TABLE III. Mixed Agglutination Test with Lung Cell Cultures and Transplantation Sera Obtained After Second Skin Homograft.

		Cell cultures					
		2192	1829	2128	2140	1831	2136
Sera of exchange-graft partners	2192 anti-2136	<50	1,250			1,250	6,000
	1829 anti-1831		<50	250		30,000	
	2128 anti-2140	250		<50	30,000		
	2140 anti-2128			150,000	<50		
	1831 anti-1829		30,000	1,250		<50	
	2136 anti-2192	30,000	6,000				<50
Sera of other recipients	2159	6,000	1,250	1,250		1,250	6,000
	2182	<50	1,250	<50		6,000	
	2184		6,000	250		30,000	
	2178	250					<50

sumption test, it was surprising to find a considerable difference. In general, positive mixed agglutination reactions did not occur until 4 to 5 weeks following the first skin graft. These reactions were weak but once present persisted throughout the study period. In contrast, positive results by antiglobulin consumption were almost always obtained 14-21 days after the first graft; however, the reaction rapidly weakened and usually disappeared within 6 weeks. No explanation for the different results obtained by these two procedures can be offered.

Summary. Mixed agglutination tests using cell cultures have been applied to the study of antibodies accompanying skin homograft in rabbits. Transplantation sera were incubated with lung or kidney cell cultures. Binding of antibody was demonstrated by an indicator consisting of sheep erythrocytes sensitized by rabbit anti-sheep erythrocyte serum and agglutinated by goat antiserum against rabbit serum. Positive reactions were obtained with sera of all recipients tested. The reactions usually became positive five weeks after the first graft and increased in strength after the second graft. Transplantation sera combined with cell cultures originating from the donor animal and some other unrelated rabbits, but they fail to act upon the recipient's own cell cultures.

1. Fagraeus, A., Espmark, A., Nature, 1961, v190, 370.

2. Högman, C., Vox Sang., 1959, v4, 12.

3. Barron, A. L., Milgrom, F., Karzon, D. T., Witebsky, E., J. Immunol., 1963, v90, 908.

4. Milgrom, F., Kano, K., Barron, A. L., Witebsky, E., ibid., 1964, v92, 8.

5. Abeyounis, C. J., Milgrom, F., Witebsky, E., Nature, 1964, v203, 313.

6. McDonald, J. C., Milgrom, F., Witebsky, E., *J.A.M.A.*, 1964, v190, 122.

7. Barron, A. L., Karzon, D. T., Proc. Soc. Exp. Biol. and Med., 1959, v100, 316.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

Fat Utilization in Rats Fed Cholestyramine, a Bile Acid Sequestrant. (29856)

ROBERT W. HARKINS, CHARLES H. WHITESIDE, HUBERT B. FLUCKIGER AND HERBERT P. SARETT

Department of Nutritional Research, Mead Johnson Research Center, Evansville, Ind.

The anion exchange resin cholestyramine is used clinically in treatment of pruritus due to partial biliary obstruction(1) and for lowering plasma cholesterol levels(2,3). Cholestyramine binds bile acids in the intestines and prevents their reabsorption *via* the enterohepatic cycle(1). Little has been done to determine whether this loss of bile acids interferes with absorption of dietary fat and fat soluble nutrients.

TABLE I. Experiment 1, Effect of Cholestyramine on Weight Gain, Caloric Efficiency and Fecal Fat Loss.

Corn oil	Cholestyramine intake		Caloric intake	Wt gain	Caloric efficiency	Fecal fat		Corrected caloric efficiency*
% of diet	% of diet	g/4 wk	cal/4 wk	g/4 wk	g gain/ 1000 cal	g/4 wk	cal loss	g gain/ 1000 cal
1	0	0	1116	108 ± 14†	97 ± 6†	3.0	27	99 ± 6†
1	2	6.1	1210	116 ± 14	96 ± 5	4.5	41	100 ± 4
10	0	0	1253	118 ± 19	94 ± 8	6.3	57	99 ± 8
10	2	5.7	1190	109 ± 16	92 ± 6	9.4	85	99 ± 7
20	0	0	1215	114 ± 18	94 ± 11	5.4	49	98 ± 12
20	2	5.5	1285	116 ± 13	90 ± 7	13.2	119	100 ± 8

* Corrected for loss of calories in feces as fat. † Standard deviations.

Hashim *et al*(4) found no increase in fecal fat in subjects receiving 15 g of cholestyramine per day, a dose which decreases plasma cholesterol levels, although the daily ingestion of 30 g produced frank steatorrhea. Hyun *et al*(5) observed that cholestyramine decreased absorption of cholesterol from the intestines and decreased lymph cholesterol. Kelley *et al*(6) reported that absorption of vit K_1 in dogs was not affected by cholestyramine when administered at dose levels (g/kg) corresponding to that used in man; with a dose level 10 times that used in man, cholestyramine significantly interfered with utilization of vit K_1.

Reported here are the effects of dietary cholestyramine on the absorption of fat fed at different levels in the diet to young, rapidly growing rats and to mature rats.

Methods. Male rats of the McCollum-Wisconsin strain were selected and housed in individual screen-bottom cages in an air conditioned animal room. Diets were supplied to all animals *ad libitum*; intakes of food and water, and weight gains were recorded. Stools were collected from all animals and pooled as group samples for fecal fat analyses(7) and fatty acid content(8).

In the first experiment, 6 groups of 10 male weanling rats each were fed synthetic diets containing 1, 10 or 20% corn oil, with 0 or 2% cholestyramine for 4 weeks. The diets contained the following materials per 100 g diet: casein 18 g, Amidex* 72.65-53.65 g, corn oil 1-20 g, non-nutritive fiber 4 g, vitamins 0.35 g(9), and minerals 4 g(10).

In the second experiment, 6 groups of young adult male rats which had been on Purina laboratory chow were given diets containing 5 or 15% hydrogenated vegetable oil or were continued on the chow diet which contained 5% fat. The synthetic diets contained the following ingredients per 100 g diet: casein 20 g, sucrose 66.65-56.65 g, Primex† 5-15 g, non-nutritive fiber 4 g, vitamins 0.35 g(9), and minerals 4 g(10). This experiment was divided into 3 feeding periods, each of 2 weeks duration. During these 3 successive periods, half of the animals on each diet received 0, 1 and 2% cholestyramine in the diet; the other half received 2, 1 and 0% cholestyramine.

Results. Experiment 1. Shown in Table I are the effects of inclusion of 2% dietary cholestyramine on the 4-week weight gains and caloric efficiency values. Weight gains of the weanling rat were not markedly affected by cholestyramine. Caloric efficiency values were slightly, but consistently, lower in the groups receiving cholestyramine than in the control groups, and averaged 1, 2 and 4 g less gain per 1000 calories on the 1, 10 and 20% corn oil diets, respectively. Caloric efficiencies were also calculated after correcting the caloric intakes for the loss of calories in the feces as fat. The corrected caloric efficiency values of the cholestyramine-treated groups were equal to those in the corresponding control groups.

The addition of cholestyramine increased fecal fat excretion on each of the diets. The

* Amidex—modified corn starch—Corn Products Co.

† Primex—Procter and Gamble.

TABLE II. Experiment 1, Fatty Acid Distribution in Fecal Fat of Weanling Rats Receiving Diets with and without Cholestyramine.

Corn oil, % of diet	1		10		20		
Cholestyramine, % of diet	0	2	0	2	0	2	Corn oil
Fatty acid	Fatty acid, %						
14:0	.8	.6	.5	.2	.3	.2	.1
16:0	27.4	31.4	26.8	26.7	28.5	22.9	9.6
16:1	1.5	1.5	.8	.8	0	0	.2
18:0	18.9	24.8	19.4	20.2	26.7	13.0	2.3
18:1	19.5	22.1	27.6	31.2	26.7	37.2	28.1
18:2	3.3	3.0	4.6	3.8	3.8	7.2	57.5
18:3	3.5	2.2	3.2	2.3	2.7	2.6	1.0
20:1	6.3	5.3	1.9	1.7	1.4	1.9	
20:3	.8	.3	.1	0	.2	0	
20:4	2.6	1.3	.8	.6	1.3	.7	.8
22:2	.6	.3	.3	.1	.4	.5	
22:4	0	2.6	1.7	.9	2.0	1.2	
24:0	4.9	0	1.8	1.5	1.0	1.0	
Others	9.9	4.6	7.5	10.0	5.0	11.6	

TABLE III. Experiment 2, Effect of Cholestyramine on Fat Retention in Adult Rats.

Group No. diet treatment	Avg fat intake	Fat retention, %					
		Period A		Period B		Period C	
		Week 1	Week 2	Week 1	Week 2	Week 1	Week 2
	(mg/day)	0% cholestyramine		1% cholestyramine		2% cholestyramine	
1. 5% Primex	869	97	97	94	94	86	92
2. 15% "	2110	95	97	98	96	92	96
3. Purina chow	917	89	87	88	86	86	88
		2% cholestyramine		1% cholestyramine		0% cholestyramine	
4. 5% Primex	807	86	92	93	94	96	96
5. 15% "	2379	81	93	96	96	96	97
6. Purina chow	917	84	84	87	84	89	94

higher the dietary fat level, the greater was the increase in fecal fat loss found with cholestyramine. Distribution of the fatty acids in the fecal fat is shown in Table II. The fecal fatty acid patterns of the groups receiving 1% corn oil were nearly identical and similar to those seen in animals receiving a fat-free diet. Addition of cholestyramine to the diets with 10 or 20% corn oil resulted in an increase in oleic acid content of the fecal fat but little change in linoleic acid content.

Experiment 2. In the first 2-week period, A, the diets of Groups 1 to 3 contained no cholestyramine while those of Groups 4 to 6 contained 2% cholestyramine (Table III). During the first week, fecal fat losses due to cholestyramine were greater in the animals switched to the synthetic diets than in those continued on the chow diet. In the second week cholestyramine had only a small effect on fecal fat loss on all diets.

In Groups 4 to 6, fat retention was successively increased in periods B and C as the level of cholestyramine in the diet was decreased to 1 and 0%, respectively. Conversely, fat retention by Groups 1 to 3 was decreased as the level of cholestyramine was raised to 1% and then to 2% of the diet during periods B and C, respectively.

In period C, patterns of dietary treatment were the reverse of those of period A. The differences in fat excretion due to cholestyramine were similar but smaller in magnitude in period C than in period A. Weight gains and food intakes of the adult rats were not affected by cholestyramine administration and are not shown.

Discussion. Cholestyramine, a bile acid sequestrant, significantly increased fecal fat excretion in the young weanling rat. The fecal fat loss was marked only with a high level of fat in the diet. The fecal fat loss in the group receiving the 20% corn oil diet

with 2% cholestyramine was 13.2 g in 4 weeks compared with a loss of 5.4 g in the control group. Only in this group were fatty acids of dietary origin found in the feces in increased amounts during cholestyramine administration.

In the mature rats, 2% cholestyramine decreased fat retention by about 5% in animals on the chow diet. In animals switched to synthetic diets, the loss of fat during the first week on cholestyramine was quite marked, but there was little effect during the second week. These results may have been due in part to adjustment to the synthetic diets, since little effect of cholestyramine on fecal fat loss was found in those groups fed the synthetic diets for 2 weeks before cholestyramine was added to the diets.

The incorporation of cholestyramine as 2% of the diet provided 2 and 1 g of cholestyramine per kg of body weight per day in the young and mature rats, respectively. In man, the daily dose of 12 to 15 g of cholestyramine (used in lowering plasma cholesterol) provides about 0.2 g of cholestyramine per kg per day. Thus, fat utilization is decreased by cholestyramine both in the young rat and in man(4) when given at high dose levels and especially with high levels of dietary fat.

Summary. In the young weanling rat, 2% cholestyramine in the diet increased the loss of fecal fat, particularly in diets of high fat content. Administration of cholestyramine to the mature rat resulted in a moderate decrease in fat retention, but after one week the effect was minimal.

1. Carey, J. B., Jr., J. Clin. Invest., 1961, v40, 1028.
2. Bergen, S. S., Jr., Van Itallie, T. B., Tennent, D. M., Sebrell, W. H., Circulation, 1959, v20, 981.
3. Tennent, D. M., Siegel, H., Zanetti, M. E., Kuron, G. W., Ott, W. H., Wolf, F. J., J. Lipid Res., 1960, v1, 469.
4. Hashim, S. A., Bergen, S. S., Jr., Van Itallie, T. B., Proc. Soc. Exp. Biol. and Med., 1961, v106, 173.
5. Hyun, S. A., Vahouny, G. V., Treadwell, C. R., ibid., 1963, v112, 496.
6. Kelley, K. L., Lehman, E. G., Robinson, H. J., Fed. Proc., 1963, v22, 434, abstr.
7. Assn. of Official Agricultural Chemists Official Methods of Analysis, edit. 9, Washington, D. C., 1960, p161.
8. Smith, L. M., J. Dairy Sci., 1961, v44, 607.
9. Saret, H. P., Snipper, L. P., J. Nutrition, 1954, v52, 525.
10. Jones, J. H., Foster, C., ibid., 1942, v24, 245.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

Effect of Aldosterone on Juxtaglomerular Index and Zona Glomerulosa of Normotensive and Hypertensive Rats.* (29857)

EDWIN R. FISHER AND M. TAMURA
Departments of Pathology, University of Pittsburgh and VA Hospital, Pittsburgh, Pa.

Evidence has been accumulated which indicates that renal hypertension results from increased activity of the renin-angiotensin-aldosterone mechanism. The precise mechanism of action of angiotensin II and aldosterone in this regard is less certain and in some instances increased secretion of both substances has not been accompanied by hypertension(1,2). Feedback control of this system is considered to be mediated by the depressor effect of aldosterone's sodium retaining properties on the juxtaglomerular apparatus of the kidney, the site of renin formation(3). However, rabbits pretreated with aldosterone exhibit a lesser pressor response to angiotensin infusions than controls(4). Although angiotensin has been noted to cause an increase in renal renin in rats, this effect is prevented when aldosterone is also administered(5). This has suggested that the latter may have a direct blocking effect on angiotensin(4). In an attempt further to explore this possibility, it was considered worthwhile

* Supported by USPHS Grant C-5195.