Effect of free fatty acid mobilization on the electrophoretic mobility of α-lipoproteins in the dog

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ABSTRACT Dogs were given infusions of norepinephrine and subsequent additional infusions of propranolol and nicotinic acid over a 4-hr period. Under different physiological conditions, α-lipoproteins of three different electrophoretic mobilities were identified by means of paper electrophoresis; they were designated α-lipoproteins X, Y, and Z. During norepinephrine infusion, α-lipoprotein Y fell from 45% (of all lipoproteins) to 14%. There was a reciprocal rise in α-lipoprotein Z. On the other hand, α-lipoprotein X was not significantly changed. There was evidence that α-lipoprotein Y was progressively transformed into α-lipoprotein Z by increasing plasma FFA concentrations. The percentages of both α-lipoproteins Y and Z returned to original values after the dogs were given either nicotinic acid or propranolol. The alterations in the α-lipoprotein peaks Y and Z were rapid, being noted within 5 min of change in plasma FFA concentration. However, there appeared to be a threshold of plasma FFA concentration of 1200 μEq/liter, below which no changes in α-lipoproteins were noted. It was concluded that α-lipoprotein Y is rapidly, progressively, but reversibly transformed into α-lipoprotein Z by binding to plasma FFA above a threshold level of 1200 μEq/liter. However, α-lipoprotein X does not appear to be involved in the binding of plasma FFA.

SUPPLEMENTARY KEY WORDS propranolol · nicotinic acid · norepinephrine · catecholamines · β-lipoproteins · atherosclerosis · lipoproteins · thrombogenesis

THE PURPOSE of this report is to describe three forms of α-lipoproteins which were found on paper electrophoresis of dog plasma and the changes which occurred in relative amounts among the various types under different physiological conditions. Although it is known that α-lipoproteins may exist in several forms (1), the factors which influence the various forms have not been described. It is known that the concentration of plasma FFA can affect the migration rate of plasma proteins and lipoproteins, but these changes have not been studied under physiological conditions (2–4). In this study, we have shown that two of three forms of α-lipoproteins described herein are affected by the concentration of plasma FFA.

MATERIALS AND METHODS

Mongrel dogs were anesthetized with pentobarbital, 30 mg/kg, with a single intravenous dose and connected to a Harvard pump respirator, arterial catheter, blood pressure recording equipment, and an intravenous catheter. Continuous blood pressure and ECG recordings were made. Blood samples were taken at frequent intervals from the arterial catheter as indicated in Tables 1 and 2. The first 5 ml of blood drawn from the arterial catheter was discarded. If needed to maintain anesthesia, additional doses of pentobarbital (30 or 60 mg) were given immediately after a blood sample was taken. Care was taken not to give pentobarbital immediately before samples were drawn because it was found that a fall in blood pressure due to pentobarbital was associated with a brief elevation of plasma FFA concentration. When several agents were infused, they were combined in the same bottle and concentrations of each agent were adjusted to give the desired rate of infusion. All infusions were given in normal saline at 30 ml/hr.

There was a 15-min base-line period for each dog; during this time two blood samples were drawn. Norepinephrine infusion was then started and continued in

TABLE 1 TREATMENTS OF DOGS

Group 1	Norepinephrine throughout (4 dogs)
Group 2	Saline throughout (6 dogs)
Group 3	Norepinephrine throughout, propranolol at 60 min + nicotinic acid at 120 min (3 dogs)
Group 4	Norepinephrine throughout, nicotinic acid at 60 min + propranolol at 150 min (3 dogs)
Group 5	Norepinephrine throughout, nicotinic acid at 60 min (3 dogs)
Group 6	Norepinephrine throughout, propranolol at 60 min (3 dogs)

Norepinephrine was given as an infusion of 1.0 μg/kg/min for 4 hr. Propranolol was given as one dose 0.07 mg/kg + infusion 0.0017 mg/kg/min. Nicotinic acid was given as one dose 1.0 mg/kg + infusion 0.0555 mg/kg/min.

all dogs (except the saline control animals) for 4 hr. As indicated in Tables 1 and 2, at 1 hr and sometimes at 2.5 hr a single dose of propranolol or nicotinic acid was given. These agents were then infused intravenously. Each dog was used only once.

The reason for the choice of propranolol and nicotinic acid was to employ two different means of inhibition of plasma FFA mobilization in order to show that only plasma FFA concentrations were primarily responsible for changes in α-lipoproteins. These dissimilar agents share few other common effects. Another reason for the choice of these particular agents was the finding, previously reported, of a synergistic action of these two

TABLE 2 EFFECT OF NOREPINEPHRINE, PROPRANOLOL, AND NICOTINIC ACID ON VARIOUS LIPOPROTEINS AND PLASMA FFA IN THE DOG*

Time (min)	−15	0	30	60	65	75	120	150	155	165	210	240
						% of total lipoproteins						
Group 1		Norepi→		Norepi→				Norepi→				
Origin	5	7	5	5		8	6	6		7	5	6
β-lipoprotein	19	15	13	12		14	14	11		15	14	14
α-lipoprotein X	16	18	14	11		11	13	10		13	15	14
α-lipoprotein Y	47	42	19	19		22	23	27		24	30	31
α-lipoprotein Z	16	16	45	50		45	47	46		41	35	35
Plasma FFA (μEq/liter)	529	692	1717	1753		1684	1662	1586		1516	1518	1540
Group 2		Saline→		Saline→				Saline→				
Origin	13	8	8	6			7	7			7	7
β-lipoprotein	14	12	14	14			14	13			14	14
α-lipoprotein X	15	15	16	18			18	17			19	18
α-lipoprotein Y	48	48	49	50			51	51			53	54
α-lipoprotein Z	13	13	12	13			13	13			13	12
Plasma FFA (μEq/liter)	464	441	603	688		666	681	605		563	484	511
Group 3		Norepi→		Norepi and Prop→				Norepi, Prop, and Nic→				
Origin	12	10	10	8	7	7	4	3	4	7	6	7
β-lipoprotein	12	13	11	11	9	11	9	9	7	9	11	10
α-lipoprotein X	15	17	12	12	16	20	14	11	14	17	15	18
α-lipoprotein Y	47	46	37	32	39	46	56	59	58	56	56	56
α-lipoprotein Z	14	14	31	37	28	18	15	17	14	11	13	14
Plasma FFA (μEq/liter)	727	671	1797	2286	2076	1149	1152	1151	946	546	398	577
Group 4		Norepi→		Norepi and Nic→				Norepi, Nic, and Prop→				
Origin	6	6	3	5	2	2	5	4	6	7	5	4
β-lipoprotein	11	11	9	9	6	9	11	8	11	10	10	10
α-lipoprotein X	18	19	9	9	17	18	16	17	18	19	18	21
α-lipoprotein Y	52	54	36	35	49	56	53	53	50	55	56	54
α-lipoprotein Z	14	14	38	38	26	16	15	16	15	9	11	11
Plasma FFA (μEq/liter)	316	297	1899	1718	1225	1009	943	1229	864	650	492	542
Group 5		Norepi→		Norepi and Prop→								
Origin	8	7	5	6	5	6	7	8		6	6	7
β-lipoprotein	12	12	12	12	11	10	11	13		11	10	11
α-lipoprotein X	10	12	9	9	11	10	11	12		13	11	14
α-lipoprotein Y	57	53	35	29	52	56	54	49		53	55	47
α-lipoprotein Z	12	15	39	44	21	18	17	18		18	21	22
Plasma FFA (μEq/liter)	727	837	2043	2328	1626	1608	1330	1394		1186	1082	1123
Group 6		Norepi→		Norepi and Nic→								
Origin	7	7	6	6	4	6	5	5		4	6	6
β-lipoprotein	16	17	16	14	16	15	15	15		17	14	14
α-lipoprotein X	16	13	13	10	13	13	14	18		14	13	16
α-lipoprotein Y	50	54	27	29	49	50	51	45		51	50	46
α-lipoprotein Z	12	10	37	41	18	15	15	15		13	16	17
Plasma FFA (μEq/liter)	760	731	1894	1881	1514	782	832	814		774	859	833

Norepi, norepinephrine; Prop, propranolol; Nic, nicotinic acid.
* See text for doses of the agents.

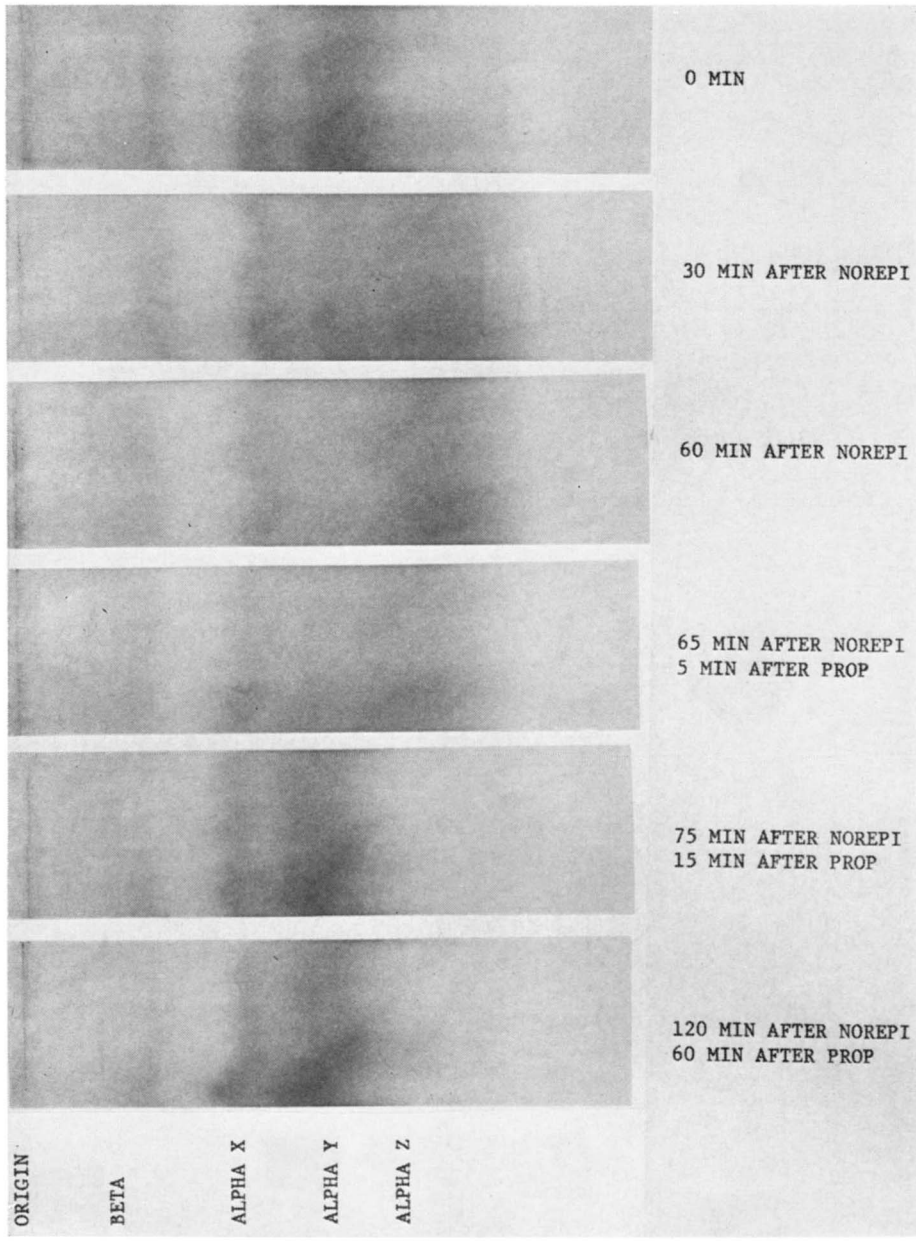

FIG. 1. Photograph of representative paper electrophoresis strips from an animal given a norepinephrine (NOREPI) infusion at 0 hr and an added propranolol (PROP) infusion at 1 hr.

agents in low concentrations on the inhibition of plasma FFA mobilization (5). The mechanism of action of propranolol is to block the receptor site on the adipose cell which mediates β-adrenergic activation of lipase (6). The mechanism of action of nicotinic acid is to inhibit the activation of adenyl cyclase in the adipose cell responsible for the activation of lipase (7).

Chemical Procedures

FFA were determined by the method of Dole (8). Serum cholesterol was determined with the digitonin method system manufactured by Hycel Co., Houston, Texas (9). 0.1 ml of serum samples and 0.1 ml of cholesterol standard (Hycel) were placed in 20 × 150 mm test tubes. To each test tube 6.0 ml of Hycel Control Reagent was added and the contents of the tube were thoroughly mixed. Tubes were placed in a water bath of 37°C for 20 min. Absorbance was read at 625 nm (red filter). Unknowns were compared to a cholesterol standard. Serum triglyceride and serum glycerol were determined using the glycerol kinase method (10–12). Reagents were obtained from the Boehringer Mannheim Corp., N.Y. 0.2 ml of serum was mixed with 0.5 ml of 0.5 N alcoholic potassium hydroxide (90% absolute

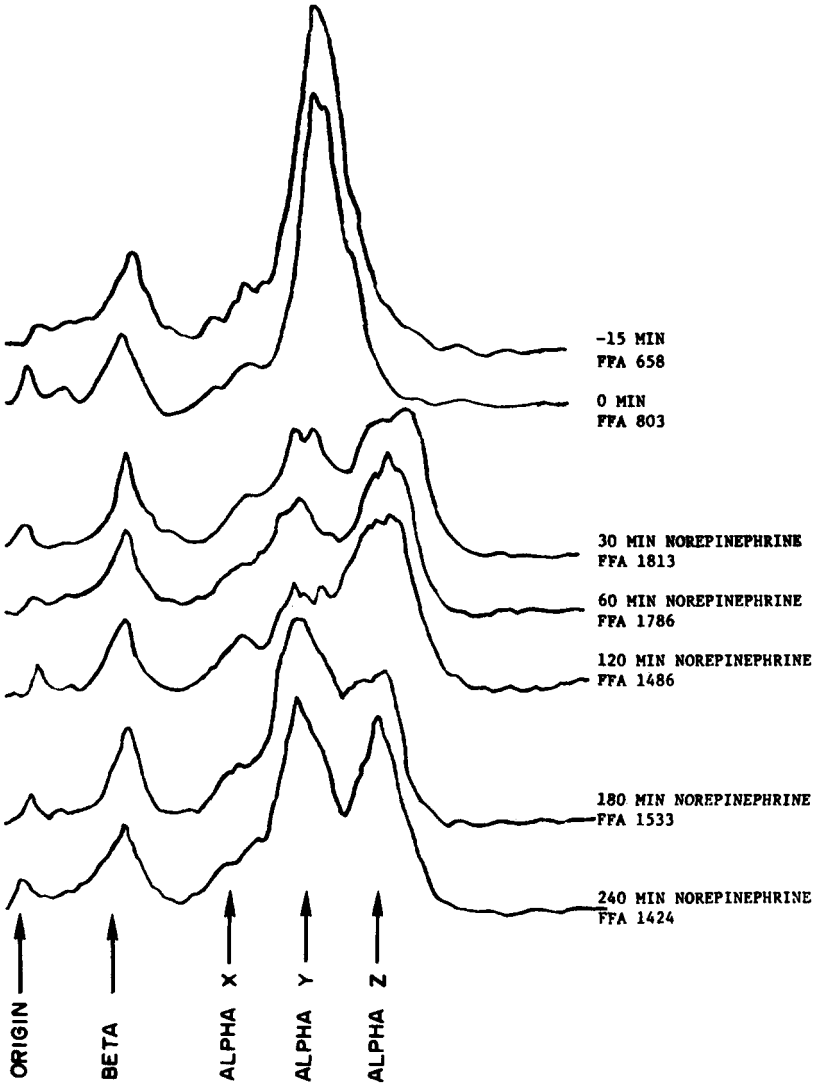

FIG. 2. Effect of norepinephrine infusion on lipoprotein electrophoresis over a 4-hr period in one dog.

alcohol, 10% redistilled water), stoppered, and incubated in a water bath for 30 min. The mixture was allowed to cool to room temperature and then 1.0 ml of 0.015 M magnesium sulfate solution was added. The solutions were mixed well and centrifuged for 10 min. 0.5 ml of the supernatant (hydrolyzed triglyceride solution) and 0.2 ml of the serum were then used to determine the amount of glycerol. The above samples were added to 2.50 ml of 0.1 M triethanolamine buffer, pH 7.6, containing 4 mM magnesium sulfate. 0.10 ml of a solution of the following composition was added: 6 mM NADH, 33 mM ATP, and 11 mM phosphoenolpyruvate. Then 0.02 ml of a solution containing 2 mg of lactic acid dehydrogenase per ml and 1 mg of pyruvic kinase per ml was added. The solutions were mixed and allowed to stand for 10 min at room temperature. Absorbance was measured at 366 nm. 0.02 ml of a solution containing 2 mg/ml of glycerokinase was added and the absorbance was remeasured. After 10 min the absorbance was again measured. The difference in absorbance between the second and third measurements was taken as the small nonspecific change in the reaction and was subtracted from the absorbance change occurring in the first 10-min period. The total glycerol in the serum (saponified) was determined by optical difference × 149. The free glycerol in the serum was determined by optical difference × 17.5. The total glycerol minus the free glycerol equaled the triglyceride glycerol, which when multiplied by 9.62 gave the neutral fat in mg per 100 ml. The use of a control reagent of highly refined triglyceride (olive oil; Sigma Chemical Co., St. Louis, Mo.) and aliquots of a serum of known triglyceride concentration with each group of tests indicated high accuracy and reproducibility.

For electrophoresis of lipoproteins the method of Jencks and Durrum (13) as modified by Fredrickson,

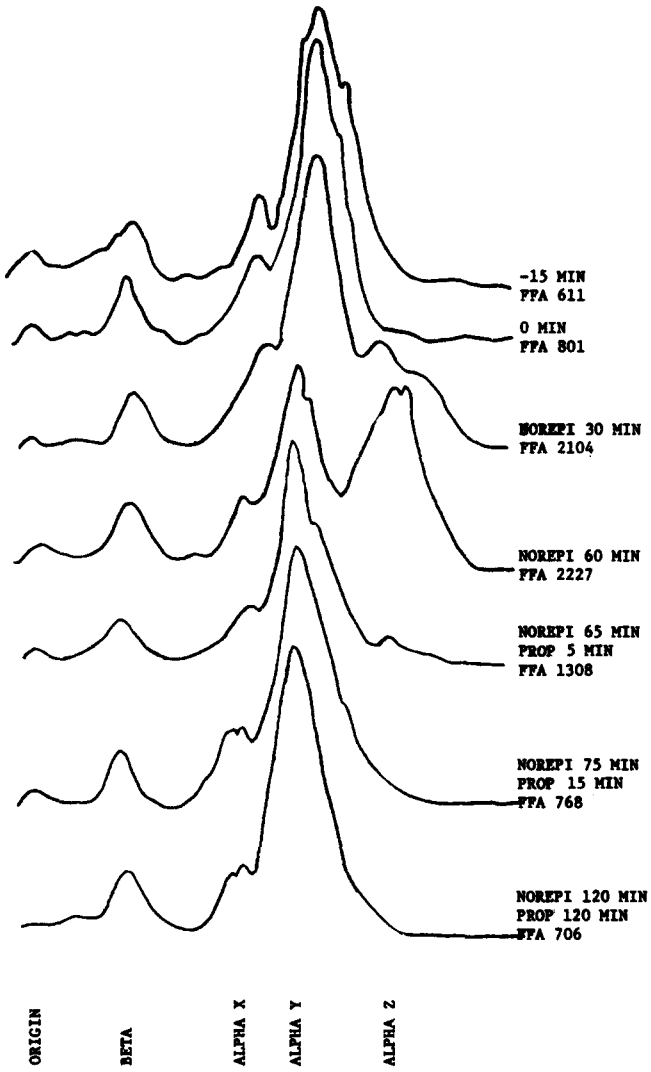

FIG. 3. Effect of norepinephrine (NOREPI) infusion and subsequent propranolol (PROP) infusion on lipoprotein electrophoresis over a 4-hr period in one dog.

Levy, and Lees (14) and used by Lees and Hatch (15) was used. The paper electrophoretograms were analyzed with a Spinco Analytrol Model RB (Beckman Instruments, Inc., Palo Alto, Calif.) with automatic integration in a manner similar to that of Noble et al. (16). In the resulting record five peaks could be identified, representing the origin, β-lipoproteins, and three forms of α-lipoproteins designated X, Y, and Z.

RESULTS

Identification of the Lipoproteins

The paper electrophoretograms of the plasma lipoproteins from fasting dogs resembled those of the human, except for the absence of a pre-β band and a lighter β band, and were similar to those described by others (17). As seen in Figs. 1–4, the origin at the cathode showed little evidence of chylomicrons in these fasting dogs. The β-lipoprotein (somewhat away from the cathode) was represented by a single peak. The α-lipoprotein (most distant from the cathode) was represented by three different peaks. The three forms of α-lipoprotein were designated X, Y, and Z. These letters were chosen in order to avoid confusion with other labels where numbers and letters designate α-lipoproteins, α-apoproteins, and subfractions separated by ultracentrifugation and other techniques (18, 19). The three forms of α-lipoprotein could always be identified. The α-lipoprotein X usually appeared as a separate, distinct peak. This is noted in Figs. 2–4 and 9. Otherwise it was seen as a notch on the ascending portion of the main peak, termed α-lipoprotein Y. The α-lipoprotein Y was the dominant peak in the electrophoretograms of plasma from fasting dogs. α-Lipoprotein Z appeared to be present in fasting specimens as a notch on the descending portion of the main peak α-lipoprotein Y. As seen in Figs. 2–4, at high levels of plasma FFA, two changes were observed in the α-lipoprotein Z band. It became larger and it moved away from the α-lipoprotein Y band. As noted below, size and migration of this band increased with increasing plasma levels of FFA. Separation of the two bands was seen in every instance (18 animals) in which norepinephrine infusion was given.

α-Lipoprotein Y

As seen in Table 2 (groups 1 and 3–6), the percentage of α-lipoprotein Y (expressed as a percentage of the total lipoproteins present) changed markedly when the various agents were infused. The α-lipoprotein Y consisted of the main α-lipoprotein peak during the fasting state, as seen in Figs. 2–4. During norepinephrine infusion and mobilization of plasma FFA, the percentage of α-lipoprotein Y fell to low levels in all animals. This change coincided with a rise in the percentage of α-lipoprotein Z, as seen in Table 2 and Fig. 6. It was also coincident with a rise in plasma FFA concentration, as noted in Table 2 and Fig. 7.

As seen in Fig. 5 and Table 2 (groups 1 and 3–6), the fall in α-lipoprotein Y during norepinephrine infusion was rapid. In a number of animals in which frequent samples were taken, this change was marked within 15 min of the beginning of norepinephrine infusion. On the average, within 1 hr the percentage of α-lipoprotein Y fell to about one-third, and occasionally to one-tenth, of its original value. In animals that received only norepinephrine, α-lipoprotein Y fell and showed only a slight rise subsequently throughout the experiment.

As noted in Table 2 (groups 3–6) and Fig. 5, when either propranolol or nicotinic acid was given, the reverse process occurred; there was a marked increase in

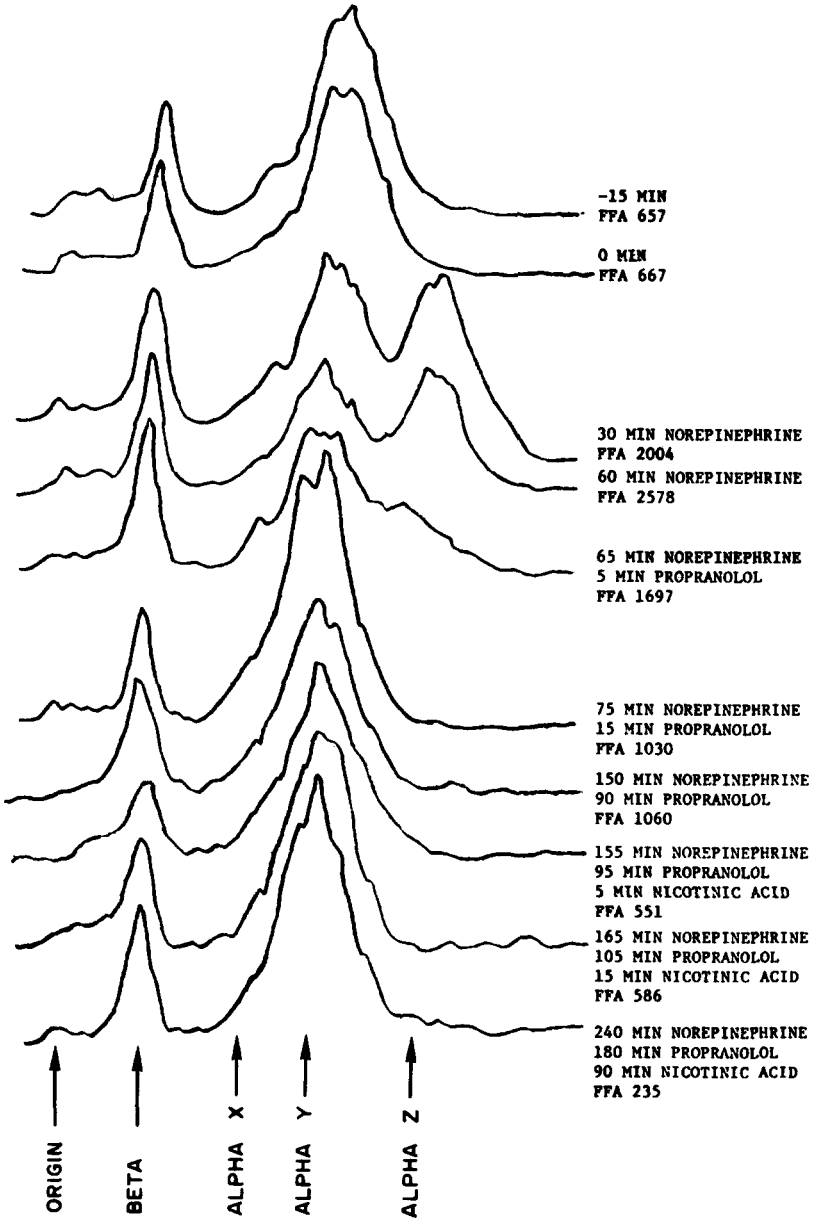

FIG. 4. Effect of norepinephrine, propranolol, and nicotinic acid infusion over a 4-hr period on lipoproteins in one dog.

α-lipoprotein Y (Fig. 5, lines *A*, *C*, *D*, and *E*). This increase was coincident with a fall in plasma FFA (Fig. 7), and it was rapid; it was first noted within 5 min and was quite marked within 15 min.

α-Lipoprotein Z

As seen in Table 2 (groups 1 and 3–6) and Figs. 6 and 8, the percentage of α-lipoprotein Z changed inversely with α-lipoprotein Y. This is also illustrated in Figs. 2–4. During the first hour of infusion of only norepinephrine, the percentage of α-lipoprotein Z rose markedly in association with a rise in plasma FFA concentrations. When either propranolol or nicotinic acid was added at 1 hr, the percentage of α-lipoprotein Z fell rapidly. The reduction was noted in all animals after 5 min of either agent. The change was well advanced at 15 min.

Threshold Effect of Plasma FFA at 1200 μEq/Liter

As noted above, a rise in plasma FFA concentrations was associated with a rapid decrease in the percentage of α-lipoprotein Y and an increase in α-lipoprotein Z. Conversely, a depression in the concentration of plasma FFA levels was associated with a rapid reversal of the percentages of these α-lipoproteins toward their original levels. However, there appeared to be a threshold con-

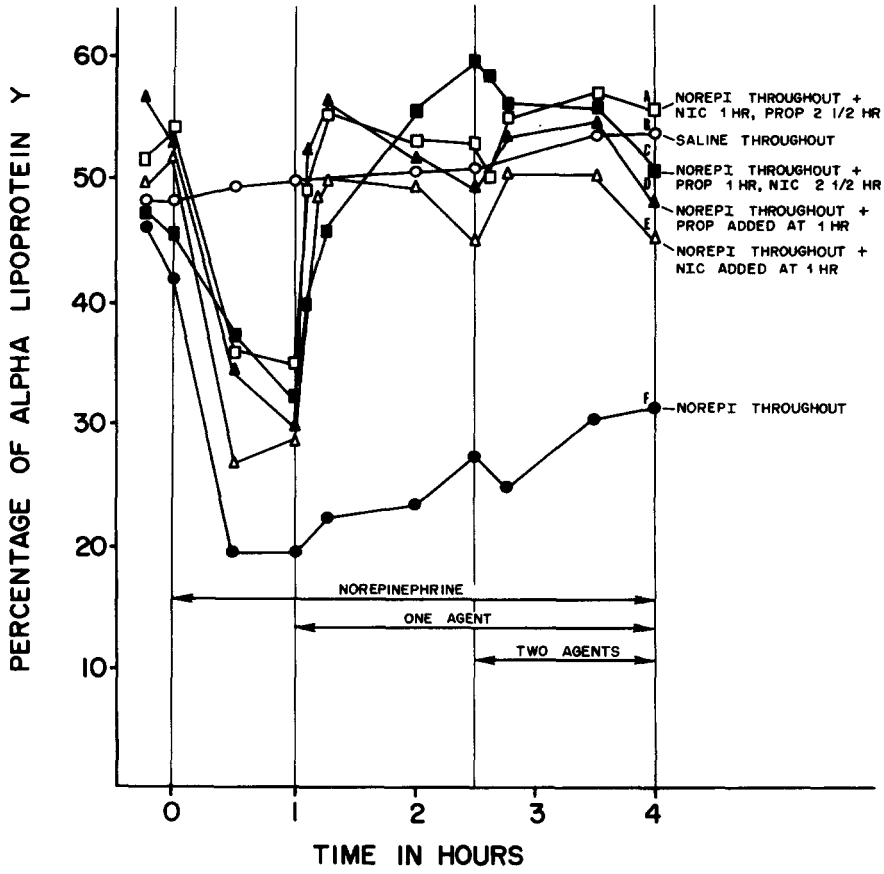

FIG. 5. Effect of norepinephrine (NOREPI) and the subsequent infusions of propranolol (PROP) and nicotinic acid (NIC) over a 4-hr period on the percentage of α-lipoprotein Y as measured on paper electrophoretograms. Line *F* represents animals given norepinephrine alone for 4 hr; line *C*, propranolol added to norepinephrine at 1 hr and nicotinic acid added at 2.5 hr; line *A*, nicotinic acid added at 1 hr and propranolol added at 2.5 hr; line *D*, propranolol added at 1 hr; line *E*, nicotinic acid added at 1 hr; and line *B*, saline controls. Each line represents the average of observations in 3–6 animals as described in Materials and Methods.

centration of 1200 μEq/liter of plasma FFA for this effect. Below this level there was little change in the α-lipoproteins, as seen in Figs. 5 and 6. When propranolol and nicotinic acid were given at 1 hr into the experiment, the addition of the other agent at 2.5 hr into the experiment had little added effect in changing the percentage of α-lipoproteins Y and Z. This was true despite the fact that the addition of the second agent, as seen in Fig. 6, caused a further reduction in the concentration of plasma FFA. This is also illustrated in Fig. 7, in which FFA concentrations are plotted against the percentage of α-lipoproteins Y and Z. The percentage of the two α-lipoproteins was generally proportional to the concentration of plasma FFA above a level of 1200 μEq/liter, but was little changed below this level.

α-Lipoprotein X

α-Lipoprotein X was noted as a separate peak in the specimens from most fasting dogs. In the others it was noted as a notch on the ascending limb of α-lipoprotein Y; examples are seen in Figs. 2–4. As seen in Table 2, norepinephrine and the subsequent addition of propranolol and nicotinic acid had little effect on the percentage of α-lipoprotein X over the 4 hr of the experiments. The percentage of α-lipoprotein X seemed to fall slightly under the influence of norepinephrine infusion, but the changes were found not to be statistically significant. Small increases in the percentage of α-lipoprotein X after propranolol or nicotinic acid infusion were also statistically not significant.

Plasma FFA

As seen in Table 2 and Fig. 7, plasma FFA concentrations rose under the influence of norepinephrine and fell under the influence of propranolol and nicotinic acid despite the continued infusion of norepinephrine. As seen in Fig. 7 (lines *B* and *C*), when one agent was used alone the plasma FFA concentrations fell below those in the animals treated with norepinephrine alone, but did not fall as low as those in animals which received both

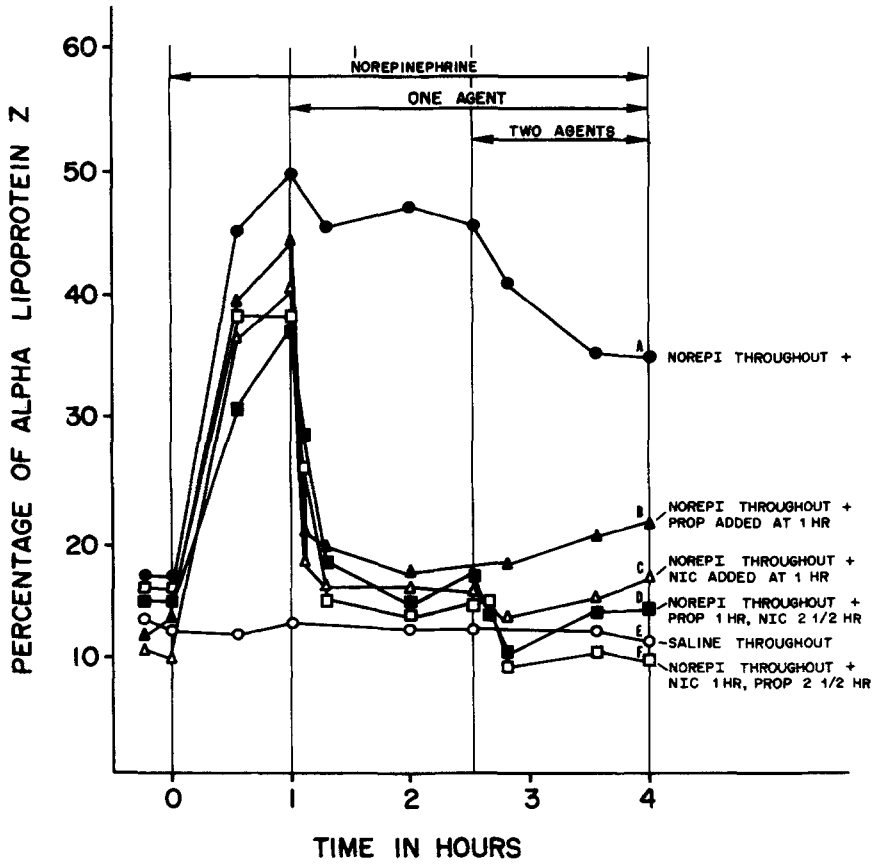

FIG. 6. Effect of norepinephrine (NOREPI) and subsequent infusions of propranolol (PROP) and nicotinic acid (NIC) over a 4-hr period on the percentage of α-lipoprotein Z as measured on paper electrophoretograms. Line *A* represents animals given norepinephrine alone over 4 hr; line *D*, propranolol added to norepinephrine at 1 hr and nicotinic acid added at 2.5 hr; line *F*, nicotinic acid added at 1 hr and propranolol at 2.5 hr; line *B*, propranolol added at 1 hr; line *C*, nicotinic acid added at 1 hr; and line *E*, saline controls.

(lines *D* and *E*). Indeed, when two agents were given, as shown in lines *D* and *E*, the plasma FFA concentrations fell to a level similar to that of the saline controls shown in line *F*. Thus, the use of propranolol and nicotinic acid in small doses was synergistic. These doses were approximately $^1/_8$ to $^1/_{20}$ the fully effective doses found in a previous study (5) to be necessary when used alone.

Effects of Variations in Processing Procedures

An experiment in which some of the variables in the standard procedure were changed was done to make sure that our findings were not an artifact of the procedure or due to the staining of a protein such as albumin heavily bound with FFA. Three sets of electrophoretic strips were made from one animal. The first set was processed in the usual way. The second set was processed in a similar fashion except the albumin was omitted from the buffer. The third set was similar to the first set, except that it was stained for proteins. More frequent samples than usual were taken. The results are seen in Fig. 9. It is seen that more frequent sampling revealed the gradual emergence of α-lipoprotein Z from α-lipoprotein Y. At the bottom of the figure is a representative electrophoretogram from serum processed without albumin in the buffer. Those processed without albumin in the buffer in this manner showed α-lipoproteins which were similar at every time interval to those processed with albumin. Also shown at the bottom of the figure is a representative electrophoretogram stained for protein. At all time intervals the protein electrophoretogram was essentially the same. It is noted that the albumin peak is well outside the α-lipoprotein peaks. In another variation not shown, palmitic acid was dissolved in albumin mixed with saline. Samples were subjected to paper lipoprotein electrophoresis. The paper strips resulting from this study were blank. Together, these variations suggested that the procedures used in this study stained only lipoproteins and that FFA bound to albumin were not affecting the staining of the paper strips or the electrophoretograms.

Serum Cholesterol and Triglyceride

Preliminary results indicate that serum cholesterol con-

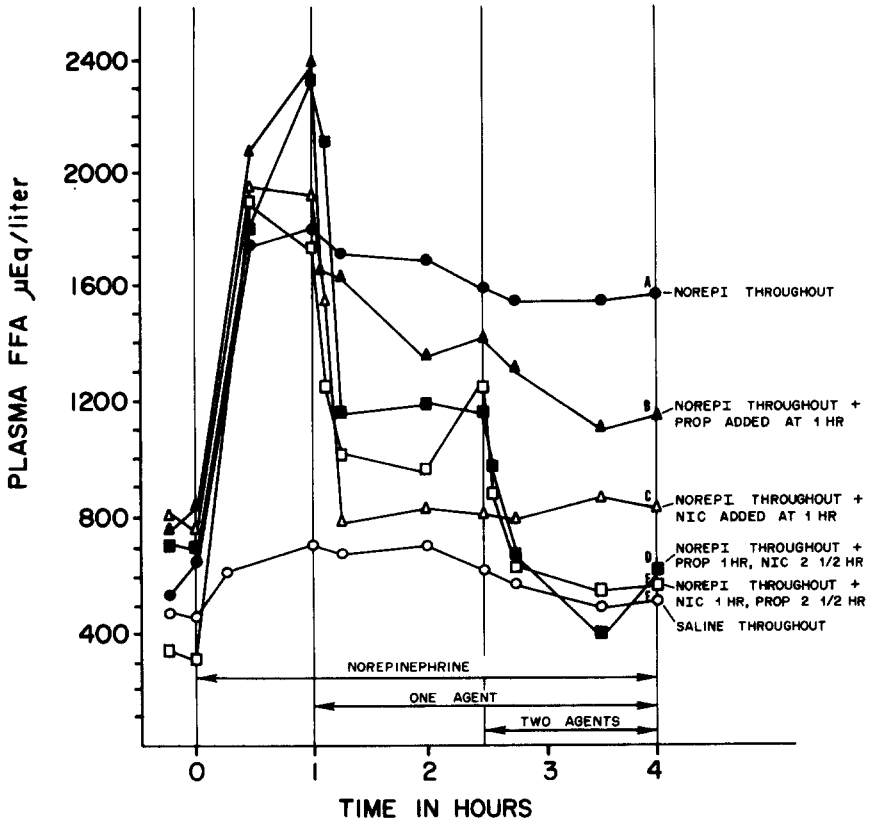

FIG. 7. Effect of norepinephrine (NOREPI) and subsequent infusions of propranolol (PROP) and nicotinic acid (NIC) on plasma FFA concentration. Line *A* represents results in animals given only norepinephrine throughout; line *B*, only propranolol added at 1 hr; line *C*, only nicotinic acid added at 1 hr; line *D*, propranolol added at 1 hr and nicotinic acid added at 2.5 hr; line *E*, nicotinic acid added at 1 hr and propranolol added at 2.5 hr; and line *F*, saline alone given for 4 hr.

centration did not change during the course of the experiment in the treated animals as compared with the controls. Serum triglyceride concentration rose 21% in the animals treated with norepinephrine alone over a 4-hr period. Conversely, serum triglyceride fell 29% in the animals treated with norepinephrine, propranolol, and nicotinic acid, but this change was noted only after 3 or 4 hr. The slow time course of the changes in serum triglycerides, therefore, appeared to be unrelated to the rapid changes noted in α-lipoproteins.

DISCUSSION

Three Forms of α-Lipoprotein

In this study it was possible to identify three peaks designated X, Y, and Z in the region of α-lipoprotein mobility. The Y peak was the dominant one in plasma from fasting animals. The X peak was also fairly distinct and preceded the Y peak; it is clearly seen in Figs. 2–4 and 9. The Z peak succeeded the Y peak but was less distinct in the fasting state. However, it emerged clearly, at times to become the dominant peak, during mobilization of FFA with norepinephrine and receded when mobilization was inhibited. This is seen in Figs. 2–5 and 9.

Effect of Plasma FFA Elevations on Changing α-Lipoprotein Bands

As noted above and seen in Figs. 2–6, mobilization of plasma FFA was associated with a rapid conversion of α-lipoprotein Y to α-lipoprotein Z. α-Lipoprotein X was only slightly affected. Elevated plasma FFA concentrations seemed to be the main factor involved in the transformation of α-lipoprotein Y to α-lipoprotein Z. This was suggested by the reversal of the process by either propranolol or nicotinic acid. One of the few physiological effects which these two agents share in common is their depression of plasma FFA concentration (20–23). This change in α-lipoproteins occurred within 5 min of the beginning of propranolol or nicotinic acid infusion and paralleled the time course of the changes in the concentration of plasma FFA. The rapid fall of α-lipoprotein Y and the reciprocal rise of Z within 5 min were not likely to be the result of changes in the composition of the proteins themselves, which have been shown to have a half-life of 4–5 days (24, 25). The rapid changes

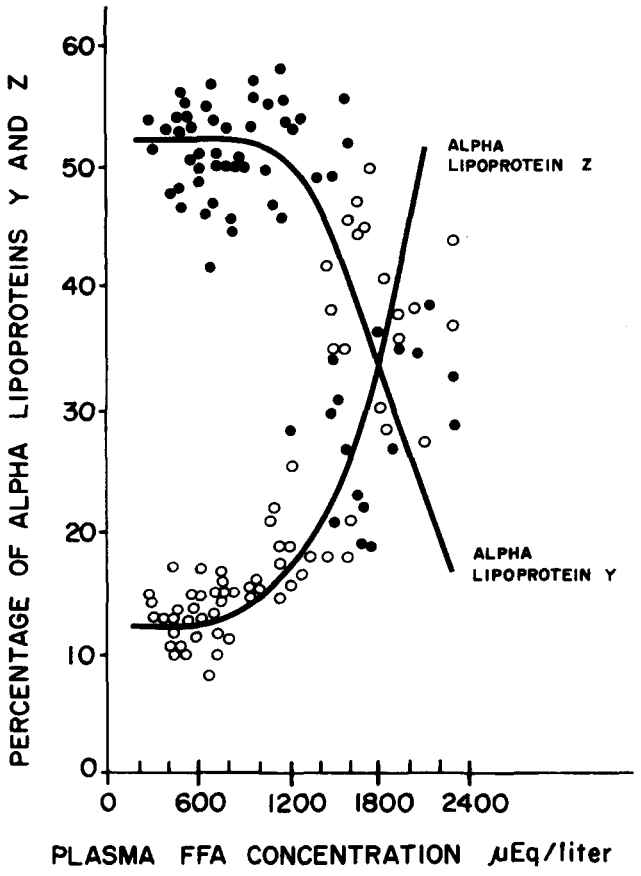

FIG. 8. Percentage of α-lipoprotein Y and α-lipoprotein Z as a function of plasma FFA concentrations. This is a composite scattergram of the results of all groups. Each point represents the average of determinations in 3–6 dogs taken at different intervals during various infusions of norepinephrine, propranolol, nicotinic acid, and saline (Table 1).

in α-lipoproteins were also not likely to be due to changes in fatty acid composition of lipoprotein triglycerides, which have been shown to have a half-life of 6 hr (26). Although in this study the concentration of triglyceride changed under the influence of norepinephrine, propranolol, and nicotinic acid, the changes took 3–4 hr and the time course was too slow to account for the rapid changes in α-lipoproteins. The slow course of the change in triglyceride has been noted by others (20, 22, 27, 28). It has also been shown that there is a reciprocal relationship between the synthesis of β-lipoproteins and α-lipoproteins, but this process also requires some time (29, 30). Thus, the changes in α-lipoproteins noted in this study were likely to be due to a loose and reversible association of these proteins with plasma FFA.

The conclusion that a change in the concentration of plasma FFA resulted in the changes in the migration of α-lipoproteins Y and Z is compatible with evidence that plasma FFA can alter the charge and electrophoretic mobility of many proteins (2–4). These three forms of α-lipoproteins and the changes in their mobilities under the influence of plasma FFA, however, have not been previously described.

As noted in Fig. 8, changes in plasma FFA concentrations below 1200 μEq/liter had little effect on the percentage of the various α-lipoproteins. This study suggested that only above 1200 μEq/liter α-lipoprotein Y acted as a carrier of plasma FFA. This is in accord with studies indicating that in the physiological range, 97% of plasma FFA are carried by serum albumin (31). It is also in accord with in vivo and in vitro studies showing that at high concentrations of plasma FFA other lipoproteins act as carriers (2–4, 32). In this study, there was evidence that the in vivo saturation point of albumin is approximately 1200 μEq/liter. However, we found that only one lipoprotein, α-lipoprotein Y, was a significant carrier of excess plasma FFA under conditions of endogenous mobilization of plasma FFA.

As seen in Table 2, the percentage of α-lipoprotein X was not greatly changed by norepinephrine, propranolol, or nicotinic acid and the associated marked variations in plasma FFA concentrations. The small differences noted were found not to be statistically significant. Thus, it appeared that α-lipoprotein X had little affinity for plasma FFA in contrast to the high affinity of α-lipoprotein Y.

This study suggests that α-lipoprotein is involved in the binding of plasma FFA when the binding capacity of albumin is exceeded. This fact may be of importance in patients with atherosclerosis and hyperlipidemia, who have been shown to have reduced levels of α-lipoproteins (29, 33). Moreover, it is known that patients with atherosclerosis have increased ability to mobilize plasma FFA (34–38). Thus, the combination of decreased binding and increased mobilization of FFA in such patients may be significant, particularly in the light of recent studies of the possible role of FFA in thrombogenesis. Excess plasma FFA have been shown to cause accelerated coagulation (39–42). In addition, plasma FFA in excess have been shown to increase platelet aggregation (43). In related studies, it has been shown that this platelet aggregation occurs even when coagulation is prevented by heparin and can lead to coronary platelet thrombi and subsequent congestive failure (44). Thus, in patients with atherosclerosis, there may be a relationship between increased mobilization of plasma FFA and decreased α-lipoproteins on the one hand, and the tendency to thrombosis on the other.

We would like to thank Miss Nancy Bresnahan, Mrs. Julie Roslund, and Mrs. Tina Braccia for their expert technical assistance.

This investigation was supported by training grants 3 T1 HE05391 and T12 HE05780 from the National Heart Institute and the U.S. Public Health Service.

Manuscript received 21 July 1970; accepted 5 January 1971.

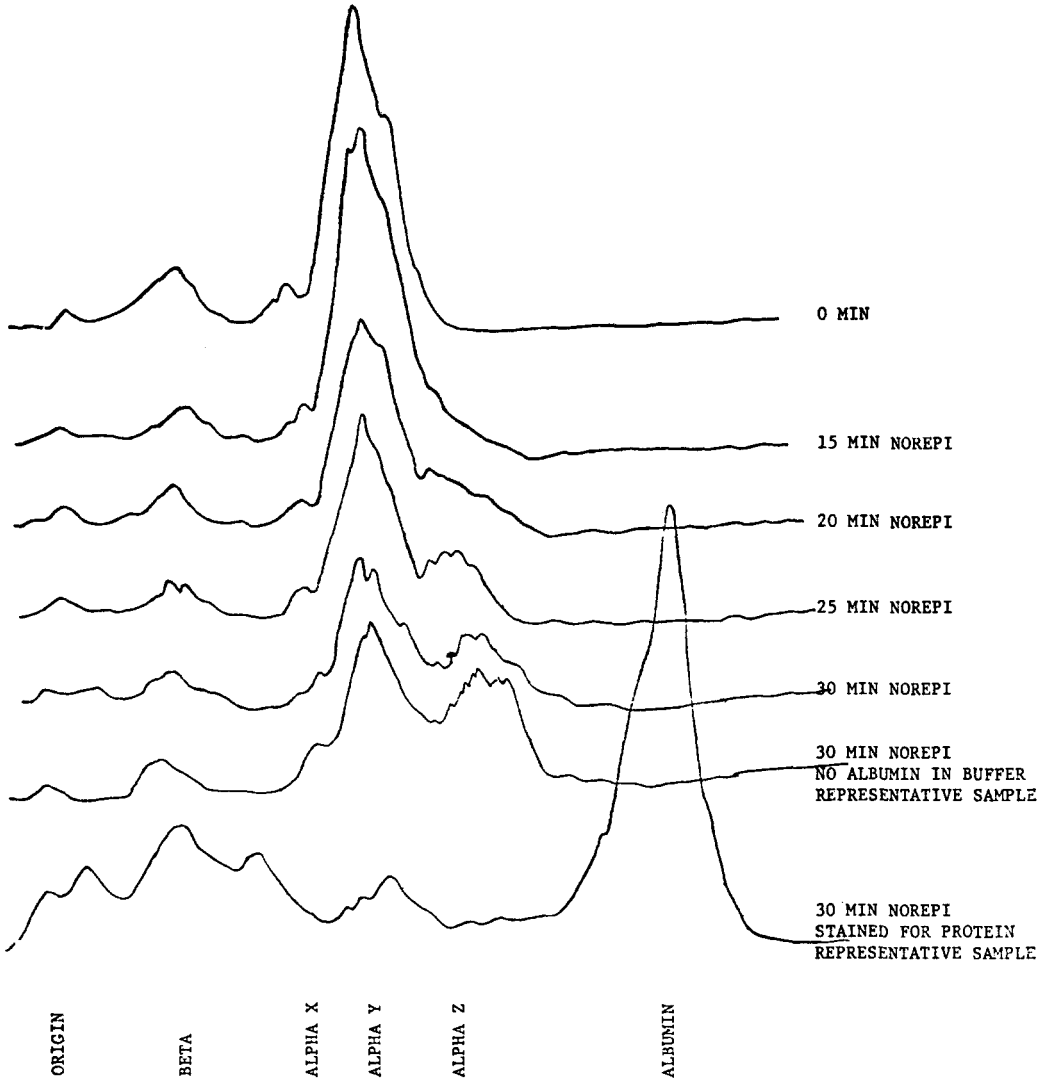

FIG. 9. Representative electrophoretograms of plasma samples of one dog taken every 5 min for 1 hr and processed in the various ways discussed under Effects of Variations in Processing Procedures. NOREPI, norepinephrine.

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