6. Since this paper was submitted for publication, nine additional sublines of cell cultures were checked for contamination with pleuropneumonialike organisms. One of the sublines yielded growth of pleuropneumonialike organisms.

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Comparative Studies of Lipoproteins by Starch and Paper Electrophoresis

Previous investigations have been concerned with the cholesterol and phospholipid content of serum lipoproteins separated either by paper electrophoresis (*1*) or by starch electrophoresis (*2–4*). This study is concerned with comparative analyses of cholesterol and phospholipid in the various serum lipoproteins separated simultaneously by starch and paper electrophoresis (*5*). Normal serum and serums from patients with idiopathic hypercholesteremia and with idiopathic hyperlipemia were analyzed.

Starch electrophoresis was performed in two parallel blocks 8 by 36 by 1.5 cm using 2.0 ml of serum for each block and applying 450 volts and a current of 18 to 35 ma for 16 to 18 hours in a cold room at a steady temperature of 5°C. Barbiturate buffer of *p*H 8.6 and of ionic strength 0.05 was used. Extraction of the

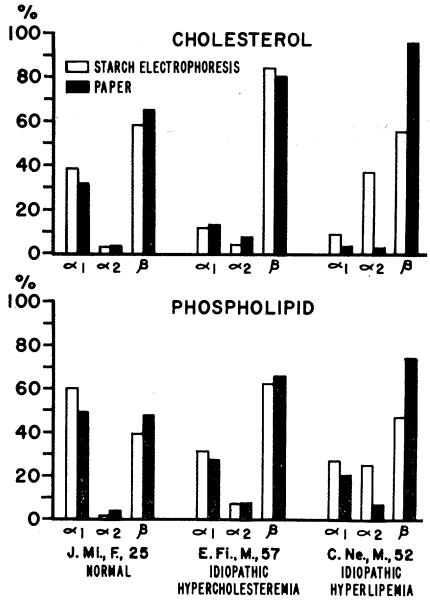

Fig. 1. Cholesterol and phospholipid contents in alpha-1, alpha-2, and beta lipoprotein fractions separated by starch and paper electrophoresis. No significant differences were observed between the two methods of electrophoresis in a normal person or, in persons with idiopathic hypercholesteremia. In idiopathic hyperlipemia, marked increase of cholesterol and phospholipid was seen in the alpha-2 lipoprotein by starch electrophoresis, whereas, by paper electrophoresis, these lipids were mainly increased in the beta lipoprotein.

starch segments (1 cm wide) was performed with a mixture of chloroform and methanol (2/1). The extracts were analyzed for total and esterified cholesterol (*6*) and phospholipid (*7*). Analogous segments of the second block were used for protein determinations by the biuret method (*8*).

Paper electrophoresis was performed in two parallel strips of Whatman 3-MM filter paper (15 by 30 cm) using 0.4 ml of serum and applying 250 volts and a current of 15 to 20 ma for 7 hours. A 2-cm-wide part of one strip was stained with Amidoblack 10B dye for localization of the protein fractions. The buffer and the lipid solvent were identical with those used in starch electrophoresis. Each paper segment (1 cm wide) was analyzed for total and esterified cholesterol and phospholipid (*6*, *7*).

The control serum showed normal lipid partition: cholesterol, 228; phospholipid, 304; triglycerides, 28; and total lipids, 560 mg percent. The lipoprotein fractions alpha-1, alpha-2, and beta that were separated either by starch or by paper electrophoresis showed no significant differences in the distribution of cholesterol and phospholipid (Fig. 1).

In idiopathic hypercholesteremia, the serum was characterized by decided elevation of both cholesterol and phospholipid with a slight elevation of triglycerides and total lipids: cholesterol, 334; phospholipid, 328; triglycerides, 118; and total lipids, 880 mg percent. Regardless of the method of electrophoresis used, the cholesterol and phospholipid contents were markedly increased in the beta lipoprotein fraction and decreased in the alpha-1 lipoprotein fraction (Fig. 1).

In idiopathic hyperlipemia, the serum was characterized by lactescence and by marked elevation of all lipid fractions: total cholesterol, 776; phospholipid, 764; triglycerides, 1460; and total lipids 3000 mg percent. Marked differences in the distribution of cholesterol and phospholipid were observed with the two methods of electrophoresis. When starch was used as the supporting medium, elevation of cholesterol and phospholipid in the alpha-2 lipoprotein fraction was the prominent feature, whereas when paper was used the elevation of these lipids was seen in the beta lipoprotein (Fig. 1). By both methods, decrease of these lipids was observed in alpha-1 lipoprotein.

Considerable adsorption of serum triglycerides (chylomicrons) to the paper was noted at the point of application (*2*). A comparison of the distribution curves of cholesterol and phospholipid by the two methods of electrophoresis showed that large amounts of cholesterol and phospholipid were present in both alpha-2 and beta lipoprotein when starch electrophoresis was used, while the major pattern of these lipids was found in the beta lipoprotein by paper electrophoresis.

The adsorption of serum lipids at the point of application in paper electrophoresis interfered with the migration of some of the cholesterol and phospholipid molecules. When starch was used as supporting medium, no accumulation and adsorption of triglycerides was observed at the point of origin. The triglycerides migrated freely (*4*) and the cholesterol and phospholipid molecules migrated with them. This observation is probably related to the easier extractability of cholesterol "enmeshed in lipids" (*9*).

FIORENZO PARONETTO*
CHUN-I WANG
DAVID ADLERSBERG
Departments of Medicine and *Chemistry, Mount Sinai Hospital, New York*

References and Notes

1. E. Nikkilä, *Scand. J. Clin. and Lab. Invest.* suppl. 8, 1 (1953); B. Swahn, *Scand. J. Clin. and Lab. Invest.* suppl. 9, 44 (1953); G. S. Boyd, *Biochem. J. (London)* 58, 680 (1954).
2. H. G. Kunkel and R. J. Slater, *Proc. Soc. Exptl. Biol. Med.* 80, 42 (1952).
3. P. G. Ackerman, G. Toro, W. B. Kountz, *J. Lab. Clin. Med.* 44, 517 (1944).
4. G. Schettler, in *Handbuch der Inneren Medizin*, 7, 713, 741 (1955).
5. Supported in part by research grant H-564 from the National Institutes of Health, U.S. Public Health Service, Department of Health, Education, and Welfare.
6. W. M. Sperry and M. Webb, *J. Biol. Chem.* 137, 97 (1950).
7. W. M. Sperry, *Ind. Eng. Chem. Anal. Ed.* 14, 88 (1942).
8. J. Goa, *Scand. J. Clin. and Lab. Invest.* 5, 218 (1953).
9. S. C. Byers and M. Friedman, *J. Clin. Invest.* 35, 405 (1956).

* Research Fellow of the National Academy of Sciences (U.S.), in cooperation with the Foreign Operation Administration (FOA), with the OECE and the CIR of Italy.

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Missing Step in Guinea Pigs Required for the Biosynthesis of L-Ascorbic Acid

Man, other primates, and guinea pigs are the only mammals known to be unable to synthesize L-ascorbic acid; thus they require vitamin C in their diet to prevent scurvy. It has not been known

Table 1. Conversion of L-gulonolactone to L-ascorbic acid by rats and guinea pigs.

Species	Conversion* (%)
Rat	9.1
Rat	7.2
Guinea pig	< 0.2
Guinea pig	< 0.2

* Estimated from the amount of C^{14}-labeled L-ascorbic acid present in the animal 24 hours after intraperitoneal administration of 12-mg doses of L-gulonolactone-1-C^{14} (*3*).

Table 2. Conversion of L-gulonolactone to L-ascorbic acid in rat and guinea pig livers. Five milligrams of L-gulonolactone-1-C^{14} (0.6 μc) was incubated for 90 minutes at 37°C under air in 5 ml of a 10-percent homogenate (or microsomes from an equivalent amount of liver) in 0.15*M* phosphate buffer (*p*H 7.20) containing 0.13*M* sucrose. L-Ascorbic acid was isolated after addition of carrier (150 mg) from a 5-percent trichloroacetic acid extract of the incubation mixture as its 2,4-dinitrophenylosazone derivative (*3*).

Species	Conversion (%)
Homogenate	
Rat	12.0
Rat	8.0
Rat	3.6
Guinea pig	< 0.05
Guinea pig	< 0.05
Microsomes	
Rat	18.6
Rat	7.7
Rat	3.8
Guinea pig	< 0.05
Guinea pig	< 0.05

why these species are unable to synthesize L-ascorbic acid. This report presents results that point out a possible missing biochemical step in guinea pigs required for the synthesis of the vitamin.

Previous work has shown that L-ascorbic acid is formed from D-glucose in the rat by the following steps: D-glucose → D-glucuronolactone → L-gulonolactone → L-ascorbic acid. Evidence for this scheme comes from experiments in which the incorporation of C^{14} into L-ascorbic acid was measured after administration of various D-glucose tracers (*1*), uniformly labeled D-glucuronolactone (*2*) and carboxyl-labeled D-glucuronolactone and L-gulonolactone (*3*). Further evidence for this pathway in rats was presented by Isherwood and coworkers (*4*), who reported increased urinary excretion of L-ascorbic acid after administration of nonlabeled D-glucuronolactone and L-gulonolactone.

In the present study the role of L-gulonolactone as a precursor for L-ascorbic acid biosynthesis in rats and guinea pigs was compared. In contrast with the appreciable conversion of L-gulonolactone to L-ascorbic acid in the rat, no detectable conversion was observed in guinea pigs (Table 1). Similarly, results of experiments *in vitro* showed considerable synthesis of L-ascorbic acid from L-gulonolactone in homogenates of rat liver but none in those of the guinea pig (Table 2) (*5*). Cellular fractionation studies (*6*) showed that the conversion of L-gulonolactone to L-ascorbic acid occurs chiefly in the microsomes of rat liver, but no activity was detected in the microsomes of guinea pig liver (Table 2). Rat liver mitochondria possess about one-fourth of the activity of microsomes in converting L-gulonolactone to L-ascorbic acid, but none was found in the mitochondria of the guinea pig liver. The system for converting L-gulonolactone to L-ascorbic acid in the rat is apparently present only in the liver, since incubation of L-gulonolactone-1-C^{14} under identical conditions in homogenates of kidney, brain, small intestines, and muscle resulted in no synthesis of the vitamin (< 0.05-percent conversion).

These results showing that microsomes of guinea pig liver are unable to convert L-gulonolactone to L-ascorbic acid point out the possible missing biochemical step in this species needed for the synthesis of L-ascorbic acid. A more definite conclusion on whether this is the only step missing in guinea pigs will be possible when information becomes available on the enzymes required for the synthesis of L-gulonolactone from D-glucose.

J. J. Burns
Pincus Peyser
Arnold Moltz
Laboratory of Chemical Pharmacology, National Heart Institute, National Institutes of Health, Bethesda, Maryland, and the *Research Service, Third (New York University) Medical Division, Goldwater Memorial Hospital, New York*

References and Notes

1. S. S. Jackel *et al.*, *J. Biol. Chem.* **186**, 569 (1950); H. H. Horowitz and C. G. King, *ibid.* **200**, 125 (1953); J. J. Burns and E. H. Mosbach, *ibid.* **221**, 107 (1956).
2. H. H. Horowitz and C. G. King, *J. Biol. Chem.* **205**, 815 (1953).
3. J. J. Burns and C. Evans, *ibid.*, in press.
4. F. A. Isherwood, T. Y. Chen, L. W. Mapson, *Biochem. J.* **56**, 1 (1954).
5. Unpublished experiments with L-ascorbic acid-1-C^{14} show that this compound is extensively metabolized in rat liver homogenates but to a lesser extent in guinea pig homogenate. The values reported in Table 2, therefore, represent minimum values for the conversion of L-gulonolactone to L-ascorbic acid in the rat liver.
6. Microsomes and mitochondria were prepared in isotonic sucrose by the method of W. C. Schneider and G. H. Hogeboom [*J. Biol. Chem.* **183**, 123 (1950)].

12 October 1956

Table 1. Accumulation of uric acid and its precursors (hypoxanthine and xanthine) in rat liver as a result of anaerobiosis. Incubation was carried out for 1 hour at 37.5°C in 100-percent oxygen or nitrogen.

Fraction	Uric acid	Hypoxanthine + xanthine
	(μmole/100 g wet weight of liver or equivalent of incubation medium)	
Incubation in oxygen		
Tissue	8.1	4.8
Medium	0.0	21.5
Total	8.1	26.3
Incubation in nitrogen		
Tissue	14.5	62.5
Medium	72.7	240.0
Total	87.2	302.5

Uric Acid Metabolism and the Mechanism of Iron Release from Hepatic Ferritin

Previous studies in our laboratory have suggested that lowered oxygen tension is a stimulus for the release of iron from ferritin stores in the liver. During the hypoxia that attends the drastic hypotension of hemorrhagic shock, increasing quantities of iron appear in the plasma, in amounts almost sufficient to saturate the iron-binding capacity of the plasma. *In vitro* studies have shown that, as ferritin iron is reduced to the ferrous state, it becomes less tightly bound to the protein and can transfer across a membrane or be removed from ferritin in the presence of an avid iron-binding compound such as α,α′-dipyridyl or the iron-binding protein of the plasma (*1*).

The results of the present study (*2*) point to the involvement of the enzyme, xanthine oxidase, as well as the product of its activity, uric acid, in the reduction of ferritin iron. Slices of normal rat liver that are incubated in a nitrogen atmosphere in Ringer-phosphate solution liberate into the medium a substance that is capable of reducing ferritin iron. This compound has been identified as uric acid by means of its absorption spectrum, reduction of the uric acid reagent, and the disappearance of these two properties after incubation with the enzyme, uricase, which is highly specific for uric acid.

Analyses of both the liver slices and the medium in which they had been incubated (Table 1) revealed an over-all accumulation of uric acid. Of the rat tissues examined, liver, spleen, kidney, and small intestine, only the liver produced more uric acid in response to lowered oxygen tension; the others produced less. An examination of aerobic and anaerobic liver slices for their content of uric acid precursors, xanthine and hypoxanthine, showed a considerable accumulation of these compounds under anaerobic conditions (Table 1). This increase is presumed to result from depletion of high-energy compounds needed for synthetic reactions involving the purines, and from the resultant increase in catabolic reactions (*3*). Uricase, which is present in rat liver, was found to be much more sensitive to inhibition by lowered oxygen tension than xanthine oxidase.

Xanthine oxidase may act as a dehydrogenase in the presence of a suitable