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Recombination with lipids of the lipid-free protein from canine serum (d 1.063-1.21, α_1) lipoprotein*

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SUMMARY

The protein (αP), prepared by delipidation of canine serum α_1-lipoprotein (αLP), when labeled with I^{131} and injected into dogs, was metabolized at the same rate as native αLP, labeled in the protein moiety with I^{131}. When αP-I^{131} was added to serum or injected into dogs, the radioactivity promptly appeared only in the αLP fraction, indicating a preferential interaction of the labeled protein with its own lipoprotein class. The nature of this interaction was not established. Mixing of αP-I^{131} with the low density lipoprotein class (βLP), in absence of serum, yielded two radioactive fractions, floating at d 1.063 and d 1.21. These two fractions had electrophoretic mobility similar to radioiodinated native βLP and αLP. In the absence of serum, αP-I^{131} reacted also with chylomicrons from serum or chyle. When the radioactive chylomicrons thus formed were injected into dogs, their disappearance from circulation paralleled that of an injected Lipomul (artificial triglyceride emulsion)-αLP-I^{131} complex. In both instances the disappearance of triglycerides was accompanied by appearance of radioactivity in the αLP fraction of plasma. When Lipomul was given intravenously to dogs injected with αLP-I^{131}, it combined with a small amount of this labeled lipoprotein. The possible participation of αLP in the metabolism of triglycerides is briefly discussed.

Studies from this laboratory have shown that the high density d 1.063–1.21 lipoprotein of human serum can be freed of practically all its lipids to yield a protein, apparently undenatured and identical from the immunochemical standpoint, with the native lipoprotein (1, 2). Further, *in vitro* experiments have shown that this lipid-free protein has high avidity for lipids and, under suitable conditions, combines with them to reconstitute the original protein-lipid complex (3).

The studies reported below deal with the *in vitro* and *in vivo* recombining capacity for lipids of the lipid-free protein from d 1.063–1.21 lipoprotein of canine serum.

MATERIAL AND METHODS

Sera from fasting normal dogs were pooled and chylomicrons removed by ultracentrifugation as previously described (4). The remainder, adjusted to d 1.063 by addition of solid NaCl (5), was centrifuged in a Spinco Model L Ultracentrifuge, 30.2 rotor, 79,420 × *g* at 16° for 18 hours. The top fraction (d 1.006–1.063 lipoproteins) was removed and the remaining serum brought to d 1.21 by addition of solid KBr (6). This serum was then spun in a Spinco 40 rotor at 105,000 × *g* at 16° for 24 hours and the top fraction (d 1.063–1.21 lipoprotein) removed.

Both d 1.006–1.063 and d 1.063–1.21 lipoproteins were purified by ultracentrifugation as reported previously (3) and dialyzed against 0.15 M NaCl. The preparations were considered satisfactory when neither albumin nor α_1-globulin was detected by starch gel electrophoresis (7, 8) and immunoelectrophoresis (9).

We will refer, for brevity, to d 1.006–1.063 and d 1.063–1.21 lipoproteins as β- and α_1-lipoproteins, respectively, according to their electrophoretic mobility.

Large aliquots of purified α_1-lipoprotein were freed of lipids by the ethanol-ether procedure of Scanu *et al.* (1) and the α_1-protein thus obtained was desiccated and stored at 0°. For use, an appropriate amount of this material was dissolved in 0.15 M NaCl.

I^{131} labeling of α_1-lipoprotein, α_1-protein, and β-lipoprotein was performed according to McFarlane (10); free iodine was removed by an ion exchange resin (Ioresin®, Abbott). The labeled protein was estimated

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to have about one atom of iodine per molecule. A molecular weight of 75,000 was assumed for the lipoprotein protein. The lipid moiety of the lipoproteins contained 4% to 5% of the total radioactivity.

Ultracentrifugal separation and purification of serum chylomicrons were performed as described previously (4), starting from pooled lipemic sera separated from dog's blood 6 hours after oral administration of 500 ml of 18% cream. Chyle chylomicrons were also used in our experiments. The chyle was obtained from two dogs in which the thoracic duct was cannulated as described by Albrink *et al.* (11) 3 hours following the administration of 500 ml of 18% cream. The separation of chyle chylomicrons was performed as for serum chylomicrons.

Total lipids were determined gravimetrically by Sperry's method (12). Total proteins were determined by Lowry's method (13). In lipemic samples, the protein content was also calculated by multiplying by the factor 6.25, the values of Kjeldahl nitrogen, obtained by the micromethod of Lang (14). In the *in vitro* studies αP-I^{131} [1] was mixed with whole serum, αLP, βLP, chylomicrons, or a cottonseed oil emulsion (Lipomul®, Upjohn). The mixtures were then incubated for 10 minutes at 24° and analyzed by ultracentrifugation and starch gel electrophoresis.

In the *in vivo* experiments six male mongrel dogs, 12 to 16 kg, were used. Throughout the course of the experiments they were kept in metabolism cages. Three days preceding the injection of the labeled lipoprotein and during the entire course of the experiments, 10 drops of Lugol's solution was added daily to the drinking water to prevent uptake of radioiodine by the thyroid. Each dog was given intravenously 1 to 2 mg[2] of αLP-I^{131} or αP-I^{131} of a specific activity of 20 to 30 μc/mg. Blood samples were taken immediately, 10, and 20 minutes after the injection of the labeled protein, and then at 24-hour intervals. Hematocrit values and αLP protein concentration were determined in all samples. αLP was separated by ultracentrifugation and its protein content determined by Lowry's method. In most of the serum samples the distribution of radioactivity was studied in the protein fractions separated by starch gel electrophoresis and in the lipoprotein fractions separated by ultracentrifugation. Starch gel electrophoresis analysis was performed according to Smithies (7), using a discontinuous system of buffers at pH 8.4; $\Gamma/2$, 0.05 (8). Amidoschwarz 10 B Bayer was used for protein staining and Spinco Oil Red O was used for lipid staining. Radioactivity was determined in a sodium iodide crystal scintillation detector (Tracerlab) with a counting efficiency of about 36%, determined by means of an I^{131} standard.

Calculations. The plasma volume was calculated by the usual isotopic dilution technique according to the formula $V_2 = C_1V_1/C_2$, where V_2 is the volume of circulating plasma in ml, C_1 the concentration of the total radioactivity of injected αLP-I^{131} or αP-I^{131} in cpm/ml, V_1 the volume of the injected material, and C_2 the radioactivity of dog's plasma in cpm/ml at 10 or 20 minutes from the injection. Half time, $(T/2)$ was calculated from the slope of the exponential line of disappearance of the radioiodinated lipoprotein protein from plasma. For the calculation of the volume of distribution, this line was extrapolated back to zero time. The curves of disappearance of the radioiodinated lipoprotein protein from plasma was obtained by plotting on a semilog paper time against per cent radioactivity retained, considering 100% the serum radioactivity at zero time. The value for total exchangeable pool size was the sum of the amounts of lipoprotein protein in the intra- and extravascular compartments. Turnover time (T_t) was calculated by the formula: $1.44 \times T/2$. The value for replacement rate was obtained by the formula: body pool in mg/g $\times$ fractional turnover time $(1/T_t)$.

RESULTS

Interaction of αP-I^{131} with Whole Serum, αLP, and βLP. αP-I^{131}, 100 μg, was mixed *in vitro* with 1 ml of fasting dog's serum and the mixtures were analyzed by ultracentrifugation at various salt densities. The data, summarized in Figure 1, indicate that αP-I^{131}, which alone sediments at d 1.21, floats at this density after in-

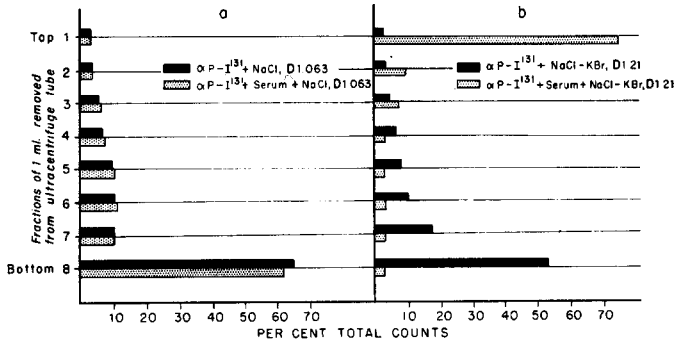

FIG. 1. Distribution of radioactivity in ultracentrifuge tubes after spinning αP-I^{131}, alone and mixed with serum, at d 1.063 (*a*) and 1.21 (*b*). Components: αP-I^{131}, 100 μg, specific activity 0.5 μc/mg, dog serum 8 ml, protein content 0.56 g. Densities were adjusted after incubation of the mixtures at 24° for 10 minutes. 30.2 rotor, 79,400 $\times$ *g*, 16°, 18 hours.

[1] Abbreviations used in the text: αLP = α_1-lipoprotein; αP = lipid-free α_1-protein; βLP = β-lipoprotein. αLP-I^{131}, αP-I^{131}, and βLP-I^{131} indicate radioiodinated αLP, αP, and βLP.

[2] In the text the weight of the lipoproteins is expressed in milligrams of lipoprotein protein.

cubation with serum. This radioactive top fraction at d 1.21 had electrophoretic and ultracentrifugal characteristics of a native αLP.

The ultracentrifugal analysis of a mixture of αP-I^{131} (100 μg) and αLP (1 mg) also showed presence of a radioactive fraction floating at d 1.21 (Fig. 2). Studies

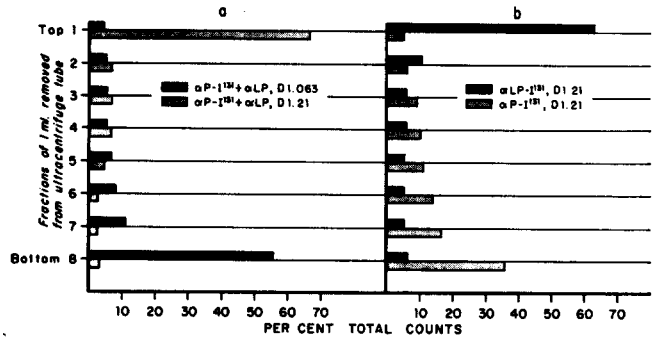

FIG. 2. Distribution of radioactivity after ultracentrifugation of a mixture of αP-I^{131} and αLP (*a*) at d 1.063 and 1.21. In (*b*) αP-I^{131} and αLP-I^{131} were spun alone at d 1.21. Components: αP-I^{131}, 100 μg, specific activity 0.5 μc/mg, αLP, protein content, 1 mg, αLP-I^{131}, 1 mg, specific activity 0.1 μc/mg. For experimental conditions see legend to Figure 1.

were also performed by starch gel electrophoresis. By this technique αLP gave three boundaries, each stainable for protein and lipids. αP also gave three boundaries, only stainable for protein, and with mobilities higher than the corresponding fractions of the native lipoprotein. The data, represented diagrammatically in Figure 3, indicate that αP-I^{131}, after incubation with αLP, has a reduced electrophoretic mobility and moves with αLP in the electrophoretic field.

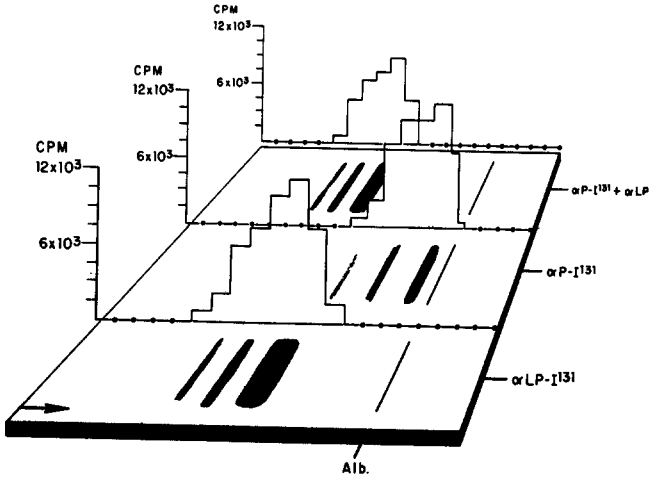

FIG. 3. Starch gel electrophoresis patterns of αLP-I^{131}, αP-I^{131}, and a mixture of αP-I^{131} and αLP. αLP-I^{131}, 2 mg, specific activity 50,000 cpm/mg. αP-I^{131}, 2 mg, specific activity 50,000 cpm/mg. The mixture contained 200 μg of αP-I^{131} and 2 mg of αLP. Staining: Amidoschwarz.

Mixtures of αP-I^{131} (100 μg) and βLP (1 mg), after ultracentrifugation at d 1.003, did not show a significant amount of radioactivity in the top layer. At d 1.063, the top fraction contained 33% of the total radioactivity (Fig. 4*a*). When this radioiodinated layer was removed and the remainder spun at d 1.21, 26% of the radioactivity contained in the ultracentrifuge tube floated (Fig. 4*b*). These labeled top fractions at d 1.063 and d 1.21, when respun at their respective densities, appeared in the top layer of the ultracentrifuge tube; when analyzed by starch gel electrophoresis, the distribution of radioactivity was the same as that of control samples of αLP-I^{131} and βLP-I^{131}.

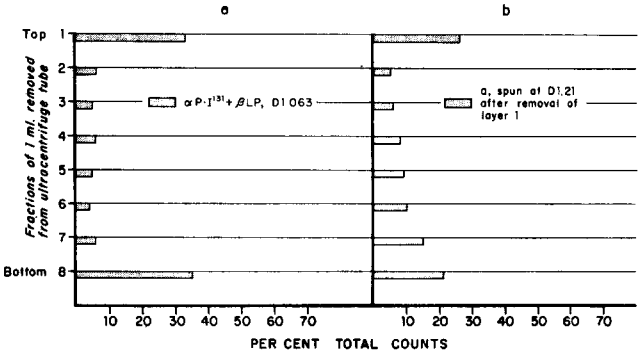

FIG. 4. Distribution of radioactivity in ultracentrifuge tubes after spinning a mixture of αP-I^{131} and βLP at d 1.063 (*a*) and at d 1.21 (*b*) after removal of the top fraction from (*a*). Components: αP-I^{131}, 100 μg, specific activity 0.5 μc/mg, βLP 1 mg. For experimental conditions see legend to Figure 1.

Addition of 1 mg of αLP to a mixture containing 100 μg of αP-I^{131} and 1 mg of βLP, prevented the interaction between αP and βLP.

When αLP-I^{131}, instead of αP-I^{131}, was mixed with βLP, the latter failed to become radioactive.

In the *in vivo* experiments, dogs were first injected with αLP-I^{131} and the rate of disappearance of the labeled protein from plasma was followed for 4 weeks. After a period of rest of 2 to 3 months, the same dogs were injected with αP-I^{131} and serum radioactivity followed again for 4 weeks. The results of a typical experiment are reported in Figure 5. Both αP-I^{131} and αLP-I^{131} had a monomial phase of metabolic degradation with a half time of 3.62 and 3.50 days, respectively. The values of half time, turnover time, and replacement rate (Table 1) for the injected αP-I^{131} and αLP-I^{131} were quite similar in all dogs studied.

αLP-I^{131} and αP-I^{131}, after intravenous administration into dogs, both migrated electrophoretically with the αLP fraction of serum (Fig. 6) and floated with the d 1.063–1.21 lipoprotein class.

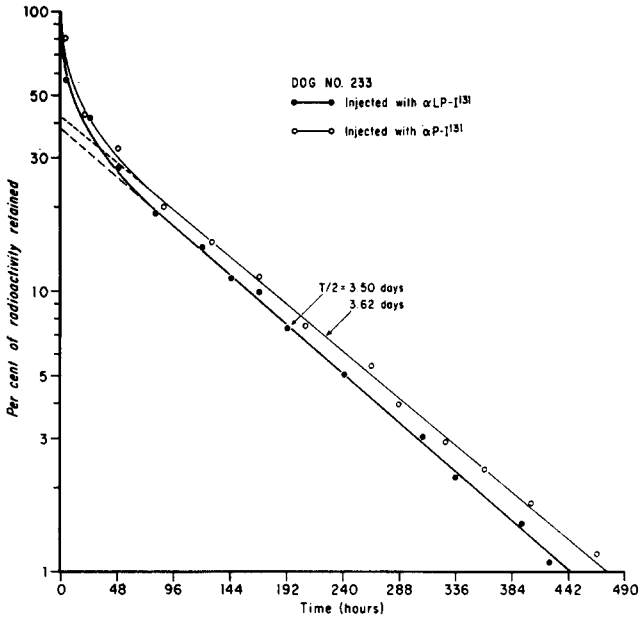

FIG. 5. Curves of disappearance of αP-I^{131} and αLP-I^{131} from circulation. Dose injected intravenously. αLP-I^{131}, 2 mg, specific activity 40 μc/mg; αP-I^{131}, 2 mg, specific activity 40 μc/mg.

Interaction of αP-I^{131} with Serum Chylomicrons, Lymph Chylomicrons, and an Artificial Fat Emulsion (Lipomul). Since fasting serum was used in the above experiments, the interaction of αP-I^{131} with chylomicrons could not be studied. Therefore *in vitro* experiments were first performed in which αP-I^{131} and αLP-I^{131} were mixed with both chyle and serum chylomicrons. These mixtures were spun at d 1.003 and the distribution of radioactivity determined. The mixtures of αP-I^{131} and chylomicrons from both chyle and serum showed a significant amount of radioactivity (36.6% and 34.2%) in the top 1 ml fraction (Table 2). This radioactive top fraction was not present when αP-I^{131} or αLP-I^{131} were spun alone at d 1.003 or when αLP-I^{131} was mixed with chylomicrons.

The problem was further investigated by studying the interactions of αP-I^{131} with Lipomul alone, or previously mixed with αLP. These results are summarized in Table 3. Formation of a labeled complex floating at d 1.003 occurred when αLP-I^{131} or αP-I^{131} were mixed with Lipomul alone. On the other hand, when Lipomul-αLP mixture was used, the labeled complex occurred after addition of αP-I^{131}, but not after addition of αLP-I^{131}. Lipomul-serum mixtures did not interact with either αLP-I^{131} or αP-I^{131}. In these experiments the mixtures of Lipomul-αLP and Lipomul-serum were purified according to the technique used for preparation of chylomicrons (10). As indicated in Table 3, lipemic sera from dogs in absorptive phase, when mixed with αP-I^{131} or αLP-I^{131}, failed to show a significant rise of radioactivity at d 1.003.

The following *in vivo* experiments, each one including two dogs, were performed. (*a*) Dogs were injected with either αP-I^{131} or αLP-I^{131}. A day later, when specific activity values for their plasma αLP was 5,000 to 10,000 cpm/mg, 500 ml of 18% cream was fed to the animals and 100 ml of blood withdrawn 6 hours later. The concentrated chylomicrons, separated and purified by ultracentrifugation at d 1.003 from the hyperlipemic sera, had a total lipid content of 20 mg/ml and contained no radioactivity. (*b*) Dogs were fed 500 ml of 18% cream and 6 hours later αP-I^{131} or αLP-I^{131} was

TABLE 1. TURNOVER OF αLP-I^{131} AND αP-I^{131} FOLLOWING INTRAVENOUS INJECTION INTO DOGS

Dog	Material Injected	Body Weight	P* Serum Conc.	Intravasc. Compartment Vol.	Intravasc. Compartment P	Extravasc. Compartment Vol.	Extravasc. Compartment P	Pool Size	T/2	Turnover Time	Replacement Rate
		kg	*mg/100 ml*	*ml*	*mg*	*ml*	*mg*	*mg*	*days*	*days*	*mg/g/day*
A	LP	14.9	293	590	1725	962	2820	4545	3.60	5.18	0.060
	P	15.0	284	538	1510	798	2266	3776	3.75	5.40	0.046
B	LP	13.0	273	495	1360	806	2200	3560	3.50	5.04	0.054
	P	13.2	262	470	1210	755	1970	3180	3.60	5.18	0.046
C	LP	16.0	248	676	1673	1057	2621	4294	3.51	5.05	0.053
	P	16.4	242	686	1660	1029	2490	4150	3.75	5.40	0.046
D	LP	13.2	272	520	1414	848	2306	3720	3.50	5.04	0.056
	P	13.4	278	538	1495	731	2032	3527	3.62	5.21	0.050
E	LP	12.6	281	510	1438	795	2223	3661	3.41	4.91	0.059
	P	13.1	282	500	1410	815	2298	3708	3.50	5.04	0.055
F	LP	12.0	260	480	1248	783	2035	3283	3.50	5.04	0.054
	P	12.6	278	486	1351	729	2026	3377	3.75	5.40	0.050

* P = αLP protein.

injected intravenously. Ten minutes later 100 ml of blood was drawn. The specific activity of this serum αLP was 5,000 to 12,000 cpm/mg. The chylomicrons, separated from these lipemic sera, had total lipid values of 25 mg/ml and no radioactivity. The results obtained in (*a*) and (*b*) did not change when 500 ml of Lipomul instead of cream was fed to animals. To make it palatable, Lipomul was flavored with vanilla.

(*c*) Dogs were injected with either αP-I^{131} or αLP-I^{131}, and a day later, when specific activity values for serum αLP were the same as in the above experiments, 40 ml of Lipomul were injected intravenously and blood taken 10 minutes later. The particles of fat emulsion, separated by ultracentrifugation at d 1.003, contained a significant amount of radioactivity. Their content of labeled protein which had a specific activity of 4,000 to 10,000 cpm/mg was 200 μg/100 mg of total lipids.

(*d*) Dogs were given 40 ml of Lipomul intravenously, and 10 minutes later αP-I^{131} or αLP-I^{131} was injected intravenously and blood withdrawn. The chylomicrons contained about 500 μg of labeled protein/100 mg of total lipids. The specific activity of this protein was approximately of the same order of the soluble αLP in plasma (8,000 to 12,000 cpm/mg).

Mode of Disappearance from Plasma of I^{131} Chylomicrons and Lipomul αLP-I^{131} Complex. For these

TABLE 2. INTERACTIONS OF αP-I^{131} AND αLP-I^{131} WITH CHYLE AND SERUM CHYLOMICRONS*

Layers of 1 ml Removed from Top No.	αLP-I^{131}	αP-I^{131}	Chyle Chylomicrons Plus αLP-I^{131}	Serum Chylomicrons Plus αLP-I^{131}	Chyle Chylomicrons Plus αP-I^{131}	Serum Chylomicrons Plus αP-I^{131}
1+	12.0	12.3	14.7	14.0	36.6	34.2
2	12.1	12.1	12.0	12.0	10.4	9.0
3	12.1	11.7	12.1	12.1	9.2	9.1
4	12.2	11.4	11.9	11.8	8.2	9.4
5	11.8	11.9	12.0	11.8	7.8	9.4
6	12.4	12.2	12.1	12.1	8.5	9.2
7	12.3	11.7	12.0	12.3	8.8	9.0
8	14.1	16.7	13.2	13.9	10.5	10.7

* The data are expressed in per cent of total radioactivity present in each layer. Each value is the mean of the results from three experiments. 100 μg of αP-I^{131} or αLP-I^{131} with a specific activity of 0.5 μc/mg was used. Chyle and serum chylomicrons had a total lipid content of 50 mg. The mixtures containing the labeled lipoprotein protein and chylomicrons, following incubation for 10 minutes at 24°, were brought up to a volume of 8 ml with NaCl solution of d 1.003, then spun at 105,000 × *g* for 18 hours in a Spinco 30.2 rotor, 16°. Layers of 1 ml were removed by aspiration.

+ Top fraction.

TABLE 3. INTERACTIONS OF αP-I^{131} AND αLP-I^{131} WITH LIPOMUL ALONE OR MIXED WITH αLP AND SERUM*

Mixtures Analyzed			Results Radioactivity in Top Fraction at d 1.003
Fat Emulsion	Unlabeled Material Added	Radioactive Material Added	
			per cent
Lipomul	—	αLP-^{131}	73.2
Lipomul	—	αP-I^{131}	72.8
Lipomul	αLP	αLP-I^{131}	10.2
Lipomul	αLP	αP-I^{131}	28.2
Lipomul	Serum	αLP-I^{131}	10.6
Lipomul	Serum	αP-I^{131}	11.2
Lipemic serum	—	αLP-I^{131}	11.2
Lipemic serum	—	αP-I^{131}	10.9

* In all these experiments a constant amount of Lipomul containing 50 mg of total lipids was used. One mg of αLP or 1 ml of serum was incubated for 10 minutes at 24° with Lipomul. The mixtures were then equilibrated with 100 μg of αLP-I^{131} or αP-I^{131} and ultracentrifuged as indicated in the legend for Table 3. Lipemic sera were obtained from dogs 6 hours after feeding Lipomul.

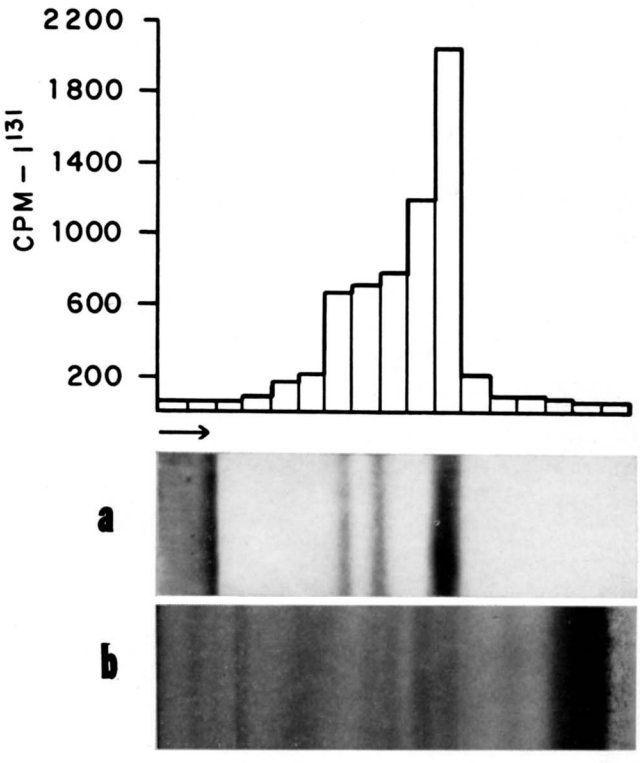

FIG. 6. Starch gel electrophoresis pattern of a serum from a dog injected with αP-I^{131}. The starch block was divided into three slices by two cuts parallel to the plane of migration. Upper curve: distribution of radioactivity in each one-half cm segment of starch; (*a*) pattern stained with Oil Red O; the broad boundary close to the origin is βLP, the other three boundaries correspond to αLP; (*b*) pattern stained with Amidoschwarz. Arrow indicates direction of migration.

experiments a large amount of chylomicrons separated from dog lipemic sera was used. Labeling of the chylomicron protein was performed according to the following technique: chylomicrons were equilibrated for 10 minutes at 24° with αP-I^{131}. About 100 μg of αP-I^{131} was added for each milligram of chylomicron protein. The mixture was then diluted with 0.15 M NaCl and spun at 9,000 × g for 10 minutes in a Spinco Model L ultracentrifuge, rotor 30.2, 16°. The top fraction obtained was layered under 0.15 M NaCl and centrifuged for an additional 18 hours at 105,000 × g in a Spinco 40 rotor. The chylomicron fraction, which floated at the top of the tube, had specific activity values up to 5 μc/mg of chylomicron protein.

The labeled chylomicrons were injected intravenously into two dogs and the rate of disappearance from plasma followed by determining chylomicron radioactivity and serum absorbancy values at 660 mμ of blood samples taken at 2-minute intervals. Fall of chylomicron radioactivity paralleled the fall in absorbancy at 660 mμ of whole serum. The half time of disappearance of chylomicrons from blood was about 10 minutes. As indicated in Figure 7, the fall of radioactivity of serum chylomicrons was accompanied by rapid appearance of

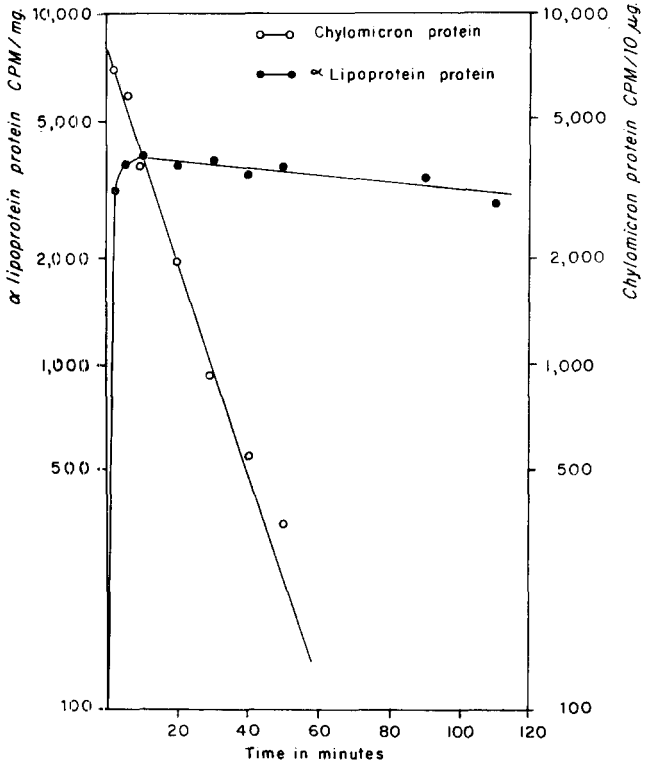

FIG. 7. Curve of disappearance from circulation of chylomicrons, labeled in the protein moiety with I^{131}. Specific activity values are expressed in cpm/mg of αLP protein and cpm/10 μg of chylomicron protein.

radioactivity in plasma αLP. No significant radioactivity appeared in plasma βLP.

Two more dogs were given Lipomul-αLP-I^{131} complex intravenously. This complex was prepared by mixing 50 to 60 ml of Lipomul with αLP-I^{131} of a specific activity of 40 μc/mg. Radioiodinated lipoprotein (100 μg) was added to each milliliter of Lipomul. After incubation for 10 minutes at 24°, the mixture was treated in the same manner as for the purification of labeled chylomicrons. When the complex Lipomul-αLP-I^{131} thus obtained was injected into dogs, its rate of disappearance from plasma was similar to the one observed with labeled chylomicrons. Also, in this case the plasma αLP became radioactive promptly.

DISCUSSION

These studies have shown that the protein (αP), prepared by delipidation of dog serum α_1-lipoprotein (αLP), when labeled with I^{131} and injected into dogs, is metabolized at the same rate as the radioiodinated protein moiety of a native αLP. The results, together with previously reported physicochemical (1) and immunochemical findings (2), strongly suggest that the αLP protein is not significantly changed by removal of lipids by the ethanol-ether procedure at −20° employed (1).

The present studies have also shown that when αP labeled with I^{131} is added to fasting or lipemic serum or is injected into dogs in absorptive or postabsorptive phase, radioactivity promptly appears only in the serum lipoproteins of density class d 1.063–1.21 having electrophoretic mobility of αLP. This indicates that the labeled αP interacts readily with its own class of high density lipoproteins, without exchanging with the protein moiety of low density lipoproteins (βLP) and chylomicrons. Similar findings were obtained by Gitlin *et al.* (15) after injection into humans of αLP, labeled in its protein moiety with I^{131}.

The exact nature of the interaction occurring between αP and αLP cannot be defined on the basis of our findings. It could be a simple process of adsorption of αP to αLP with formation of a complex with electrophoretic and ultracentrifugal properties similar to native αLP. Another possibility is that an actual transfer of a small amount of lipid occurs from αLP to αP, a transfer which may give a full complement of lipid to αP (which becomes αLP) at the expense of the donor native αLP. A third possibility is that of an exchange reaction between αLP and αP. In previous studies (3), samples of human serum αLP were equilibrated with various amounts of human serum αP-I^{131} and the mixtures spun at d 1.21. The distribution of radioac-

tivity between top and bottom fractions varied in a linear fashion with the mole fractions of the components. These results, and the fact that the radioactive top fractions had the electrophoretic and ultracentrifugal properties of a native human αLP, were interpreted as evidence for an exchange reaction between αP-I^{131} and αLP. Whether those findings can be applied to canine serum αP remains to be established.

It has been reported, and our *in vitro* and *in vivo* data are in agreement, that no exchange occurs between the protein moieties of αLP and βLP (15, 16). An exchange of lipids, to isotopic equilibrium, however, takes place involving cholesterol (17) and phospholipids (18, 19). Our present studies have shown that when αP-I^{131} is mixed with βLP, in absence of αLP, the mixture analyzed by ultracentrifugation at d 1.21, shows a radioactive top layer, which migrates electrophoretically in the αLP zone. These results, which are similar to the ones obtained with mixtures of human αP-I^{131} and βLP (3), indicate that either an actual transfer of lipids occurs from βLP to αP, to provide αP with enough lipid to re-form a lipoprotein complex similar to a native αLP_1 or an αP-I^{131}-βLP complex forms with a density between that of βLP and that of αP-I^{131}. Some reservations must be made until chemical and immunochemical characterization of the radioactive fraction floating at d 1.21 has been performed.

We have already mentioned that αP-I^{131}, when mixed with lipemic serum or plasma, fails to interact with the chylomicron fraction. Yet, when αP-I^{131} is mixed with purified samples of chylomicrons, in absence of serum, a radioactive top layer appears after ultracentrifugation of the mixture at d 1.003. It seems, therefore, that serum prevents the interaction between αP-I^{131} and chylomicrons. This is probably related to the fact that αP-I^{131} interacts preferentially with the αLP of serum to yield a product, αLP-I^{131}, which, as shown by our findings *in vitro* and *in vivo*, does not interact with chylomicrons.

It has been reported that αLP is one of the components of the chylomicron protein (4, 20). In view of the preferential interaction which occurs between αP and αLP, the question arises as to whether the αLP of chylomicrons is involved in the interaction chylomicron-αP-I^{131}. If this could be proved, it may furnish a basis for labeling specifically, *in vitro*, the αLP moiety of chylomicrons.

Our experiments have shown that by mixing an artificial triglyceride emulsion (Lipomul) with αLP, a Lipomul-αLP complex forms, which floats at d 1.003. This complex, when mixed with αP-I^{131}, showed the same type of interaction as exhibited by chylomicrons. Further, when the labeled Lipomul-αLP-I^{131} complex was injected into dogs, the rate of disappearance of the radioactivity from plasma was similar to that obtained by injecting chylomicrons, labeled in the protein moiety through the interaction chylomicron-αP-I^{131}. In both cases, the rapid process of removal of the triglycerides from circulation was accompanied by the appearance of labeled αLP in plasma, indicating a transfer of the labeled protein moiety from Lipomul or chylomicrons to plasma αLP. Whether this transfer of a labeled protein involves also lipids, cannot be established by these experiments. This possibility is, however, suggested by the experiments of McCandless and Zilversmit (21) and Fredrickson *et al.* (17), which have shown that phospholipids and cholesterol remain in circulation long after chylomicron triglycerides have disappeared from plasma. The similarity in behavior between Lipomul-αLP-I^{131} and labeled chylomicrons, shown by our experiments, may offer indirect evidence that the portion of chylomicrons, which is labeled by interaction with αP-I^{131}, is αLP.

Our results have shown that Lipomul, given intravenously to dogs, combines with a small amount of circulating αLP. Chylomicrons, as we already mentioned, also contain αLP. The question arises as to whether αLP may have any importance in the metabolism of triglycerides. We know, for instance, that αLP, when mixed with an artificial fat emulsion, renders it suitable for hydrolysis by lipoprotein lipase (22, 23), a property which is not exhibited by other serum proteins (4). It seems also established that hydrolysis of triglycerides accompanies their removal from the blood stream (24). It is possible, therefore, that lipoprotein lipase and, consequently, αLP actively participate in the removal of triglycerides from circulation.

From the radioisotope data it appears that αLP and αP are removed from circulation according to a monomial exponential curve suggesting homogeneity of the αLP protein. On the other hand, the starch gel electrophoresis patterns of αLP showed three boundaries each stainable for protein and lipid. Since the electrophoretic mobility of a lipoprotein may vary according to the amount of lipid bound to the protein, it might be assumed that in αLP there are three subfractions containing the same protein but different amount of lipids. However, this hypothesis is ruled out by the fact that three boundaries were also present with αP. Here absence of lipids enhanced significantly the electrophoretic mobility of αP. The presence of three boundaries in αLP and αP may be related to dissociation *in vitro* or *in vivo* of a larger molecule into smaller units and exhibited by starch gel. Another possibility is that the three boundaries are due to protein with different net charge and amino acid composition indicating non-

homogeneity of αLP. Biological homogeneity of αLP may have resulted only because the method we employed was not sensitive enough to detect small differences among similar, but not identical, proteins. This problem is at present under investigation.

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