# Serum Lipids, Dietary Factors and Ischemic Heart Disease<sup>1</sup>

S. L. MALHOTRA, B.SC., M.B., M.R.C.P., D.C.H., D.P.H., D.I.H., F.C.C.P., F.A.SC.<sup>2</sup>

**M**<sup>UCH</sup> EVIDENCE based on several careful studies indicates that the serum cholesterol levels are lower in countries where the incidence of ischemic heart disease is lower than in those where it is higher; also that serum cholesterol levels are lower in people consuming largely oils containing small amounts of unsaturated fatty acids than those eating largely saturated fats. (For references see review by Begg (1).)

On the other hand, there are several reports contrary to these findings. Walker and Arvidsson (2) found no significant difference between the Bantus on higher fat diet compared with the Bantus on their usual low fat diets. Our own investigations showed that the occurrence rates of acute myocardial infarction were seven times higher in the South Indians as compared with the North Indians, even though the North Indians consumed nine times more fat, most of which was animal fat derived from milk and ghee, with a preponderance of saturated fatty acids (3). The Albany-Afro-Asian team (4) and the Vanderbilt team (5), in fact, found much lower serum lipid values and an immunity to ischemic heart disease in the Samburu tribe in Kenya and the milk- and meateating Masai of Tanganyika than those in corresponding Americans even though the fat content of their diet would seem to

be at least as high and probably higher than that of most Americans. A high degree of exercise or freedom from competitive stress among the Samburu and the Masai tribes has been credited with a hypolipidemic effect as well as protection against ischemic heart disease (4, 6). The results of the South African investigations, on the other hand, have been attributed to the pattern of diet, particularly the high fiber content of the Bantu diet; and it has been suggested that these "fiber rich" diets have an effect on the intestinal motility or an increase in the excretion of fecal fats or perhaps on intestinal microorganisms (2).

Although it is well-known that bile in the intestinal lumen has a profound effect on the digestion and absorption of fats; in the absence of bile there is a definite depression of absorption (7-9), in epidemiological studies of populations with disparate serum lipid levels, widely differing incidence rates of ischemic heart disease and contrasting dietary habits, it has not been given the importance it deserves. There is an apparent disparity between the diets of residents of North India as compared with those of South India and the incidence of coronary heart disease for these two regions (3, 10). Because the frequency of coronary disease failed to parallel the intake of saturated fats, the present study was undertaken to determine the interrelationship between serum lipid levels and fecal urobilinogen in two population groups, similar in their physical activity and socioeconomic status but showing big differences in the frequency of ischemic

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<sup>&</sup>lt;sup>2</sup> Chief Medical Officer and Head of Medical Department, Western Railway, Bombay, India.

heart disease and in their dietary patterns. The mortality from ischemic heart disease is 15 times higher among the railroad employees in the trade of sweepers, in South Indians as compared with the North Indians (10). We, therefore, decided to draw our subjects from these disparate geographical areas.

#### MATERIALS AND METHODS

#### Subjects

Twenty-eight pairs of healthy, age-matched railroad employees, all men, from the trade of sweepers between the ages of 35–45 years, were included in this study. A preliminary screening which included an ECG, hemoglobin estimation, microscopic examination of feces, and a Benedict's test for urine sugar, was done on all the subjects, to exclude diabetes, anemia, parasitic infestation, and ischemic heart disease. The method of inclusion in the lists of subjects was to draw names serially from the official rosters; the element of selection being only their age group. One set of subjects lived in Madras (South India) and the other set in Udaipur (North India) (Fig. 1).

The work of sweepers on the railways is manual cleansing of roads, colonies and drains, and lifting of heavy loads and involves a great deal of physical exercise. In this respect, therefore, our two groups are identical.

#### Diet

Data on diet were obtained individually from each subject in both groups by the survey method of cooked food (12).

#### Lipid Studies

All serum estimations were done separately at Udaipur and Madras in an identical way with the same person observing the end points at both the places. We used the same colorimeter, glassware, and stock reagents for both groups. Fasting blood was collected in the morning at 7 AM every day, when none of the subjects had undertaken any physical exertion, nor had smoked before being bled. Blood from an antecubital vein, with the subjects reclining in the supine position, was drawn into nonsiliconized glass syringes, using an 18-gauge steel needle, and immediately transferred as follows:



UDAIPUR

FIG. 1. Map of India showing the location of Udaipur and Madras.

5 ml to a heparinized vial for free and esterified fatty acids; 5 ml to an oxalated vial for total and ester cholesterol, and 3 ml to a plain glass test tube for separating serum. Plasma was separated within 10 min by centrifugation. Serum for the turbidimetric tests was obtained from clotted blood in the usual manner (13). From the same venipuncture and needles blood samples were also obtained by a two-syringe technique (13) for coagulation studies, reported elsewhere (14).

Total serum lipids were estimated by the method of Kunkel et al. (15); total cholesterol by three different methods, namely, Sackett's (16) method using a known solution as control; Zurkowski's (17) method and the method of Sperry and Webb (18); ester cholesterol by the method of Schoenheimer and Sperry (19), as modified by Sperry and Webb (18). Free fatty acids were measured by titration in duplicate using the method of Trout et al. (20) and the esterified fatty acids by the method of Stern and Shapiro (21).

#### Fecal Studies

Fecal urobilinogen was estimated in duplicate by the method of Maclagan (22) on specimens uncontaminated with urine, obtained on arising in the morning after overnight fasting on the same day as the serum studies. The subjects were on their habitual dietary regimes and had been asked not to eat anything after 8 PM on the day preceding the tests. Feces were passed directly into marked and specially designed containers so that contamination with urine could not take place while passing stools into them. The subjects were also specifically instructed to avoid any contamination of the feces with urine. The tests were done in a darkened room in an identical way, using the same weighing scales and glassware, the same colorimeter and stock reagents and the same observer noted the end points in both groups. All batches included a known fecal specimen of our staff as a control. Each specimen of feces was also examined microscopically for the presence of vegetable cells and fibers.

Urine urobilinogen was estimated by the method of Watson (23) and the results expressed in milligrams per 24 hr. Due to certain technical difficulties urine urobilinogen studies could not be done on all the subjects and for this we selected 12 subjects from Udaipur and 10 from Madras.

Apart from this survey of the sweepers we carried out feeding experiments on 12 healthy adult males who were admitted to the metabolic wards of the Jagjivan Ram Hospital and were given the fat-rich North Indian-style diet, basically the same as our Udaipur group for 15 days and then changed to the lipid-poor rice diet basically the same as our Madras group for another 15 days. To eliminate the effect of energy outputs the two diets were isocaloric with one another at 2271 cal in each diet. Fecal urobilinogen, urine urobilinogen, and serum cholesterol determinations were done on each subject on the last 3 days of the two dietary regimes.

#### RESULTS

A description of age, height, weight and smoking habits of the men is summarized in Fig. 2. There were twice as many biri (a country-made cheroot) smokers in the Udaipur group as in Madras.

# Diet

The data on diet are summarized in Table I. The consumption of fats is much higher in the Udaipur group (North Indians) as compared with Madras (South Indians); also fats in the Udaipur group are derived mostly from milk, ghee and fermented milk products which contain mostly short-chain saturated fatty acids, the polyunsaturated fatty acids being hardly 2%; whereas the fats in the diets of the Madras group are derived from seed oils,



FIG. 2. Age, weight, height, and smoking habits (biris) of 28 sweepers from Madras (MAS) and 28 sweepers from Udaipur (UD).

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chiefly ground-nut oil or sesame oil which have about 44% of polyunsaturated fatty acids (24). The proportion of short-chain fatty acids, however, is much higher in the ghee, milk, or fermented milk products of the Udaipur group (milk and ghee have 45% of the short-chain fatty acids: Hilditch (25)); whereas the Madras group consumes mostly seed oils which contain long-chain fatty acids such as oleic, linoleic, and palmitic acids. Our dietary data are consistent with the surveys carried out by the Indian Council of Medical Research (26) which showed that the consumption of fats was 8-19 times higher in the north (Punjab) than in the south. Apart from the differences in the pattern of diet there are important differences in methods of cooking and the manner of eating which have been published (3). The South Indian diets are "sloppy" and are usually bolted down without much chewing whereas the North Indian diets require thorough chewing (27). The methods of cooking such as long frying of ghee with turmeric powder (Curcuma domestica), the method of preparing the fermented milk drinks, and the practice of boiling milk, prevalent in the north, reduce the size of the fat globules manyfold as a result of emulsification (3).

#### Serum Lipids

The results of lipid studies are summarized in Table II. There were no significant differences in the mean serum cholesterol values between the two groups, and all three tests applied by us showed normal values in both groups. Zurkowski's method (17) showed a slightly higher range than the other two methods because this method usually gives higher normal values. Although the mean values for free fatty acids were higher in the Udaipur group than in Madras, the differences are not statistically significant (t = 0.93). Similarly, the mean values for esterified fatty acids, cholesterol esters, and total triglycerides showed no significant differences.

<b>FABLE I</b>	
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Pattern of Food Consumption of the Sweepers in the Madras and the Udaipur Groups: Grams per Consumption Unit

Name of Food	Madras, g	Udaipur, g
Rice	425	0
Wheat	0	440
Other cereals	140 (Ragi)	40 (Barley or maize)*
Pulses	15	62
Leafy vegetables	0	50
Nonleafy vegetables	122 (Onions or brinjal)	88
Seed oils	7 (Ground-	20 (Mustard
	nut)	or sesame)
Ghee	0	55
Milk, buttermilk or curd	12	150
Meat and fish	30 (Beef)	5
Condiments	15	5
Sugar and jaggery	12	50
Total calories	2,334	2,786
Total fats, g	7	75
Calories from fats, %	31/2	23
Composition of fats	Chiefly	Chiefly
	long-	short-
	chain	chain
	fatty acids	fatty acids
	of seed oils	of ghee
		and milk
		fats
Saturated fatty acids, %	2	44

Fats and oils are the amounts of these foodstuffs available for consumption as such, not the total amount of fat derived from all dietary constituents. \* Optional.

Tests of significance indicate t values of 0.10, 0.14, and 0.81, respectively. The results for total triglycerides have been expressed as direct turbidity readings on a Klett-Summerson colorimeter without applying the conversion factor since our aim principally was a comparison between the two groups. These differences also are not statistically significant.

# Fecal Urobilinogen

Results of fecal urobilinogen concentration (mg/100 g stool) displayed in Table

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# TABLE II

Mean Values for Serum Lipids of 28 Pairs of Railwaymen Sweepers from Madras and Udaipur

	Total Cholesterol, mg/100	/100 ml	Cholesterol Acid	Free Fatty Acids, mean	Esterified	Total Fats,	
Group	Sackett	Sperry and Webb	Zurkowski	Ester, mg/100 ml	values per liter, mmoles	Fatty Acids, mg/100 ml	Summerson Readings
Madras							
Mean	174.3	186.0	207.0	113.0	698.0	230.0	41.8
SE	± 4.71	$\pm 9.34$	± 5.89	± 7.36	± 73.79	$\pm 12.04$	$\pm$ 3.2
Udaipur							
Mean	169.2	165.0	222.6	114.4	794.0	228.0	38.6
SE	± 7.85	± 9.32	± 10.07	$\pm 6.6$	± 72.22	± 11.94	± 2.17
Significance of differ-							
1	0.55	1.57	1 34	0.14	0.93	0.10	0.81
P	NS	NS	NS	NS	NS	NS	NS

## Table III

Comparison of Mean Values of Fecal Urobilinogen Concentration in mg/100 g of Feces in 28 Pairs of Age-matched Railway Sweepers All Men; and 16 Pairs of Women, from Madras and Udaipur

Group	Mean, mg/100 g	Standard Frror	Significance of Difference	
	mg/ 100 g	Liidi	t	Р
Madras, men Udaipur, men	64.4 19.8	$\pm 4.64 \\ \pm 2.52$	8.47	<0.001
Madras, women Udaipur, women	$35.1 \\ 12.3$	$\pm 3.18 \\ \pm 1.49$	6.67	<0.001

III show highly significant differences in the mean fecal urobilinogen levels between the two groups (t = 8.47, P < 0.001) the mean values being 64.4 mg and 19.8 mg for Madras and Udaipur, respectively. Fecal urobilinogen was also estimated in 16 pairs of women from these two geographical areas and the results showed that while the fecal urobilinogen levels were much lower in women as compared with men, there were differences even between women in the two groups, the mean values being lower in the case of Udaipur than Madras (28).

Twenty-two of the fecal specimens of men in Udaipur showed an abundance of vegetable fibers whereas only six of the samples in Madras showed vegetable fibers although in their case these were not so plentiful. These trends were similar for women in the two groups.

#### **Results of Feeding Studies**

The total and ester cholesterol levels (Sperry and Webb) showed no significant differences between the two different dietary regimes, the respective mean values being 166.3 mg/100 ml  $\pm$  10.46 and 168.9  $mg/100 ml \pm 11.08$  for total cholesterol and  $104.8 \text{ mg}/100 \text{ ml} \pm 4.33 \text{ and } 112.0 \text{ mg}/$  $100 \text{ ml} \pm 7.20$  for ester cholesterol for the rice versus the wheat regime; tests of significance indicating t values of 0.01 and 1.00, respectively. Fecal urobilinogen concentration (mg/100 g of stool) was significantly higher on the South Indian-style rice diet as compared with the North Indian-style wheat diet (Table IV). The marked rise in fecal urobilinogen concentration (mg/100 g of stool) in these feeding

experiments, when the North Indianstyle fiber- and fat-rich wheat diet was changed to the North Indian-style cellulose- and lipid-poor rice diet, is partly due to dilution because the mean weight of feces was lower on the rice diet as compared with the wheat diet (Table v). But there is an actual increase in fecal urobilinogen excretion (mg/24 hr) on rice diet as compared with wheat diet because the total mean excretion (mg/24 hr) showed a striking rise in 11 out of 12 individuals when wheat regime was changed to rice diet. The respective mean value rose from 60.58 mg/ 24 hr to 115.15 mg/24 hr (P approximately 0.001) (Table vi). These differences were also reflected in the urine urobilinogen (mg/24 hr) in our feeding experiments, the respective values being 1.28 mg/24 hr and 0.22 mg/24 hr on the South Indian versus the North Indian regimes (P < 0.01) (Table VII).

# Interpretation and Validity of Results of Fecal Urobilinogen

Since urobilinogen is formed in the intestine by the reduction of bilirubin and

		Та	BLE 1	v			
Mean	Fecal	Urob	ilinog	gen	Co	ncentrati	or
(mg/	/100 g	Stool)	with	Wł	neat	Diet (A)	
an	d Rice	e Diet	(B)	in	12	Adults	

Subject	A Wheat Diet	B Rice Diet
1	53.10	85.5
2	12.13	68.3
3	27.70	117.8
4	10.50	67.6
5	38.43	82.7
6	14.63	57.7
7	44.60	117.4
8	26.00	118.4
9	8.40	95.8
10	37.10	107.6
11	16.80	63.3
12	28.36	61.7
Mean	26.48	86.98
SE	4.28	6.84

TABLE	v
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Mean Weight of Feces Excreted (g/24 hr) with Wheat Diet (A) and Rice Diet (B) in 12 Adults

Subjects	A Wheat Diet	B Rice Diet
1	220	192
2	225	140
3	325	163
4	164	71
5	153	54
6	214	132
7	253	144
8	391	184
9	188	88
10	231	54
11	216	265
12	203	135
Mean	232	135
SE	19.27	18.04

TABLE	VI
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Mean Fecal Urobilinogen Excretion (mg/24 hr) with Wheat Diet (A) and Rice Diet (B) in 12 Adults

Subject	A Wheat Diet	B Rice Diet
1	117.06	163.67
2	26.65	97.91
3	86.20	152.12
4	15.81	51.20
5	53.24	45.18
6	29.75	105.84
7	116.38	169.08
8	91.66	203.94
9	15.79	84.30
10	85.70	58.10
11	35.28	167.75
12	53.52	82.75
Mean	60.58	115.15
SE	10.82	15.56

its amount in feces depends primarily on the amount of bilirubin entering the intestine (29), the findings recorded here point to greater amounts of bile entering the intestinal lumen in our Madras group as compared with our Udaipur group; and greater in men than women. This is fur ther supported by the results of the 24-hr urine urobilinogen excretion in our two groups. The Madras group showed higher mean values than the Udaipur group, the respective mean urine urobilinogen values (mg of urobilinogen/24 hr) being 1.39 mg  $\pm$  0.3386 and 0.18 mg  $\pm$  0.0665 for Madras and Udaipur, respectively (P < 0.01). Since urine urobilinogen is derived from that part of the urobilinogen absorbed in the intestine which is not excreted by the liver, the amount present thus depending upon the amount of bilirubin entering the intestine (29), these results also indicate that higher amounts of bilirubin are present in the intestine of our Madras group as compared with our Udaipur group. These results viewed side by side with the results of our feeding experiments under metabolic conditions, show unequivocally that our South Indian group on a carbohydrate-rich, lipid-poor regime of boiled rice and lentil soups had significantly larger amounts of bile in their intestinal lumen, as compared with our North Indian group on fat-roughage and

TABLE VII Mean Urine Urobilinogen Excretion (mg/24 hr) on Wheat Diet (A) and Rice Diet (B)

Subject	А	В
1	0.0386	1.4000
2	0.0534	1.1327
3	0.5830	0.8262
4	0.0653	2.4725
5	0.1600	2.6724
6	0.0760	0.3223
7	0.0620	0.9160
8	0.3610	1.3479
9	0.8300	3.7024
10	0.0960	0.3668
11	0.1220	0.0817
12	0.2390	0.1312
Mean	0.2238	1.2810
± se	0.072	0.393

cellulose-rich wheat diet; and that these differences are dependent upon the pattern of diet and eating.

# DISCUSSION

## Fat Consumption and Serum Lipid Levels

The present investigation demonstrates that serum lipid levels may be normal in people showing big differences both in their consumption of fats, including animal fats, and in the incidence of ischemic heart disease. The association of a high intake of animal fats, a comparative freedom from ischemic heart disease, and normal lipid values in our Udaipur group (North Indians) on the one hand, and the inverse association of a low intake of animal fats, higher incidence of ischemic heart disease, and normal lipid values in our Madras group (South Indians), is at variance with the established concepts of the role of fats in ischemic heart disease as well as serum lipid levels. But this supports the findings of several other workers (4-6) that serum lipid levels are not dependent upon the total quantities of fats consumed, or even on the proportion of saturated versus unsaturated fatty acids.

The data in Table 1 show that 44% of the fats in our Udaipur group were composed of saturated fatty acids while these formed only 2% of the dietary fats in our Madras group. On the basis of the prediction equations of Keys et al. (30), one would have expected much higher serum cholesterol levels in our Udaipur group than in our Madras group. But this is not the case, which may indicate, as pointed out by Ahrens et al. (31), that the reason for the observations that animal fats elevated serum lipid and vegetable fats lowered the plasma cholesterol may be due to some other factors not yet determined, rather than the preponderance of unsaturated fatty acids in certain types of fats. The diets of our Madras group consisted mainly of boiled rice and in view of the finding of Ahrens et al. (31) that when

dietary fats were isocalorically exchanged with carbohydrate, the highest levels of all serum lipids occurred on the lowest fat intake: total cholesterol concentrations were lowest on the 40% intake of corn oil calories, and were significantly higher on both the fat-free and 70% intakes. It is, therefore, interesting to speculate if the carbohydrate-rich lipid-poor regimes of South Indians have raised their serum lipid levels, and the high-fat carbohydrate-poor and cellulose-rich diets of the North Indians have lowered their serum lipid levels in relation to what should have been expected on the basis of the prediction equation of Keys et al. (30). Even if this has happened, the mechanism of such a response is totally unclear.

## Physical Activity and Serum Lipid Levels

Exercise has been credited with the role of lowering both the serum lipids and the tendency to ischemic heart disease by some (4, 6, 32, 33); while others believe that it has no such effect (34, 35). Both our groups are habituated to an identical amount of a high degree of physical exercise at work and it is therefore difficult to explain how this could have brought about an equalization of the levels of serum lipids in our two groups, with disparate fat consumption and belonging to two different population groups with disparate experience of ischemic heart disease. As a second, and perhaps a more convincing test of the absence of any effect of exercise on serum cholesterol, we compared the mean cholesterol values of our Madras group with 13 age-matched professional blood donors from the Railway Hospital at Madras, who were all engaged in sedentary work. This comparison showed no differences in the mean cholesterol values between the sedentary blood-donors on the one hand, and the physically active sweepers on the other; the mean values, using Zurkowski's method for estimation, being 207 mg/100 ml  $\pm$  8.70 for blood

donors and 207 mg/100 ml  $\pm$  5.89 for sweepers. Since the diets and age groups of the blood donors were comparable with the sweepers, the only apparent difference being in the amount of their physical activity, it is not unreasonable to conclude that the theory of lipid-lowering action of physical exercise fails to exclude other possible factors. This is further supported by the lack of any significant differences in the levels of serum cholesterol or mean cholesterol ester levels in the 12 subjects included in our feeding experiments under metabolic conditions, the respective values being  $166.3 \text{ mg}/100 \text{ ml} \pm 10.46 \text{ and } 168.9 \text{ mg}/100$ ml  $\pm$  11.08 for total cholesterol; and 104.8 mg/100 ml  $\pm$  4.33 and 112.0 mg %  $\pm$  7.20 for ester cholesterol for the rice versus the wheat regime, tests of significance indicating t values of 0.01 and 1.00, respectively.

#### Smoking and Serum Lipid Levels

The effect of smoking on serum lipids has been studied by several investigators and it has been shown that smoking increases the fatty acid content of plasma (36). Page et al. (37), Mustard and Murphy (38), and Hernberg (33) have, however, not found this to happen. Their findings together with the evidence from the present study indicate that the higher mean serum cholesterol reported in smokers as compared with nonsmokers (39) is perhaps not due to smoking.

#### Role of Bile in Serum Lipid Levels

There is good evidence which indicates that bile is important in the digestion and absorption of fats (7, 8); it has been observed that the rate of absorption is directly related to the amount of bile salts in the intestinal lumen (9).

The highly significant differences in the fecal urobilinogen concentration in our two groups which depend primarily on the amount of bile entering the intestinal lumen are, therefore, of particular concern. Although we have no evidence on which to base a distinction between a)

mobilization of adipose tissue fat, b) new synthesis of fat from dietary carbohydrate, or c) possible retardation of fat utilization, the balance of evidence would seem to indicate that because of the lower amounts of bile in the intestinal lumen, less fat may have been absorbed in the case of our Udaipur group despite higher amounts being available in their diets, and because of the higher amounts of bile in the intestinal lumen more fat may have been absorbed in the case of the Madras group, although less was available in their diet.

In order to test this hypothesis, we estimated fecal fat excretion by the technique of Kamer et al. (40), in 11 of our 12 subjects included in the feeding experiments. The results are expressed in grams of fat excreted per 24 hr. Fecal fat excretion showed a rise in 9 out of 11 subjects on fat-rich, carbohydrate-poor North Indian diet, as compared with the carbohydraterich lipid-poor South Indian diet (Table VIII) (P < 0.02); and this correlates inversely with the amount of bilirubin in the intestine as indicated by the fecal urobilinogen concentration and excretion.

#### TABLE VIII

Mean Fecal Fat Extraction (g/24 hr) with Wheat Diet (A) and Rice Diet (B) in 11 Adults

Subject	A Wheat Diet	B Rice Diet
1	1.158	1.473
2	3.467	2.215
3	1.687	1.229
4	4.519	2.195
5	1.049	0.561
6	3.127	1.403
7	2.354	1.304
8	2.700	2.195
9	4.824	1.290
10	2.884	3.572
11	3.671	2.744
Mean	2.858	1.834
SE	0.38	0.25
	1	

The recent data of Wastell and Ellis (41) show a similar situation.

This may indicate that the amount of fat absorbed from the intestine does not depend so much upon the amount of fed fats but is rather a function of the amount of bilirubin in the intestinal lumen. While the mechanism that produces these variations in the amount of bilirubin entering the intestinal lumen is less clear and demands investigation, the pattern of diet and eating seems to bear responsibility for the differences in fecal urobilinogen recorded here, probably due to its effect on gall-bladder evacuation (42).

In relevant publications very little attention has been devoted to studies on the amounts of bile and the patterns of eating in relation to serum lipids or the liability to develop ischemic heart disease. It seems that these may be profitable fields of future enquiry, as not only has bile a regulatory influence on the level of the absorption of fats but it also determines the route of absorption of the fatty acids (7-9, 43).

# Serum Lipids and Ischemic Heart Disease

It is difficult to explain the higher death rates from ischemic heart disease among the South Indian railroad sweepers as compared with the North Indians on the basis of the serum levels of cholesterol or triglycerides, because we found no significant differences between the mean cholesterol concentrations and no significant differences for triglyceride concentrations nor for the free and esterified fatty acid levels in the two groups of subjects. It is not improbable that some other possible variations in the composition of plasma triglyceride acids may be present despite no differences in the total serum levels of lipids, and may account for the disparate mortality rates from ischemic heart disease between the North Indians versus the South Indians. There is good evidence that the chain length of the triglyceride acids in the plasma depends upon the fatty acid composition of the fed fats (31, 44). (For further discussion see Malhotra (3).) Moreover, the amount of bile salts in the intestinal lumen determines not only the rate (9) and the route (43) of absorption of fed fats, but also the composition of the plasma triglyceride acids. Thus, ordinarily the food fats are hydrolyzed by bile salts into fatty acids which are then resynthesized into complex fatty acid triglycerides, partly characteristic of the species, whereas short-chain fatty acid triglycerides are absorbed directly into the blood stream (45). Thus Borgström et al. (46) have computed that some 40% of absorbed neutral fat was absorbed as triglyceride, some 40% was completely hydrolyzed, while the remainder was only partly hydrolyzed. The data of Lough et al. (44) and Ahrens et al. (31) show unequivocally that the triglyceride acid composition of plasma depends upon the fatty acid composition of fed fats. Furthermore, the virtual absence of cellulose and vegetable fibers from the South Indian dietary regimes and their presence in abundance in the North Indian diets may be responsible for liberating large quantities of acetic acid, a precursor of short-chain fatty acids in the case of North Indians as compared with South Indians. Popjack et al. (47) have shown that the fermentation of cellulose and vegetable fibers liberates large quantities of acetic acid in the rumens. It is easy to see, therefore, that the triglyceride acids in the North Indians have a preponderance of short-chain, directly absorbed fatty acids versus the complex fatty acids partly characteristic of the species, not only because of the fatty acid composition of the fed fats and the differences in their diet of cellulose and fiber content, but also because of a deficiency of the bile salts in the intestinal lumen of the North Indians as compared with the South Indians. In the case of the South Indians, on the other hand the situation will be just the opposite. This is of relevance because Connor and Poole

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(48) and Connor et al. (49) have shown in experimental systems that thrombosis was enhanced by long-chain fatty acids of not less than 16 carbon atoms, whereas shortchain fatty acids ( $C_6$ - $C_7$ ) hardly produced any enhancement. This may, thus, explain why the liability to develop acute myocardial infarction is neither related to the dietary fat intakes, including animal fats, nor to the serum levels of lipids but may be a function rather of the chain length of fatty acids (3, 10, 50).

Elsewhere (3), we have discussed the effect of certain culinary practices on the particle size of the fed fats of the North Indians versus the South Indians. The methods of cooking and eating reduce the particle size of the fat globules more than 100-fold in the case of the North Indians as compared with the South Indians. While the implications of this are less clear and need further investigation, these may be of relevance because, as shown by Frazer (51) and Daniel et al. (52), direct absorption of neutral fat droplets into the epithelial cells and thence into lymph may occur if the fat particles are finely emulsified, and also because the coagulation mechanisms are influenced by the particle size of fats in solution (53).

#### SUMMARY

Serum lipid levels in 28 pairs of agematched railwaymen from two geographically different population groups with disparate consumption of fats and showing big differences in mortality rates from ischemic heart disease showed no significant differences in total and ester cholesterol, free and esterified fatty acids, and total serum triglycerides. Although the consumption of fats, most of which were of animal origin, was 10 times more in North Indians as compared with South Indians, there were no differences in the levels of their serum lipids. These differences in diet were, on the other hand, related inversely to the fecal and urine urobilinogen excretion, which may indicate that in the presence of a deficiency of bilirubin in the intestinal lumen less fat is absorbed even though more may be present in the food, while in the case of a higher bilirubin content more fat is absorbed even though less was present in the food. The role of physical activity, smoking, and the amount and kind of fat in these results is unproved. These results can be explained entirely on the basis of the amount of bilirubin available in the intestine, which in turn seems to depend upon the pattern of diet and eating.

Evidence pertaining to the possible differences in the chain length of plasma triglyceride acids, despite there being no differences in the serum lipid levels in our two groups, is discussed and this may explain the discrepancy that mortality from ischemic heart disease is 15 times higher among our South Indian population as compared with our North Indian population. This is true even though the South Indians eat one-tenth as much fat, most of which is from seed oils and is composed of long-chain fatty acids, in contrast to the North Indians whose dietary fats are mostly of animal origin but have a preponderance of short-chain fatty acid triglycerides.

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