TABLE III. Total Sucrose Excretion, Urine Volume and Urinary Sucrose Concentration During the First Hour After Loading (NA, Nonadapted Rats; A, Adapted Rats).

Sucrose load (ml/100 g b.w.)	Sucrose excreted 0-60 min (mM/100 g)		Urine volume 0-60 min (ml/100 g)		Urine sucrose concentration (mM/l)	
	NA	A	NA	A	NA	A
2.0	2.11 (13)	1.74 (13)	7.4	5.1	285	341
2.5	2.16 (13)	1.93 (8)	6.9	5.6	313	345
3.0	2.44 (8)	2.05 (8)	7.4	6.0	330	342
3.5	2.11 (7)	2.08 (8)	6.3	6.0	335	347
4.0	2.19 (10)	2.42 (9)	6.2	6.8	353	356
4.5	1.85 (7)	2.52 (9)	5.5	7.0	336	360

Values are means. Figures in parentheses are numbers of animals per group. Sucrose infused as a 1.46 M (50% $^{w}/_{v}$) solution through chronically indwelling arterial catheters.

total solute excretion) in adapted animals during the second control interval (Table I).

Summary. Two series of unanesthetized rats were infused intraarterially with standardized volumes of 1.46 M (50% w/v) sucrose over the range 2.0 to 5.0 ml/100 g body weight. Animals in one of these series had been exposed 5 days earlier to the same concentration of sucrose at 2.0 ml/100 g body weight. These latter animals constituted the adapted groups. Comparison of the sucrose tolerance of adapted animals with nonadapted revealed that previous exposure to hypertonic sucrose significantly increased the resistance of twice-loaded animals to the damaging effects of the hypertonic solution. Evaluation of the excretion patterns of urinary solutes and the relationships between urine flow and solute concentrations suggest that a reduction in GFR may occur in nonadapted animals as load of hypertonic sucrose is increased from 2.0 to 4.5 ml/100 g b.w. Similar loads given to adapted animals appear less effective in their GFR depressing actions.

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Carbohydrate and Lipid Metabolism in Animals Treated with Pyrrolidinomethyl Tetracycline. (31217)

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Utilization of glucose is disturbed in animals after prolonged administration of penicillin(1), dihydrostreptomycin(2), oxytetracycline(3), chlortetracycline(4), and chloramphenicol(5). A mixture of penicillin and streptomycin when administered to rats and rabbits did not affect fasting blood sugar or glucose tolerance(6). Deposition of glycogen in liver was diminished in rats after treatment with oxytetracycline(3) and chlortetra-

* Work was most carried out at S. M. S. Medical College, Jaipur, India.

cycline(7,8) and in mice after puromycin(9). A higher fasting blood sugar with a higher blood glucose level 15 minutes after glucose administration had been reported in pigs after chlortetracycline(10) and in rabbits after oxytetracycline(11) treatments.

There are numerous reports on the alterations of lipid metabolism in animals after administration of antibiotics. When administered for prolonged periods, chloramphenicol produced changes in the pattern of tissue and plasma lipids in rabbits(5). Oxytetracycline and tetracycline produced marked increase in the cholesterol and phospholipids of different tissues in rats and rabbits(3). Chlortetracycline-fed rats incorporated more acetate-C^{14} and showed tendency to atherosclerosis(13, 14).

Pyrrolidinomethyl tetracycline, which is highly soluble in water, is widely used in medicine. Like other tetracyclines the drug is often used indiscriminately. It was, therefore, our interest to study the deleterious effect, if any, of continued use of the antibiotic on carbohydrate and lipid metabolism. Rhesus monkeys were used so that results obtained might simulate the effect of the drug in humans. Rats and rabbits were also used for comparison of the effects of the drug on these animals with those of monkeys.

The dose of the drug used in monkeys, 15 mg/kg/day, simulated that usually used in a human adult. The dosage was increased in rats, 50 mg/kg/day, and in rabbits, 25 mg/kg/day, in order to aggravate any toxic effect of the drug. When rabbits received a dose of 50 mg/kg/day, the animals stopped eating their ration. The dose, therefore, was reduced to 25 mg/kg in these animals.

Glucose tolerance, serum lipids and tissue content of lipids and glycogen were estimated in rats, rabbits and monkeys before and after administration of the antibiotic for 10 days.

Materials and methods. Male albino rats weighing about 200 g were divided into 2 groups. Animals of one of the groups were injected intraperitoneally with pyrrolidinomethyl tetracycline, 5 mg/100 g/day for 10 days. The other group of animals served as normal controls. Oral glucose tolerance test (3) was performed on the eleventh day in both normal and treated animals. The animals were subsequently sacrificed on the same day by decapitation after collecting a sample of blood by cardiac puncture. Aliquots of liver and skeletal muscle were weighed, adherent blood removed and used for precipitation of glycogen(15) which was estimated after hydrolysis as glucose(16). Weighed portions of tissues such as liver, brain, kidney, adrenals, small intestine and the whole of the carcass were dried at 85°C, extracted with petroleum ether in a soxhlet apparatus and the extract was used for estimation of lipids(17). Blood was used for estimation of glucose(16) and total cholesterol(18).

Male albino rabbits weighing about 2 kg were divided into 2 groups. The animals of one of the groups were fasted overnight, blood was collected from the marginal ear vein for estimation of glucose(16) and serum lipids (18) and oral glucose tolerance test was performed(5). Each rabbit was subsequently given an intraperitoneal injection of 50 mg pyrrolidinomethyl tetracycline per day for 10 days. On the eleventh day, after collecting a fasting blood sample and performing the oral glucose tolerance test, animals of both the groups were sacrificed by stunning and decapitation. Weighed portions of liver, brain, kidney, adrenals, small intestine and skin were dried and extracted with petroleum ether for the estimation of lipids(17).

Rhesus monkeys weighing on an average 4 kg were fasted overnight. After collecting a fasting blood sample from the femoral vein each animal was given by intravenous injection 25% glucose, 1 g glucose per kg. Samples of blood were collected every half hour for 2 hours after injection of glucose. From the following day pyrrolidinomethyl tetracycline, 15 mg/kg, was injected intravenously daily for 10 days. Fasting blood sample was collected on the eleventh day and glucose tolerance was repeated. Glucose was estimated in the different blood samples(16) and serum was used for estimation of different fractions of lipids(18).

Results. After treatment with pyrrolidinomethyl tetracycline the following changes were observed. *Rats:* The fasting blood sugar

level increased. Rise in the blood sugar level after glucose load was slower in the first half hour, increased subsequently above normal levels and had not come to the basal level by 3½ hours after the glucose feeding (Table I). Liver and muscle glycogen diminished considerably (Table II). Total cholesterol of liver and adrenals, phospholipids of brain and kidney and total lipids of liver and brain increased (Table IV). *Rabbits:* Although the

TABLE I. Glucose Tolerance Test in Animals Before and After Treatment with Pyrrolidinomethyl Tetracycline for 10 Days.

		Fasting blood sugar (mg /100 ml)	Rise in blood sugar (mg/100 ml) from fasting level — Minutes after administration of glucose							
			30	45	60	90	120	135	150	180
Rats (6) (oral tolerance)	B†	84 ± 3	36 ± 7		40 ± 8	24 ± 4	13 ± 3		8 ± 1	0
	A†	111 ± 5	14 ± 5		23 ± 5	51 ± 3	43 ± 7		32 ± 7	7 ± 5
	t	5.9*	2.5*		1.7	4.9*	4.8*		3.6*	
Rabbits (5) (oral tolerance)	B	112 ± 3		68 ± 7		93 ± 6		54 ± 10		12 ± 3
	A	119 ± 4		116 ± 7		116 ± 7		78 ± 13		43 ± 9
	t	1.3		4.4*		2.6*		1.4		3.3*
Monkeys (4) (intravenous tolerance)	B	133 ± 7	115 ± 7		11 ± 9	−12 ± 7	−16 ± 5			
	A	138 ±14	142 ± 6		42 ± 9	−30 ±17	−37 ±16			
	t	.31	3.05*		2.5*	1.01	1.28			

Values = Mean ± S.E. Figures in parentheses indicate number of animals.
* Significant at 5% level. † B, before treatment; A, after treatment.

TABLE II. Glycogen Content of Tissues of Animals Treated with Pyrrolidinomethyl Tetracycline for 10 Days.

	Rabbits (5)			Rats (6)		
	Before treatment	After treatment	*t*	Before treatment	After treatment	*t*
Liver glycogen (mg/100 g wet wt)	3100 ± 100	830 ± 300	6.53*	248 ± 33	145 ± 20	2.62*
Muscle glycogen (mg/100 g wet wt)	124 ± 27	157 ± 30	.81	111 ± 16	55 ± 10	2.95*

Values = Mean ± S.E. Figures in parentheses indicate number of animals.
* Significant at 5% level.

TABLE III. Different Fractions of Serum Lipids in Rabbits and Rhesus Monkeys, Before and After Treatment with Pyrrolidinomethyl Tetracycline.

	Rabbits (6)			Rhesus monkeys (7)		
Serum lipids	Before treatment with antibiotic	After intraperitoneal inj of antibiotic, 50 mg/day for 10 days	*t*	Before treatment with antibiotic	After intravenous inj of antibiotic, 15 mg/kg/day for 10 days	*t*
Cholesterol (mg/100 ml)	42 ± 2	86 ± 8	5.36*	94 ± 6	119 ± 9	2.34*
Phospholipids (mg/100 ml)	75 ± 3	122 ± 4	9.77*	206 ±12	258 ±21	2.41*
Triglycerides (mg/100 ml)	80 ± 6	187 ±25	4.2 *	81 ± 4	93 ± 7	1.56
Free fatty acids (μEq/l)	310 ±31	759 ±72	5.75*	346 ±48	567 ±41	3.50*
β-lipoprotein cholesterol (mg/100 ml)	16 ± 2	51 ± 5	7.7 *	63 ± 4	60 ± 2	.63
Lipoproteins (%): α	27 ± 2	16 ± 1	3.95*	34 ± 1	36 ± 1	1.23
β	73 ± 2	84 ± 1	3.95*	66 ± 1	64 ± 1	1.23

Values = Mean ± S.E. Figures in parentheses indicate number of animals.
* Significant at 5% level.

TABLE IV. Tissue Lipids of Rats Treated with Pyrrolidinomethyl Tetracycline, 5 mg/100 g/Day, for 10 Days.

	Total cholesterol (mg/100 g wet wt)			Phospholipids (as mg lecithin/100 g wet wt)			Total lipid (g/100 g wet wt)		
	Normal control	Treated with antibiotic for 10 days	t	Normal control	Treated with antibiotic for 10 days	t	Normal control	Treated with antibiotic for 10 days	t
Liver	197 ± 12	368 ± 30	5.29*	1336 ±115	1344 ± 56	.06	5.0 ± .5	13.0 ± .6	4.23*
Brain	1647 ±144	1712 ±136	.03	3510 ±220	4108 ±148	2.24*	7.8 ± .7	17.8 ±1.3	2.79*
Kidney	200 ± 31	173 ± 35	.59	790 ± 59	942 ± 40	2.14*	—	—	
Adrenals	1959 ±193	2573 ±211	2.15*	1402 ±106	1550 ±237	.57	—	—	
Intestine	109 ± 11	100 ± 21	.41	137 ± 19	96 ± 9	1.92	—	—	
Skin	185 ± 40	254 ± 9	1.66	113 ± 17	120 ± 11	.14	10.8 ±1.5	11.3 ±1.6	.22
Carcass	159 ± 16	172 ± 22	.48	446 ± 38	452 ± 15	.16	6.7 ± .7	7.8 ± .4	1.24
Blood	63 ± 1	70 ± 4	1.46	—	—		—	—	

Values = Mean ± S.E. Six animals in each group. * Significant at 5% level.

TABLE V. Tissue Lipids of Rabbits Treated with Pyrrolidinomethyl Tetracycline, 50 mg/Day, for 10 Days.

	Total cholesterol (mg/100 g wet wt)			Phospholipids (as mg lecithin/100 g wet wt)			Total lipid (g/100 g wet wt)		
	Normal control	Treated with antibiotic for 10 days	t	Normal control	Treated with antibiotic for 10 days	t	Normal control	Treated with antibiotic for 10 days	t
Liver	235 ± 10	347 ± 17	5.76*	991 ±105	1361 ±194	1.64	2.63 ±.7	5.50 ±.3	7.33*
Brain	2415 ± 38	2284 ± 84	1.17	4962 ±263	4962 ±242	—	10.10 ±.7	8.43 ±.8	1.58
Kidney	256 ± 11	272 ± 14	.89	367 ± 18	388 ± 34	.54			
Intestine	185 ± 14	175 ± 15	.41	461 ± 45	513 ± 35	.91			
Adrenals	3805 ±352	4538 ±824	.81	2173 ± 85	2533 ± 84	3.02*			
Skin	63 ± 8	63 ± 10	—	71 ± 9	57 ± 13	.88	6.35 ± 1.5	2.24 ±.5	2.54*

Values = Mean ± S.E. Six animals in each group.
* These values of t are significant only at 5% level.

fasting blood sugar did not change, blood sugar levels were higher in all the blood samples collected up to 3 hours after the glucose feeding (Table I). Liver glycogen diminished (Table II). In the serum total cholesterol, phospholipids, triglycerides, free fatty acids, β-lipoprotein cholesterol and β-lipoprotein percentage increased while α-lipoprotein percentage decreased (Table III). Liver cholesterol, adrenal phospholipids and total lipids of liver increased. Skin total lipids decreased (Table V). *Monkeys:* Blood sugar levels one-half and one hour after glucose load were higher, without any change in the fasting blood samples and samples collected 1½ and 2 hours after glucose (Table I). Total cholesterol, phospholipids and free fatty acids of serum increased (Table III).

Discussion. Serum cholesterol increased in rabbits and monkeys after treatment with pyrrolidinomethyl tetracycline. Serum cholesterol is mainly derived from liver cholesterol(19) which increased after treatment with the antibiotic. It is probable that the hypercholesteremia might be a combined effect of increased synthesis of cholesterol in the liver, its increased absorption from the intestine and/or diminished catabolism in the tissues. An increase in plasma β-lipoprotein cholesterol and β-lipoprotein percentage usually observed in atherosclerosis were also observed in animals treated with the antibiotic. This antibiotic, therefore, might have atherogenic properties like other tetracyclines(20, 21) after prolonged use.

The increased phospholipids of serum after treatment with antibiotic might be due to increased synthesis in the liver and its transport through the blood stream to the tissues such as brain, kidney and adrenals where the concentration of phospholipids increased. Plasma phospholipids are mostly transported in combination with α-lipoprotein which was relatively diminished after administration of the

antibiotic. It seems, therefore, that phospholipids were present in forms other than in combination with α-lipoprotein.

Antilipotropic(22) and lipogenic(23) properties of chlortetracycline have been reported. Chlortetracycline inhibits lipolysis *in vitro* (24). Tetracyclines inhibit lipolysis by tissue lipoprotein lipase(25). Both oxytetracycline and chlortetracycline inhibit fatty acid oxidation in the rat liver mitochondria(26). Marked increase in the serum triglycerides and total lipids of liver after treatment with pyrrolidinomethyl tetracycline seems to be due to impaired lipid oxidation, lipogenesis, antilipotropic property of the antibiotic or inhibition of lipolysis or a combination of the above processes. Serum triglycerides are mainly carried with the β-globulins of plasma. In the antibiotic-treated rabbits plasma β-lipoprotein possibly contained large portions of the triglycerides, accounting for the apparent increase in the β-lipoprotein fraction.

Diminished glucose tolerance with decreased liver glycogen in the antibiotic-treated animals might be due to dysfunction of the liver as a result of increased deposition of lipids and faulty utilization of glucose in the tissues. The increase in the free fatty acids of plasma in the antibiotic-treated animals also indicates diminished carbohydrate utilization(27). Hampered carbohydrate utilization induced increased release of triglycerides representing an auxiliary mechanism for transporting fatty acids for caloric demands (28).

Both chlortetracycline and oxytetracycline uncouple oxidative phosphorylation, but in different degrees(26). These two antibiotics are comparable in their metabolic effects, antibacterial potency and chemical structure (3,4,29). Changes in the carbohydrate and lipid metabolism brought about by pyrrolidinomethyl tetracycline are, therefore, likely to be due to the tetracycline structure. Tetracycline, therefore, should be used with caution as the therapeutic effects might disturb the normal metabolic patterns in the body.

Summary. Pyrrolidinomethyl tetracycline was administered to rats, rabbits, and monkeys for 10 days and changes in the utilization of glucose and distribution of lipids in the tissues were studied. Treated animals showed diminished glucose tolerance. They had decreased glycogen and increased cholesterol and total lipids in the liver. There was a rise in plasma levels of lipids such as cholesterol, phospholipids, triglycerides and free fatty acids. Changes indicated impaired metabolism of carbohydrate and lipids. Tetracycline moiety of the antibiotic seemed responsible for the changes observed. The drug should be used with caution as its therapeutic effect might disturb the normal metabolic patterns in the body.

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Effects of Fluoride Intake on Disuse Atrophy of Bone in Rats. (31218)

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Disuse atrophy of bone is characterized by a reduction of the mass of bone tissue accompanied by hypercalcemia and increased excretion of calcium in the urine(1).

The influence of nutrition and various hormones on degree of bone atrophy has been studied in rats(2,3). It was found that increasing the calcium content of the diet resulted in an increase in the ash content of the humerus from a paralyzed extremity, whereas an increase of the phosphorus content of the diet had no demonstrable effect. Administration of estradiol propionate reduced or prevented bony atrophy, whereas testosterone propionate increased it(3).

It has been reported that negative calcium balances which occur in human skeletal diseases such as osteoporosis and Paget's disease (4), or in hypervitaminosis D in rats(5) can be improved by administration of fluoride. The purpose of this investigation was to study the effect of fluoride administration on atrophy of bones in rats.

Materials and methods. Forty rats of the Hebrew University strain Sabra weighing 150-170 g were used in this experiment. Rats of this size were expected to be able to withstand a nerve severance operation (4 rats died from hemorrhage during the operation). In addition the bones of these animals were still in an actively growing metabolic state and able to incorporate fluoride rapidly(6). The rats were maintained on a standard mixed diet containing 3.2 ppm F and were provided with drinking water containing 0.55 ppm F until the beginning of the experiment. This diet is considered to be low in fluoride for rats(6).

The rats were divided into 2 main groups:

Group 1—15 rats received distilled water as drinking water.

Group 2—21 rats received distilled water containing 25 ppm F as drinking water.

In 6 rats from Group 1 and 9 rats from Group 2, the right sciatic and femoral nerves were severed to permit the development of disuse atrophy. Using aseptic technique, the femoral nerve was severed in the area of the femoral triangle. The sciatic nerve was severed in the area below the gluteal region. The nerve ends were separated from each other. Operated and non-operated rats were fed *ad libitum* and killed with ether 2 months after starting the experiment. Before sacrificing, blood samples were taken from each rat for calcium analysis. The left and right femora were dissected out. X-ray radiograms of the femora were made to detect bone rarefication resulting from paralysis. After defatting and drying both femora, their specific gravity,

* Founded by the Alpha-Omega Fraternity.