TABLE I. Analysis of Amphiuma Liver Melanoprotein Granules in % Dry Weight.

	Claude(3)	This paper
Phosphorus	.09	.59
Sulfur	2.16	1.30
Iron	.24	.10
Copper	.02	.08
Lipid	2.1	—

amine, tryptophane and phenylalanine in the H_2O_2 soluble fraction than in the bleached particles. The bleached granules from amphiuma liver and from the melanoma were both positive for the PAS (periodic acid Schiff) reaction in confirmation of the corresponding histochemical observations on cryostat sections(1).

The elementary analysis of the amphiuma liver sample is compared with Claude's(3) results in Table I; no comment concerning their differences is useful since the isolation techniques were different and because of expected normal variation in the chemical properties of the granules(1). The lipid paper chromatographic analysis of a chloroform-methanol extract of amphiuma liver melanoprotein showed sphingomyelin and monophosphoinositide and very little neutral lipid.

A preliminary series of histochemical observations were made on another human melanoma obtained as a surgical specimen and on a number of S-91, B-12, and H.P. mouse melanomas. The histochemical properties of the various granules in these melanomas, particularly as detected by the PAS reaction, resembled very closely those in amphiuma liver(1) and provide very encouraging evidence that the melanin pigment cell system described for amphiuma liver(1) is also operating in melanomas. Details will be reported later.

We have supplied Professor R. A. Nicolaus of the Institute of Organic Chemistry, University of Naples, with freeze-dried samples of amphiuma liver. According to Professor Nicholaus' analyses, amphiuma liver melanin is of the indole type; a report of his work will appear in Tetrahedron.

Mason *et al*(4) have reported 12 amino acids (not named) in acid hydrolysates of *Sepia* melanin assayed by paper chromatography. *Sepia* melanin has been extensively studied by Piattelli and associates(5).

Summary. Acid hydrolysates of melanoprotein isolated by differential centrifugation from amphiuma liver and from a human liver melanoma show them to be exceedingly similar with 20 amino acids identified. Bleaching of the melanoprotein by H_2O_2 fragmented both the melanin and the protein into a large number of unidentified ninhydrin reactive components. The bleached particle was positive to the PAS reaction.

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An Assessment of the Role of Alpha and Beta Chylomicra in Hyperlipemic States.* (29245)

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In alimentary lipemia and in the fasting hyperlipemia occurring in various disease states, chylomicra have been found to vary by electrophoresis, fatty acid analysis, and

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polyvinylpyrrolidone (PVP) gradient flocculation(1-5).

Swahn(1) found that chylomicra were separable into two classes according to their migration with α_2 or β globulins on free electrophoresis. He found that chylomicra from a case each of essential hyperlipemia, nephrotic syndrome, and diabetic lipemia consisted of both α and β types while alimentary lipemia had only the α type and myxedema with lipemia had only the β type. Bierman, Gordis and Hamlin(5) succeeded in separating the chylomicra of alimentary lipemia into α and β types by starch granule electrophoresis and PVP gradient flocculation. Their finding of α chylomicra alone in thoracic duct lymph and the close similarity of the triglyceride fatty acids (TGFA) of α chylomicra to those of ingested fat at the peak of alimentary lipemia points to the intestinal origin of these particles. The extra-intestinal origin of β chylomicra is favored by their absence from thoracic duct lymph and by the dissimilarity, early in fat absorption, of their TGFA to those of ingested fat. The perfused liver of the fed rat is the only organ, except for intestine, that has been demonstrated to synthesize chylomicra(6). In the human, the liver is assumed, but not proven, to be the source of β chylomicra.

The present investigation was designed to study the relative roles of accumulation of α and β chylomicra in production of various hyperlipemic states and to assess the influence of varying levels of one type of particle upon the retention of the other type.

Materials and methods. Heparinized plasma, grossly lactescent in each case, was obtained from subjects who had been fasting for 12 hours, or longer as noted in Table II. Lipids were extracted from plasma and quantitated by silicic acid column chromatography (7). Separation of the chylomicra of plasma into α and β classes was performed using a modification of the PVP gradient column of Gordis(4). PVP of average M.W. 40,000, obtained from Mann Research Laboratories, Inc., New York, was used to make stock solutions of these concentrations: A) 10% PVP in 19% NaCl and B) 5%, 4%, 3%, 2%, 1% and 0% PVP, all in 10% NaCl. All other reagents were of analytical grade, used as obtained from the manufacturer. One ml heparinized plasma was pipetted into the bottom of a 16 × 150 mm glass test tube, and 1 ml of soln. A was added and mixed with the plasma. Three ml of 5% PVP in 10% NaCl was layered carefully on top of the plasma-PVP-NaCl solution, followed by 3 ml portions of the 4%, 3%, 2%, 1%, and 0% PVP in 10% NaCl solutions. Interfaces were visible at each change in concentration. The tubes were then placed in a 37°C room. After 16 hours, zones of turbidity were recorded as being either in the 0% layer (α), the 5% layer (β) or in both. In some instances the turbid zones were removed by capillary pipette from the tubes, and were placed in 150 ml CH_3OH-$CHCl_3$ 1:1 (V/V). After standing at room temperature for one hour, the liquid was filtered through a coarse sintered glass funnel into a flask, 90 ml H_2O was added, the flask swirled, and the resulting emulsion broken by centrifugation at 1000 rpm for 30 minutes. The CH_3OH-H_2O layer was removed to just above the interface. The bottom phase and interface were transferred to a flask and the solvent was removed, with the aid of small amounts of benzene, with a rotary vacuum evaporator at 40°C. To the dry material was added 10 ml CH_3OH-$CHCl_3$ (1:1 V/V), which usually dissolved it but occasionally produced a fine suspension. Fifty ml petroleum ether and 50 ml H_2O were then added. The petroleum ether phase was removed down to the interface and the bottom layer was extracted twice more with 50 ml portions of petroleum ether. The petroleum ether phases were combined, dried on a rotary vacuum evaporator at 40°C, and the lipid was taken up in $CHCl_3$ and transferred to a vial where it was dried and weighed.

One cm columns of .15 M NaCl were layered over 2 plasma samples, one containing predominantly α (subject EB) and one containing predominantly β chylomicra (subject HM), as determined by PVP flocculation. These were then centrifuged for 10^6 G-minutes(8). The chylomicra were harvested and were subjected to starch granule electrophoresis and subsequent elution and quantitation

TABLE I.

Subject	Clinical diagnosis	PVP	Total lipid	Lipid P	TG	CE*	Chol†	Fat intake
			mg %					
		Group 1						
M.D.	Diabetes	β	1950	18.3				Normal
H.M.	"	$\beta > \alpha$	1800	13.7	1020	192	72	"
T.B.	Nephrotic syndrome	"	1780	17.1				"
R.F.	Diabetes	β	3540	30.3	1680	371		50 g
B.S.	CHO-induced hyperlipemia	"	1530	12.0	884	119	73	29 g
		Group 2						
R.S.	Fat-induced hyperlipemia & recurrent abdomen pain	$\alpha > \beta$	6000	38.4	3700	459	224	Normal
E.B.	*Idem*	"	8620	39.6	6300	438	251	"
W.J.	"	"	3420	15.0	2420	229	140	"
A.M.	Alcoholism, probable pancreatitis	"	7100	44.7	4280	452	495	NPO 24 hr

* Cholesterol in cholesterol esters. † Cholesterol.

of lipid by the method of Kunkel and Trautman(9). The separation into predominantly α or β migrating lipids by electrophoresis, using a normal plasma sample as reference, agreed with the results obtained by PVP flocculation.

Under the same experimental conditions employed to flocculate the chylomicra of lactescent plasma samples, clear fasting plasma samples from normal subjects with normal triglyceride (TG) levels show a thin ring of very faint turbidity in the 5% zone, much less than was noted in any of the lipemic samples. One cm columns of .15 M NaCl were layered over 2 samples of clear plasma. These were then centrifuged for 10^6 G-minutes. Aliquots of the clear NaCl supernate and of the clear infranate were subjected to PVP flocculation, and a ring of very faint turbidity was noted in the 5% zone of each sample. Similar turbidity was noted in ultracentrifugal fractions D 1.063-1.019 and D 1.019-1.005 of a lactescent sample of subject BS. Since α and β particles are spun to the top by the first procedure(8), and are not present in D 1.063-1.005, the faint turbidity noted is not due to flocculation of particulate fat.

Results and discussion. The results of PVP gradient flocculation of plasma from subjects with hyperlipemia are presented in Table I, and are arranged in two groups. In Group 1, β chylomicra predominate or are present exclusively, while in Group 2, α chylomicra predominate. The presence of small quantities of β chylomicra in Group 2 subjects was substantiated by lipid analysis of the PVP zones of subject WJ and by electrophoresis of the sample of subject EB.

The 2 subjects of Group 1 whose fat intakes were restricted had β chylomicra alone in their fasting plasma. Three subjects of Group 1 were eating normal amounts of fat. One of these also had β chylomicra alone, while the other 2 had small quantities of α chylomicra in addition. To investigate this apparent impedance of α-chylomicron clearing by β-chylomicronemia, subject BS was studied serially during dietary induction of hyperlipemia and during subsequent remission (Table II). At first, β chylomicra alone were present. As TG levels rose on a 619 g carbohydrate (CHO), 29 g fat diet, subject BS accumulated α chylomicra due, probably, to saturation of the removal mechanism. A change in diet to 235 g CHO, 186 g fat was at first associated with continued retention of α chylomicra, due perhaps to the increase in dietary fat. After 4 days on the same diet, TG levels had decreased markedly, and α chylomicra had been completely cleared. It appears that α-chylomicron clearance can occur normally in this subject on diets which provoke as well as ameliorate hyperlipemia, and that α-chylomicron retention depends upon the degree of β-chylomicronemia itself. Unless β and α chylomicra clear by different mechanisms, the primary cause of CHO-induced hyperlipemia would seem to be an increase in β-chylomicron synthesis.

TABLE II.

Subject	Date	Diet	Length of fast, hr	PVP Qual.	PVP Quant., mg %	Total lipid	Lipid P	TG	CE	Chol
						mg %				
W.J.	1/ 5/63	*Ad lib*		α-sl.β		3420	15.0	2420	229	140
	1/15/63	113 g Pr., 145 g fat, 416 g CHO, 3421 Cal. (for 5 days)	16	"	α-2130 β-410	2930	18.6	2150	164	104
			21	"	α-1200 β-220	2270	13.2	1560	137	58
	1/22/63	113 g Pr., 32 g fat, 683 g CHO, 3472 Cal. (for 7 days)	15	β-sl.α	α-150 β-490	1070	9.7	626	156	47
			16	"	α-140 β-340					
			16½	"	α-90 β-290					
			17½	"	α-80 β-190					
			18½	"	α-100 β-130	1130	10.7	611	82	62
			21	β only		1090	11.2	608	86	60
B.S.	9/26/62	69 g Pr., 29 g fat, 619 g CHO, 3013 Cal.	12	Clear		770	8.3	267	133	28
	10/ 1/62		"	β only		1530	12.0	884	119	73
	10/ 2/62		"	"		1880	15.2			
	10/ 4/62		"	Not done		2450	18.1	1490	178	116
	10/ 5/62	91 g Pr., 186 g fat, 235 g CHO, 2978 Cal.	"	α>β		3160	19.5	2100	178	139
	10/ 6/62		"	"		4160	20.1	2630	480	140
	10/ 8/62		"	β only		1910	14.6	971	214	104
	10/10/62		"	"		1630	13.3	814	232	64
	2/ 7/63		"	Not done		775	9.2	129	174	57

The presence of a preponderance of α particles in the patients of Group 2 is compatible with the defects in clearing which have been demonstrated in fat-induced hyperlipemia (10) and in alcohol-induced hyperlipemia with pancreatitis(11). The presence of β particles in addition is, at first glance, most simply explained by the mechanism postulated as operative in subject BS, namely removal mechanism blockade or saturation by high levels of TG, leading, in these subjects, to secondary accumulation of β particles. However, when α chylomicra are cleared completely from the plasma of subject WJ by a low-fat diet and a 21 hour fast (Table II), β chylomicra remain, suggesting the additional presence of an abnormality in β-chylomicron metabolism.

I postulate that hyperlipemia may arise primarily either from failure of clearance of normally-produced α chylomicra (Group 2) or from excessive production of β chylomicra (Group 1). The observations presented above show that primary failure of clearance of α chylomicra is accompanied by accumulation of β particles. From the time sequence of disappearance of α and β particles during a fast, it appears likely that an abnormality in β chylomicron metabolism accompanies this defect in α chylomicron removal. On the other hand, accumulation of β chylomicra may have no associated α chylomicron retention with a normal dietary intake of fat indicating that, in some subjects, grossly normal clearing is possible, and that overproduction of β chylomicra is alone responsible for the hyperlipemic state.

Summary. Plasma chylomicra of fasting subjects with hyperlipemia were separated into α and β types by PVP gradient flocculation. Subjects with diabetes, CHO-induced hyperlipemia and the nephrotic syndrome had predominantly or exclusively β chylomicra (Group 1), while those with fat-induced hyperlipemia and severe hyperlipemia associated with alcoholism and probable pancrea-

titis had predominantly α chylomicra (Group 2). Consideration of the diet of each subject and of serial observations of a subject from each group during dietary manipulation leads to these conclusions: β chylomicronemia occurs primarily because of increased synthesis. Clearing may be grossly normal or moderately decreased at moderately elevated TG levels, and may become severely impaired at high TG levels, due to these levels *per se*. α-Chylomicronemia occurs primarily because of impaired clearing but is regularly accompanied by retention of small quantities of β chylomicra.

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Applicability of the HeLa (Gey) Strain of Human Malignant Epithelial Cells to the Propagation of Arboviruses.* (29246)

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Scherer and Syverton(1), while investigating the viral range of the HeLa strain of human malignant epithelial cells(2), obtained encouraging results with some arthropod-borne (arbo) viruses of groups A and B. Since it seemed desirable to study one *in vitro* host system extensively, efforts have been continued to determine the practical value of HeLa cells for propagation of arboviruses. A total of 94 distinct arbovirus strains have now been tested in these laboratories for their ability to produce a cytopathic effect (CPE) in HeLa cells. The purpose of this report is to provide an up-to-date list of these viruses and to discuss their cytopathology in HeLa cells.

Materials and methods. Viruses. The viruses tested are listed in Table I, grouped according to the classification in use at the Rockefeller Foundation Virus Laboratories at the end of 1962. For the initial *in vitro* passage, infected newborn mouse brain suspensions, liver suspensions and sera were sources of virus. Inocula consisted of liquid or lyophilized virus stocks stored in glass-sealed ampules at —70° and —20°C, respectively. Occasionally, fresh virus preparations were used.

Cellular cultivation and inoculation and incubation of cultures. The procedures for growing and maintaining HeLa cells have varied somewhat through the years(3,4); however, standard methods have been developed and described in detail(5). It has been the experience in these laboratories that a pH of 7.2 $\pm$ 0.2 in the inoculated cultures is optimal for growth of arboviruses at an incubation temperature of 37°C.

Assays for CPE of viruses. Increasing 10-fold dilutions of each strain were inoculated

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