

standard deviation of the method: Hexose 1.05%, glucose 0.035%, galactose 0.051%. Since urine and GBM specimens were pooled, composition of the groups were compared but intra-group variability could not be evaluated.

Results. The amino acid composition of MUPpg and GBM from both experimental groups and the controls showed no significant differences. We have previously reported the amino acid composition of GBM and MUPpg in detail⁶. The Table summarizes quantitative differences in the carbohydrate composition of GBM and MUPpg from experimental and control groups. The total hexose, glucose and galactose in MUPpg were increased about 20% in the cyclophosphamide rats as compared to untreated (control) and saline treated animals. The quantity of MUPpg excreted was similar in the 3 groups (Table).

Similar increases (15% in total hexose, glucose and galactose) were observed in the GBM isolated from cyclophosphamide treated rats compared to controls and saline treated rats without significant changes in the glucose: galactose ratio (Table).

Discussion. The pathogenesis of lipid nephrosis remains an enigma but remissions commonly accompany corticosteroid or cyclophosphamide therapy. There is evidence that proteinuria regardless of pathogenic mechanism is probably a function of increased glomerular permeability⁹⁻¹³ which may depend on the molecular arrangement of the basement membrane, related basement membrane glycoproteins³ or epithelial or endothelial lining¹⁴⁻¹⁶. The alterations in carbohydrates in GBM and MUPpg associated with treatment with cyclophosphamide suggest that cyclophosphamide may alter the chemical composition of this membrane related glycoprotein both in situ on GBM and in the urine. MISRA³ has indicated that prednisone may decrease proteinuria in the nephrotic syndrome in man by alteration of the carbohydrate composition of GBM.

Quantitative differences in carbohydrates of GBM and MUPpg in control and experimental groups

	Control group	Group I ^a Cyclophosphamide treated	Group II ^b Saline treated
Total hexose MUPpg (%)	15	18	14.7
Glucose MUPpg (%)	4.4	5.2	4.38
Galactose MUPpg (%)	1.05	1.53	1.06
Average MUPpg (mg animal/day)	0.6	0.7	0.75
Total hexose GBM (%)	5.6	6.8	5.65
Galactose GBM (%)	2.3	3.1	2.31
Glucose GBM (%)	2.0	2.7	1.92

^a Received cyclophosphamide 10 mg/kg i.p. 5 days a week for 6 weeks.

^b Received saline 1 cm³ i.p. 5 days a week for 6 weeks.

These studies suggest that one of the mechanisms by which cyclophosphamide may bring about improvement in the effectiveness of the protein filtering capability of the glomerular capillary wall is through modification of the chemistry of this class of constituents of the wall. The precise role of these components in the maintenance of GBM integrity to protein and the mode of synthesis and degradation remain unknown. Further evaluation of these important questions seem indicated on the basis of the observations reported in these studies. Also, the beneficial effect of cyclophosphamide on other diseases affecting membranes may be due to a similar mechanism.

Résumé. La cyclophosphamide est un agent efficace dans le traitement de la néphrose lipidique et de plusieurs maladies rhumatoïdes qui attaquent la membrane basale glomérulaire (MBG). Des analyses chimiques ont été effectuées sur la MBG et sur la glycoprotéine urinaire chez des rats traités et non traités. Entre les deux groupes on n'a pas constaté de différence dans la composition des amino-acides, mais de grands changements dans le glucose et le galactose de la MBG et la glycoprotéine urinaire ont été observés chez les rats traités à la cyclophosphamide; cette drogue semble donc capable d'altérer l'intégrité de la MBG.

R.M. McINTOSH^{18,19}, H. KIHARA,
D.B. KAUFMAN and C. KULVINSKAS

Gwynne Hazen Cherry Renal Research Laboratory,
University of California, Los Angeles and the Research
Department for Mental Retardation, Pacific State Hospital,
Pomona (California, USA), 9 August 1971.

- ⁹ R. C. GRAHAM and M. J. KARNOVSKY, *J. exp. Med.* 124, 1123 (1966).
- ¹⁰ A. Y. K. CHOW and K. N. DRUMMOND, *Lab. Invest.* 20, 213 (1969).
- ¹¹ M. A. VENKATACHALAM, M. J. KARNOVSKY and R. S. COTRAN, *J. exp. Med.* 130, 381 (1969).
- ¹² M. A. VENKATACHALAM, M. J. KARNOVSKY, H. D. FAHIMI and R. S. COTRAN, *J. exp. Med.* 132, 1153 (1970).
- ¹³ M. A. VENKATACHALAM, R. S. COTRAN and M. J. KARNOVSKY, *J. exp. Med.* 132, 1168 (1970).
- ¹⁴ A. F. MICHAEL, E. BLAU and R. L. V. VERNIER, *Lab. Invest.* 23, 649 (1970).
- ¹⁵ M. G. FARQUAR, S. L. WISSIG and G. E. PALADE, *J. exp. Med.* 113, 47 (1961).
- ¹⁶ M. G. FARQUAR and G. E. PALADE, *J. exp. Med.* 114, 699 (1961).
- ¹⁷ Supported by Government Research Service Grant USPHS No. 1-501 FR 0532, University of California School of Medicine, Los Angeles, California USPHS training grant HD 0060 and the Westwood Hills Juniors Foundation.
- ¹⁸ Assistant Professor of Pediatrics, University of California School of Medicine, Los Angeles (California 90024, USA). Present address: Department of Pediatrics, College of Physicians and Surgeons of Columbia University 630 W. 168th Street, New York (N.Y. 10032, USA).
- ¹⁹ Address communications to R. M. McIntosh, M.D., Room 474, William Black Research Building, College of Physicians and Surgeons of Columbia University, New York (New York 10032, USA).

Triglycerides and Other Lipid Classes in Human Atherosclerosis

Data so far available do not make clear whether triglycerides, or cholesterol, or both these lipid classes, are significantly involved in changes in lipid metabolism brought about by atherosclerosis¹⁻⁷. The purpose of this

investigation was to define the role played by individual plasma lipid classes in atherosclerosis.

Materials and methods. The investigation was carried out on plasma from 25 atherosclerotic patients in compar-

Total lipids and lipid classes in plasma of normal and atherosclerotic subjects

Number		Normal subjects	Atherosclerotic subjects	Normal value range ¹⁶
		15	25	—
Total lipids (mg/100 ml of plasma)		533±21	714±53 ^a	450–1000
Total cholesterol	mg of cholesterol/100 ml of plasma	157±6	185±12	130–250
	% of total lipids	46	42	—
Esterified cholesterol/free cholesterol		5.78±0.41	4.86±0.27	—
Triglycerides	mg of tristearin/100 ml of plasma	99±17	190±20 ^b	29–134
	% of total lipids	18	27	—
Phospholipids	mg of P/100 ml of plasma	7.31±0.27	7.62±0.53	5–12
	% of total lipids	34	28	—
Free fatty acids	mg of stearic acid/100 ml of plasma	13.10±1.84	18.78±2.04	12–25
	% of total lipids	2	3	—

Mean values ± standard error. ^a *P* 0.05. ^b *P* 0.01.

ison with 15 healthy subjects. The atherosclerotic subjects were suffering from angina pectoris, cerebrovascular disease, peripheral artery occlusion or myocardial infarction. All these diseases definitely originated from atherosclerosis. The control subjects were not suffering from any vascular atherosclerotic disease, or diabetes, liver disease or any other ailments capable of altering lipid metabolism. Diagnoses were obtained from a series of clinical tests and anamnestic data. The mean age of all the subjects was 43 years.

Blood samples were taken after at least 15 days' stay in hospital. During this period, all subjects received the same diet. Total lipids were extracted, purified and quantitatively determined by weight according to FOLCH et al.⁸.

The following substances were determined on the purified extract: total cholesterol (VANZETTI and GATTI⁹), phospholipids (ALLEN¹⁰), triglycerides, free fatty acids and free cholesterol (MARZO et al.¹¹).

Results and discussion. Plasma levels of total lipids and triglycerides proved to be higher to a statistically significant degree in the atherosclerotic patients than in our healthy subjects. Phospholipid, total cholesterol and FFA levels were higher in the atherosclerotic patients to differing extents but not to any statistically significant degree.

The ratio between esterified and free cholesterol was lower in the atherosclerotic patients but again not to a statistically significant extent. When each lipid class was expressed as a percentage of total lipids, only the percentage of triglycerides proved to be higher in these patients. Our data suggest that triglycerides should be regarded as the lipid class which is most involved in change in lipid metabolism elicited by atherosclerosis. In effect, triglycerides are the only lipid class showing a marked increase both if expressed as a plasma level and as a percentage of total lipids. The importance of changes in triglycerides is still more evident if we consider that they are the only parameter having markedly higher levels than those in the normal range reported in the literature; total lipid levels and those of the other lipid classes being included in the normal value range in the clinical literature.

However, we feel that triglycerides alone do not provide diagnostic indication of atherosclerosis. More precise diagnostic data may be obtained by evaluation triglycerides^{4–7}, serum lipoproteins^{12,13} and serum total fatty acid pattern¹⁴ simultaneously.

Riassunto. I lipidi totali e le singole classi lipidiche sono stati determinati sul plasma di 15 soggetti normali e di 25 aterosclerotici. I lipidi totali e i trigliceridi plasmatici sono risultati, in modo statisticamente significativo, più alti nei soggetti aterosclerotici. I trigliceridi inoltre sono risultati più elevati negli aterosclerotici, anche quando erano espressi come percentuale dei lipidi totali. I trigliceridi sembrano essere la classe lipidica più significativa nella malattia aterosclerotica.

P. GHIRARDI, A. MARZO, B. BRUSONI and D. SARDINI

Biochemistry Division, Simes S.p.A., Via Bellerio 41, I-20161 Milano (Italy),
Biochemistry Institute, State University, Milano, and
'De Gasperis' Department, Ospedale Maggiore 'Cà Granda', Milano (Italy), 26 July 1971.

¹ H. L. FALSETTI, J. D. SCHNATZ, D. G. GREENE and J. L. BONNELL, *Circulation* 37, 184 (1968).

² J. V. KRIVORUCHENKO, *Klin. Med.*, Moscow, 48, 108 (1970), *Chem. Abstr.* 73, 107448m (1970).

³ H. O. BANG, E. HESS THAYSEN and J. THYGESEN, *Acta med. scand.* 184, 241 (1968).

⁴ A. FORESTI, R. MILANI, S. MARCHI and D. RIVA, *Boll. Soc. ital. Cardiol.* 15, 65 (1970).

⁵ J. MACDONALD and J. OBEYESEKERE, *J. atheroscler. Res.* 7, 530 (1967).

⁶ B. M. RIFKIND, D. LAWSON and Morna GALE, *J. Atheroscler. Res.* 8, 167 (1968).

⁷ L. V. CROWLEY, *Clin. Chem.* 17, 206 (1971).

⁸ J. FOLCH, M. LESS and G. H. SLOANE STANLEY, *J. biol. Chem.* 226, 497 (1957).

⁹ G. VANZETTI and E. GATTI, *Biochem. appli.* 10, 34 (1963).

¹⁰ R. J. L. ALLEN, *Biochem. J.* 34, 858 (1940).

¹¹ A. MARZO, P. GHIRARDI, D. SARDINI and G. MERONI, *Clin. Chem.* 17, 145 (1971).

¹² D. S. FREDRIKSON, R. J. LEVY and R. S. LEES, *New Engl. J. Med.* 276, 34, 94, 148, 215, 273 (1967).

¹³ C. ALLARD and C. GOULET, *Clin. chim. Acta* 20, 534 (1968).

¹⁴ P. GHIRARDI, A. MARZO, B. BRUSONI and D. SARDINI, *Boll. Soc. it. biol. sper.*, 1972, in press.

¹⁵ R. J. HENRY, *Clinical Chemistry* (Hoeber-Harper, New York 1966).