is probably utilized preferentially to protein. The findings in the carbohydrate fed phlorhizinized rats are in accord with this reasoning. On the basis of our results in the animals fed fatty acids during phlorhizinization, it appears that fat likewise is utilized in preference to endogenous protein for energy yielding reactions. However, since fat is not thought to be converted to carbohydrate, the use of protein for carbohydrate formation should cause fat fed phlorhizinized animals to excrete more nitrogen than carbohydrate fed ones. Our results are in agreement with this possibility since the N.P.N. excretions of the animals fed fat were slightly greater than the excretions of the ones fed carbohydrate.

Summary. Rats were adapted to the force feeding of a moderate or high carbohydrate or high fat diet for several weeks before phlorhizinization. During the second day of phlorhizin injections, the animals were fed their respective diets, rendered protein free, and the urinary excretions of N.P.N. measured and compared to the outputs of starving animals. The feeding of either neutral fat or carbohydrate reduced N.P.N. losses. A similar sparing of endogenous protein occurred when animals, fed the high fat diet for 3 weeks, were given oleic acid, rather than mazola oil, on the second day of phlorhizinization.

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Received August 31, 1956. P.S.E.B.M., 1956, v93.

Ergotamine, Oxytocin and Milk Let-Down in Lactating Rat.* (22789)

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It has been demonstrated(1-4) that adrenalin in appropriate doses can block the milk let-down response to both endogenous and injected oxytocin. Adrenalin presumably acts by constricting mammary blood vessels thus blocking passage of oxytocin to mammary myoepithelium(5-6). Ergotamine, also a peripheral vasoconstrictor(7), has recently been shown to inhibit milk let-down in lactating rats(8). The present paper is concerned with effects of exogenous oxytocin on ergotamine-inhibition of milk let-down in lactating rats. Evidence is presented to indicate that ergotamine exerts its inhibition by means other than through vasoconstrictive effects.

Materials and methods. Docile lactating albino rats, each with its first litter and weighing 200-260 g, were housed in individual cages and given free access to food and water. Each litter was reduced to 8 young shortly after birth and, whenever possible, these were equally distributed as to sex. Rats with less than 8 young were discarded. Each of 42 litters when 14 days old was isolated from their mother for 10 hours. During this time they were kept warm to prevent any undesirable effects from exposure. Each litter was then divided equally. One half was weighed as a group, killed and their stomachs examined for the presence of milk. The other half was replaced with the mother and allowed to suck for exactly 20 minutes. Length of time before each mother began nursing was recorded. Young were then removed, weighed as a group and immediately killed by decapitation. Their stomachs were opened and semisolid curds removed and weighed (Table I). The mothers received single subcutaneous injections as follows: (a) 17 received 1 mg/kg ergotamine tartrate.‡ Of these 10 were injected 10 minutes, the rest, 2 minutes before replacement of litters. (b) 10 received 1 mg/kg ergotamine 10 minutes before replacement of litters followed by oxytocin ('Pitocin', Parke Davis & Co.) 8 minutes later. Oxytocin was given to 5 rats in dose of .4 I.U./kg and to remaining 5 in dose of .2 I.U./kg. (c) 5 were treated with .2 I.U./kg oxytocin 10 minutes before replacement of litters followed by 1 mg/kg ergotamine 8 minutes later. (d) 10 served as controls of which 5 were uninjected. The remaining received comparable injections of distilled water 10 minutes prior to replacement of litters. Topical effects of saline solutions of 1:2000 ergotamine and .2 I.U./ml oxytocin alone, and in combination, were studied in 5 lactating rats. Each mother was separated 4-6 hours from its litter on the 14th day postpartum. Nembutal anaesthesia (3 mg/

* Contribution from Department of Dairy Husbandry, Mo. Agr. Exp. Station. Journal Series No. 1664. Approved by Director.

† Postdoctoral Fellow of the National Institutes of Health.

‡ Kindly supplied by Sandoz Chemicals, N. Y.