Effects of high sucrose diets and 4-aminopyrazolopyrimidine on serum lipids and lipoproteins in the rat

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ABSTRACT The effect of feeding a semipurified diet high in sucrose on serum lipid and lipoprotein concentrations was studied. In rats fed this diet the serum triglyceride concentration doubled, and liver triglyceride concentration increased by 30%. A fivefold increase in VLDL protein concentration and a small but significant increase of HDL protein concentration was also observed. In these rats there was increased incorporation of labeled amino acids into the proteins of plasma VLDL and HDL.

Fatty livers developed in the animals receiving 4-aminopyrazolopyrimidine, and levels of serum triglyceride and cholesterol fell markedly. The concentration of all lipoprotein classes decreased, with VLDL showing the most marked effect. Incorporation of labeled amino acids into lipoproteins and other plasma proteins was depressed.

SUPPLEMENTARY KEY WORDS fatty liver · serum triglycerides · serum cholesterol · adenine · hyperlipemia · synthesis of serum lipoproteins

In 1956, Scholler, Philips, and Sternberg (1) showed that the administration of the adenine analog 4-aminopyrazolopyrimidine (4-APP) to rats and dogs produces fatty livers. Subsequently, Henderson (2) showed that the administration of 4-APP to mice resulted in a marked increase in the neutral lipid content of the liver and a decrease in the concentrations of plasma triglyceride and plasma cholesterol. He measured the incorporation of labeled amino acids into the plasma and liver proteins and found it to be unaltered by prior administration of 4-APP. He therefore concluded that the decreased transport of triglyceride from the liver which occurs after administration of 4-APP was probably not due to inhibition of protein synthesis. The synthesis of the plasma lipoproteins was not measured in this study. We have previously studied the mechanism of production of fatty livers caused by administration of orotic acid to rats (3). In those studies we found that synthesis of total plasma and liver proteins was not inhibited by administration of orotic acid, but that the formation of lipoproteins, especially the very low density lipoproteins, was markedly decreased. It seemed possible that an adenine analog might exert its effect by a mechanism similar to that of orotic acid. Therefore, we have undertaken to study the synthesis of lipoproteins in 4-APP-treated animals.

Our studies of orotic acid-induced fatty livers confirmed earlier studies which showed (4) that a fatty liver could be produced only in rats fed a semipurified diet. Accordingly, we studied the effect of 4-APP in rats fed commercial pellets and a semipurified diet. Since the semipurified diet per se affected serum and liver lipids, it was necessary to compare first the levels of lipoproteins and their rates of synthesis in untreated rats fed commercial pellets or a semipurified diet.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Holtzman Laboratories, Madison, Wis.) weighing 350–400 g were used. The control diet consisted of Rockland Farms mouse pellets (Teklad, Inc., Monmouth, Ill.); the semipurified diet was obtained from General Biochemicals, Chagrin Falls, Ohio, and contained 21% vitamin-free casein, 60% sucrose, 4% corn oil, 1% vitamin mixture (Wooley and Sebrell) (5), 4% salt mixture (USP XIV), and 10%

Abbreviations: 4-APP, 4-aminopyrazolopyrimidine; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

cellulose. Rats received this diet for 8–14 days prior to administration of 4-APP. Unless otherwise indicated, the rats were given food and water ad lib. throughout the experiment. In order to minimize diurnal variation, all experiments were done at the same time of day.

4-APP was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis., and from the Cancer Chemotherapy National Service Center, National Cancer Institute. Adenine sulfate was obtained from Mann Research Laboratories Inc., New York. L-Lysine-U-^{14}C and L-leucine-4,5-^{3}H were obtained from New England Nuclear Corp., Boston, Mass.

Tissue and serum lipids were extracted by the method of Folch, Lees, and Sloane Stanley (6). Triglycerides were measured according to Van Handel's modification (7) of the method of Van Handel and Zilversmit (8). Cholesterol was determined by the method of Abell, Levy, Brodie, and Kendall (9) or by the method of Zak et al. (10). This latter method gave slightly higher values. Free fatty acids were determined by the method of Dole (11) as modified by Trout, Estes, and Friedberg (12).

4-APP and adenine sulfate were administered by intraperitoneal injection. The labeled amino acids were administered intravenously into the tail vein. Blood samples were obtained by cardiac puncture of ether-anesthetized rats.

The lipoproteins were separated by the method of Havel, Eder, and Bragdon (13) into VLDL (d < 1.006), LDL (d 1.006–1.063), and HDL (d 1.063–1.21). The VLDL fraction also included chylomicrons, since the animals were not fasted. Ultracentrifugation was performed in the Spinco L ultracentrifuge using the 40.3 rotor. For separation of VLDL and LDL, centrifugation was carried out for 20 hr; for HDL the period of centrifugation was 40 hr. The supernatant lipoproteins present in the upper third of the tube were separated using a Spinco tube cutter, and the lipoprotein fractions were washed by resuspending them in a salt solution of appropriate density and repeating the centrifugation. Proteins were prepared for counting and chemical analysis as described by Roheim, Miller, and Eder (14). Aliquots were taken for protein determination, which was by the method of Lowry et al. (15); for the determination of radioactivity, aliquots were suspended in a Cab-O-Sil mixture (Cabot Co., Boston, Mass.) for counting in a Tri-Carb liquid scintillation counter (16). 35 g of Cab-O-Sil was suspended in 380 ml of 100% ethanol and 600 ml of redistilled toluene which contained 0.5% PPO and 0.05% dimethyl POPOP. The incorporation of labeled amino acids into lipoproteins was measured at two time intervals in the same animal. Leucine-^{3}H was administered and 2 hr later lysine-^{14}C was injected; the animals were killed 1 hr later. This technique permitted measurement of radioactivity in the lipoprotein fractions at 60 and 180 min after the injection of the precursor. These time intervals were chosen because the maximum specific activity of VLDL is attained between 30 and 60 min after administration of the precursor, whereas the maximum specific activity for LDL and HDL is attained between 60 and 180 min.[1]

RESULTS

Effects of Semipurified Diet on Serum Lipids and Lipoproteins

Animals of similar age and weight were randomly divided between control and semipurified diet groups at the beginning of feeding periods. At the end of the feeding period (8–14 days) the animals were weighed again. No difference was found between the weights of the animals on semipurified diet and those on Purina Chow, suggesting that there was no appreciable difference in the food intake or in the growth of the two groups of animals.

The feeding of the semipurified diet resulted in a doubling of the serum triglyceride concentrations, an increase in the serum cholesterol, and a slight decrease in serum free fatty acid concentrations (Table 1); it also caused a significant increase in liver triglyceride concentrations. When different serum lipoproteins were analyzed, it was found that in the rats fed the semipurified diet the concentration of VLDL protein increased 5-fold, that of the LDL protein was unchanged, and the HDL protein concentration was increased (Table 2).

After administration of ^{14}C-labeled lysine and ^{3}H-labeled leucine, their incorporation into lipoproteins was measured at both 60 and 180 min (Table 3). The total incorporation of labeled amino acids into the VLDL and HDL proteins was increased significantly at both 60 and 180 min in the rats fed the semipurified diets. At the same time, specific activity of the VLDL in the rats on the semipurified diet decreased. The specific activity of the HDL fraction was increased slightly at 60 min and appreciably at 180 min. For LDL, both total incorporation and specific activity were unchanged in animals fed the semipurified diet.

The effect of orotic acid on serum and liver lipids cannot be observed in animals fed semipurified diets when adenine is added (4). Since the semipurified diet does not contain adenine, we wanted to determine whether the lack of purines or purine ribosides could be responsible for the observed hyperlipemia after feeding the semipurified diet. Therefore, to the semipurified diet, 0.25% adenine sulfate or 0.1% inosine was added. Serum triglycerides and cholesterol of animals fed these diets for 2 wk were compared with the sera of animals maintained on

[1] Roheim, P. S. Unpublished observations.

TABLE 1 EFFECT OF DIET ON SERUM AND LIVER LIPIDS

Diet	Serum			Liver
	Triglyceride	Cholesterol*	Free Fatty Acids†	Triglyceride
	mg/100 ml		*mmoles/liter*	*mg/100 g (wet wt)*
Mouse pellets (9)‡	48 ± 2.5§	74 ± 5.8	324 ± 28	612 ± 33
Semipurified diet (12)	119 ± 6	97 ± 8	204 ± 23	804 ± 40
	$P < 0.001$	$P < 0.05$	$P < 0.005$	$P < 0.005$

Male rats weighing 350–400 g were maintained on the diet for 10 days and fed ad lib. up to the time of bleeding.

* Determined by method of Abell, Levy, Brodie, and Kendall (9).

† Mean of six animals in each dietary group.

‡ Number of animals in group.

§ Values are means ± SE.

TABLE 2 EFFECT OF DIET ON SERUM LIPOPROTEINS AND PROTEIN CONCENTRATIONS

Diet	VLDL	LDL	HDL	d > 1.21
		mg protein/100 ml		
Mouse pellets (7)*	0.75	7.20 ± 0.60	43.65 ± 3.21	5571 ± 316
Semipurified diet (13)	4.50 ± 0.87	6.37 ± 0.83	55.04 ± 2.27	5759 ± 220
	$P < 0.001$	NS†	$P < 0.01$	NS

Male rats weighing 350–400 g were maintained on diet for 10 days and fed ad lib. up to the time of bleeding. Lipoproteins and proteins were separated by ultracentrifugation. Each value is a mean (± SE) of separate protein determinations on each fraction in each animal.

* Number of animals in group.

† NS, not significant.

TABLE 3 EFFECT OF DIET ON AMINO ACID INCORPORATION INTO PROTEINS

Diet	VLDL		LDL		HDL		d > 1.21		Liver	
	Specific Activity	Total Counts	Specific Activity	Total Counts	Specific Activity	Total Counts	Specific Activity	Total Counts	Specific Activity	Total dpm/Liver
	dpm/mg	*dpm/ml*	*dpm/mg*	*dpm/ml*	*dpm/mg*	*dpm/ml*	*dpm/mg*	*dpm/ml*	*dpm/mg*	
Incorporation of L-lysine-^{14}C (60 min after injection)										
Mouse pellets (7)*	5,615 ± 481†	44 ± 9	3,225 ± 158	232 ± 20	1,225 ± 107	524 ± 36	453 ± 39	25,654 ± 3,337	468 ± 27	728,000 ± 43,000
Purified diet (13)	2,636 ± 124	118 ± 13	2,724 ± 227	174 ± 17	1,465 ± 67	806 ± 48	507 ± 27	29,046 ± 987	501 ± 22	
Incorporation of leucine-4,5-^{3}H (180 min after injection)										
Mouse pellets (7)	40,380 ± 2,931	288 ± 47	39,096 ± 5,075	2,794 ± 164	16,186 ± 1,429	6,902 ± 451	6,903 ± 363	380,000 ± 30,000	3,591 ± 185	5,590,000 ± 330,000
Purified diet (13)	22,635 ± 840	1,018 ± 119	42,597 ± 2,045	2,713 ± 120	23,289 ± 519	12,818 ± 515	7,680 ± 306	439,987 ± 14,689	4,200 ± 120	

Treatment of animals is the same as given in Table 2. 180 min prior to sacrifice each rat received an intravenous injection of 70 μCi of leucine-4,5-^{3}H, and 60 min prior to sacrifice the same animal received 5 μCi of L-lysine-U-^{14}C.

* Number of animals in group.

† Values are means ± SE.

semipurified diets and with that of animals fed mouse pellets. It can be seen (Table 4) that animals fed adenine or inosine had serum lipid values similar to those of the animals fed the semipurified diet. These values are very similar to the values presented in Table 1.

Effects of the Administration of 4-APP on Serum and Liver Lipid Concentrations

18 hr after the administration of 4-APP to rats (Table 5) there was a marked fall in serum triglyceride concentrations in rats fed either the semipurified diet or the pellet diet. Serum cholesterol values decreased in both groups, but to a lesser extent in the rats fed the semipurified diet. Liver triglyceride concentrations increased markedly in both groups. These values should be compared with their corresponding controls in Table 1.

The responses to different amounts of 4-APP are shown in Fig. 1. With doses up to 10 mg/kg, liver triglyceride concentrations increased sharply and then more gradually as the dose was increased to 50 mg/kg. At every dose

TABLE 4 EFFECTS OF ADENINE AND INOSINE ON SERUM LIPID CONCENTRATIONS

Diet	Triglyceride	Cholesterol
	mg/100 ml of serum	
Mouse pellets (6)*	54.9 ± 9.4†	60.5 ± 2.4
Semipurified diet (6)	128.6 ± 14.4	93.2 ± 5.6
	$P < 0.005$‡	$P < 0.001$
Semipurified diet + 0.25% adenine sulfate (6)	161.8 ± 37.2	95.5 ± 2.9
	$P < 0.025$	$P < 0.001$
Semipurified diet + 0.1% Inosine (6)	128.3 ± 19.1	78.3 ± 2.9
	$P < 0.01$	$P < 0.005$

* Number of animals in group.
† Values are means ± SE.
‡ As compared with mouse pellets in all cases.

TABLE 5 EFFECT OF 4-APP ON SERUM AND LIVER LIPIDS

Diet	Serum		Liver
	Triglyceride	Cholesterol*	Triglyceride
	mg/100 ml		*mg/100 g (wet wt)*
Mouse pellets (6)†	7.4 ± 0.75	19.7 ± 1.1	2094 ± 359
Semipurified diet (6)	6.4 ± 1.8	50.5 ± 2.8	2995 ± 210

Each male rat, 350–400 g, received an intraperitoneal injection of 4-APP (50 mg/kg) in 0.8% NaCl. Control rats received injections of saline. Each value is the mean (± SE) of separate measurements done on each rat 18 hr after the injections. Each rat was maintained on the diet for 10 days and fed ad lib. up to the time of bleeding.

* Determined by method of Abell, Levy, Brodie, and Kendall (9).

† Number of animals in group.

the concentrations of triglyceride in the livers of rats fed the semipurified diet were higher. Serum triglyceride and cholesterol decreased markedly (Fig. 2) at a dose of 5 mg/kg of APP and fell only slightly when larger amounts were administered. In the rats fed the semipurified diet the concentrations of serum cholesterol again did not decrease to the same extent as in rats maintained on the pellet diet.

The time course of the response to APP administration in rats fed the semipurified diet is shown in Fig. 3. Serum triglyceride concentrations decreased markedly within 90 min after 4-APP administration; the liver triglyceride concentrations had not increased by 6 hr but were markedly increased at 18 hr.

Effects of 4-APP on Serum Lipoprotein Concentrations and Synthesis

The administration of 4-APP (Table 6) resulted in the disappearance of VLDL from the plasma of rats irrespective of which diet was fed. The LDL virtually disappeared in the rats fed the pellet diet; in the rats fed the semipurified diet it decreased by 50%. The concentration of HDL also decreased greatly. (See Table 2 for comparisons.)

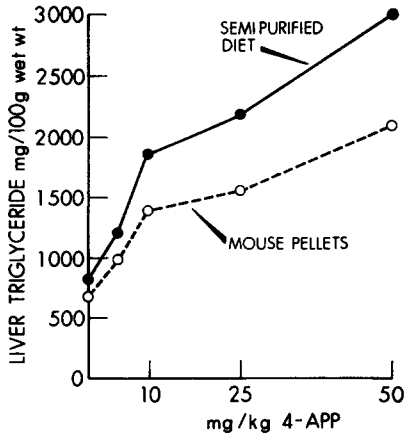

FIG. 1. Effect of different doses of 4-APP on liver triglyceride concentration. 18 hr prior to being killed each rat received intraperitoneally 2 ml of 0.85% NaCl solution containing 4-APP. Control rats received only the salt solution. Each point is derived from measurements on three rats.

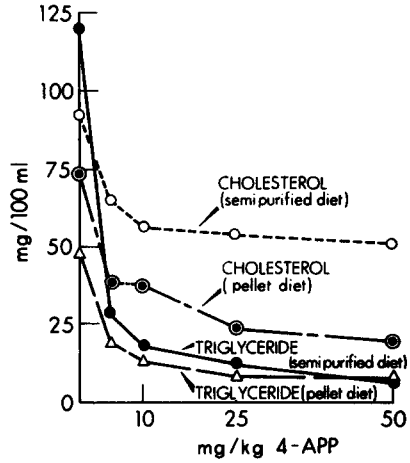

FIG. 2. Effect of different doses of 4-APP on serum triglyceride and cholesterol concentration. The treatment of the animals is the same as given in Fig. 1.

The incorporation of ^{14}C-labeled lysine and ^{3}H-labeled leucine into lipoproteins was measured in rats treated with 4-APP and maintained on either the semipurified or pellet diet (Table 7). These values should be compared with the data in Table 3. In no instance was there radioactivity found in VLDL, nor was there incorporation of radioactivity into the LDL of rats fed the control diet. However, in rats fed the semipurified diet, administration of 4-APP had little effect on the incorporation of radioactivity into LDL. Since the concentration of LDL decreased in these rats, the specific activities were markedly increased. The total incorporation of radioactivity into HDL decreased markedly in both groups. The total incorporation and specific activities of the remaining plasma proteins (d > 1.21) decreased considerably in the 4-APP-treated rats when measured 180 min

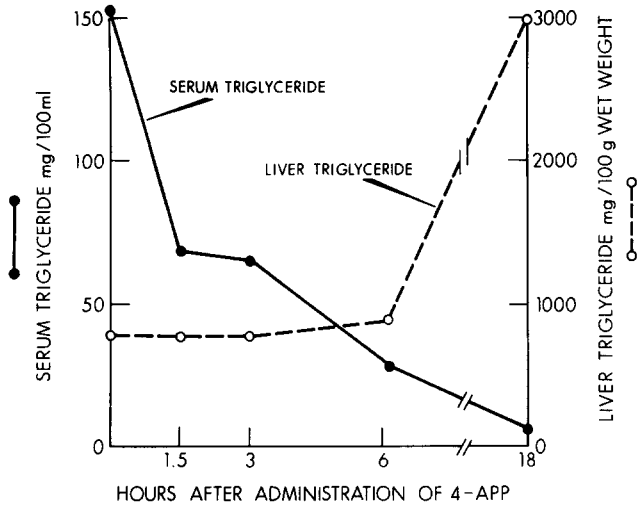

FIG. 3. Serum and liver triglycerides at various times after administration of 4-APP. 50 mg/kg of 4-APP was administered intraperitoneally to each rat. Three rats were killed at each time interval. Each point is the mean of separate determinations on each rat.

after the administration of ^{3}H-labeled leucine. However, the incorporation of label into the mixed liver proteins was not altered in most cases by administration of 4-APP.

It has been shown that the administration of adenine can prevent the formation of fatty livers by a number of agents such as ethionine (17) and orotic acid (4, 18, 19). We have, therefore, studied the effects of the simultaneous administration of adenine and 4-APP (Table 8). In rats maintained either on pellets or on the semipurified diet, the administration of adenine with 4-APP prevented the rise in the triglyceride content of the livers. With both diets it partially prevented the decrease in serum triglyceride concentrations and prevented the decrease in serum cholesterol concentrations. The administration of adenine alone to rats on the semipurified diet had no significant effects on serum or liver lipids.

TABLE 6 EFFECT OF 4-APP ON SERUM LIPOPROTEIN AND SERUM PROTEIN CONCENTRATION

Diet	VLDL	LDL	HDL	d > 1.21
		mg/100 ml		
Mouse pellets (7)*	0	0.10 ± 1.52	5.68 ± 2.78	4925
Semipurified (9)	0	3.29 ± 0.40	13.15 ± 2.11	5821 ± 351

Male rats, 350–400 g, were maintained on diet for 10 days and fed ad lib. up to the time of bleeding. Each received an intraperitoneal injection of 4-APP (50 mg/kg) 18 hr before being killed; control rats received saline. Lipoproteins and proteins were separated by ultracentrifugation. Each value is a mean (± SE) of separate protein determinations done on each fraction in each animal.
* Number of animals in group.

DISCUSSION

In the studies of Handschumacher et al. (4) it was found that administration of orotic acid to rats produced fatty livers only when the animals were maintained on a semipurified diet. Since 4-APP may operate through changes in adenine metabolism in the liver analogous to those produced by orotic acid, we felt it desirable to carry out studies of the effect of 4-APP on animals fed pellet diets and semipurified diets. Initially we studied the effects of the semipurified diet in rats not treated with 4-APP. The feeding of this diet produced a marked increase in the levels of serum triglyceride and smaller but consistent increases in liver triglyceride concentrations. These changes are comparable to those observed by others (20–24) in rats fed diets high in sugars. In those studies the sugars were administered in the drinking water as a

TABLE 7 EFFECT OF 4-APP ON AMINO ACID INCORPORATION INTO PROTEINS

Diet	VLDL		LDL		HDL		d > 1.21		Liver	
	Specific Activity	Total Counts	Specific Activity	Total Counts	Specific Activity	Total Counts	Specific Activity	Total Counts	Specific Activity	Total dpm/Liver
	dpm/mg	*dpm/ml*	*dpm/mg*	*dpm/ml*	*dpm/mg*	*dpm/ml*	*dpm/mg*	*dpm/ml*	*dpm/mg*	
Incorporation of L-lysine-^{14}C (60 min after injection)										
Mouse pellets (7)*	0	0	0	0	1,869 ± 248†	95 ± 20	324 ± 19	15,805 ± 825	631 ± 49	1,007,000 ± 79,000
Purified diet (9)	0	0	5,995 ± 701	209 ± 49	2,077 ± 311	246 ± 68	522 ± 39	30,075 ± 1,955	1,135 ± 145	
Incorporation of leucine-4,5-^{3}H (180 min after injection)										
Mouse pellets (7)	0	0	0	0	21,612 ± 2,723	1,128 ± 267	3,556 ± 272	175,945 ± 18,360	3,448 ± 325	5,470,000 ± 420,000
Purified diet (9)	0	0	58,460 ± 2,453	2,035 ± 116	14,230 ± 888	1,965 ± 301	4,595 ± 325	264,740 ± 15,076	5,110 ± 766	

Animals were treated as described in Table 6. Labeled amino acids were administered as in Table 3.
* Number of animals in group.
† Values are means ± SE.

TABLE 8 EFFECTS OF ADENINE ON 4-APP-INDUCED LIPID CHANGES

Diet	4-APP	Adenine Sulfate	Serum		Liver
			Triglyceride	Cholesterol	Triglyceride
			mg/100 ml		*mg/100 g*
Mouse pellets	−	−	57 ± 7.3*	90 ± 12	659 ± 48
Mouse pellets	+	−	9 ± 0.6	34 ± 1.5	1607 ± 252
Mouse pellets	+	+	19 ± 1.3	76 ± 9.7	604 ± 12
Semipurified diet	−	−	100 ± 7.2	118 ± 7.5	845 ± 119
Semipurified diet	−	+	85 ± 7.1	130 ± 11.6	885 ± 84
Semipurified diet	+	−	11 ± 1.4	70 ± 7.8	1545 ± 125
Semipurified diet	+	+	32 ± 1.8	120 ± 9.6	698 ± 60

Adenine sulfate (75 mg/kg) was given intraperitoneally simultaneously with 4-APP (25 mg/kg) 18 hr prior to killing the rats. Each group consisted of six rats. Cholesterol was determined by the method of Zak et al. (10).

* Values are means ± SE.

supplement to pellet diets. In the studies of Zakim et al. (23) diets high in glucose or fructose were given to rats that had been starved for 48 hr and then refed. Our studies differ from those studies in that the sucrose was incorporated into the diet. This has the advantage that the sucrose constitutes a fixed proportion of the diet.

The possibility that the semipurified diet alters serum and liver lipids because it is deficient in purines or purine ribosides is excluded by data in Table 4, in which it is shown that addition of adenine sulfate or inosine to the semipurified diet does not result in significant alteration in serum lipid concentrations.

In our studies the lipoproteins were separated by preparative ultracentrifugation and the protein content of each lipoprotein fraction was measured. We have shown[1] that in the normal rat VLDL is separated by flotation in the ultracentrifuge at d < 1.006. The LDL first appears at d 1.030 in the top fraction after ultracentrifugation. However, the bulk of the LDL is separated by flotation between d 1.040 and 1.055. Most of the HDL appears above d 1.070. For measurement of LDL it is necessary to use densities higher than 1.040 despite the possible minimal contamination with HDL. We found a marked increase in the concentration of VLDL, which is consistent with the increase in pre-β-lipoprotein observed by Eaton and Kipnis (25). Contrary to their finding of decreased α-lipoprotein by paper electrophoresis, we observed an increase in HDL protein. It should be noted that the amount of protein found in the combined VLDL–LDL fraction obtained by heparin–Mn^{2+} precipitation by these authors is markedly different from that observed in this present study and in previous studies by Havel et al. (13).

The mechanism of the hyperlipoproteinemia induced by feeding the semipurified diet was studied by measuring the incorporation of labeled amino acids into various lipoproteins. In the HDL we found higher specific activity and total incorporation, clearly suggesting increased synthesis of HDL. In the rats fed a semipurified diet the VLDL specific activity decreased by 50% while the total incorporation increased fourfold, suggesting increased synthesis of VLDL. However, these data do not exclude the possibility that the high sucrose diet also decreased the rate of turnover of VLDL. Therefore, a decreased rate of VLDL turnover could be an additional factor responsible for the increased VLDL concentration after feeding a semipurified diet.

While the cause of this increased synthesis of VLDL is not known, the following sequence of events might occur. The addition of fructose and glucose to the diet results in increased synthesis of fatty acids (22, 23) and also increased triglyceride synthesis due to the increased activity of L-α-glycerophosphate acyltransferase (26). These changes might be expected to provide increased levels of liver and serum triglycerides, and these were consistently found by us. We believe that the increase in liver triglyceride concentration is the stimulus for increased synthesis of VLDL, leading to increased transport of lipoproteins into the serum. It is likely that a new steady state is reached between triglyceride synthesis, the level of liver triglyceride, and VLDL formation. The cause of the increased synthesis of HDL is less apparent. The finding that HDL and VLDL have certain peptides in common (27, 28) suggests that there may be a relationship between the increased rates of formation of VLDL and HDL.

In the interpretation of the data, the possibility of a change in the liver precursor pool of free leucine and lysine should be considered. However, it seems unlikely that this factor is responsible for the changes found, since differences in the incorporation of these amino acids were observed in some but not in all of the proteins measured. There was no marked difference between incorporation into liver proteins and d > 1.21 plasma proteins.

The effects of 4-APP on rat serum lipids were similar to those observed by Henderson (2) on mice. With a dose of 50 mg/kg in both species there was a considerably

greater decrease in serum triglyceride in rats than in mice. Liver triglycerides were somewhat more elevated in mice receiving 4-APP.

The administration of 4-APP resulted in complete disappearance of VLDL from the sera of rats maintained either on pellets or on the semipurified diet. In both groups there was complete cessation of the synthesis of the protein of the VLDL. The administration of 4-APP also resulted in the lowering of the concentration of HDL and depression of the synthesis of this lipoprotein. Puddu et al. (29), using paper electrophoresis, have also observed a marked decrease in α- and β-lipoproteins in rats treated with 4-APP. The response of LDL to 4-APP administration varied with the diet; in rats fed the pellet diet, the LDL disappeared, while in rats fed the semipurified diet, the LDL concentration decreased by only one-half. The different responses of the two groups of animals to 4-APP might be related to the possible differences in the peptide components of the lipoproteins, and these in turn might be the result of feeding different diets.

Henderson (2) speculated that the changes in serum and liver lipids produced by 4-APP were not the result of altered protein synthesis because he found no reduction in plasma protein synthesis after the administration of 4-APP to mice. This finding is probably explained by our observations that changes in incorporation of labeled amino acid into the $d > 1.21$ proteins are not apparent after 60 min, the time interval used by Henderson, whereas after 180 min both specific activity and total incorporation into plasma proteins are depressed by 4-APP administration. At neither of these times was there a decrease in the synthesis of the total proteins of the liver. These findings can be compared with those of Lombardi and Oler (30), who found that after the administration of ^{14}C-labeled leucine to choline-deficient rats there was decreased radioactivity in plasma proteins and slightly increased radioactivity in the liver. They suggested that this could be the result of decreased transport of labeled plasma proteins from the liver. It should be noted that Robinson and Seakins (31) administered puromycin, which markedly inhibited the synthesis of plasma proteins but caused only a slight decrease in the synthesis of liver proteins. We believe the accumulation of fat in the liver and the decrease in serum lipoprotein concentrations resulting from administration of 4-APP could be due to inhibition of the synthesis of the lipoproteins or to decreased transport, or both. No impairment of incorporation into liver proteins was observed, but there was a decrease in the synthesis of the $d > 1.21$ plasma proteins. It is possible that 4-APP exerts its effect through inhibition of protein synthesis, as found with inhibitors such as puromycin or ethionine (31, 32), or its action could be more similar to choline deficiency (30, 33). The effect of 4-APP differs from the inhibition of lipoprotein formation seen after orotic acid administration in that orotic acid does not inhibit protein synthesis, either in the plasma or in the liver (3).

It is of interest that adenine completely reverses these effects of orotic acid administration on LDL and VLDL (18). When adenine was administered with 4-APP it prevented the accumulation of triglyceride in the liver and the decrease in plasma cholesterol concentration, but it only partially prevented the drop in plasma triglyceride levels produced by 4-APP administration. These differences in the action of adenine are in accord with our hypothesis that 4-APP and orotic acid exert their effects by different mechanisms.

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