PLASMA PROTEIN METABOLISM—ELECTROPHORETIC STUDIES

THE INFLUENCE OF PLASMA LIPIDS ON ELECTROPHORETIC PATTERNS OF HUMAN AND DOG PLASMA

BY L. J. ZELDIS, M.D., E. L. ALLING, M.D., A. B. McCOORD, PH.D., AND J. P. KULKA, M.D.

(From the Departments of Pathology, Radiology, and Pediatrics, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

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Electrophoretic patterns of plasma before and after the extraction of a large part of the plasma lipids are compared in these experiments. Marked alterations in total electrophoretic area, in the relative areas of individual electrophoretic components, and in electrophoretic albumin:globulin ratios are disclosed by such comparisons. Applied to samples of human plasma, this procedure confirms the observation of Blix, Tiselius, and Svensson (2) that a particularly rich content of lipid materials characterizes human beta globulin. Abnormally large beta globulin peaks regularly occur in the presence of elevated plasma lipids. Marked increases in the areas of other globulin components, particularly of gamma globulins, however, are also found to be due in large part to elevated plasma lipid levels in certain abnormal human plasmas.

The greatest relative amount of lipid in dog plasma, in contrast to human plasma, is associated not with the beta component, but with globulins whose mobilities are intermediate between those of albumin and beta globulin, that is, with electrophoretic components usually designated as alpha globulins. Not only the areas, but the configuration and even the number of the alpha globulin peaks in dog plasma are altered by the extraction of plasma lipids.

These studies are an outgrowth of previous investigations (17, 18) of alterations in the plasma electrophoretic patterns of dogs in experimental hypoproteinemia. During such hypoproteinemia, particularly when induced by long continued low protein feeding (18), there is an apparently paradoxical increase in the areas of the alpha globulin peaks. For several reasons, it appeared that such increases might be due to materials other than protein. Nitrogen analysis of the plasma for example, frequently yielded lower values for total protein concentration than those indicated by electrophoretic areas. Increased plasma cholesterol and total plasma lipid levels were regularly found to be associated with these discrepancies. Since Longsworth and others (10, 11) had reported decreases in the beta globulin component in the ether-extracted plasma of human cases of nephrosis, it seemed likely that the increased alpha globulin peaks of hypoproteinemic dogs might be due to elevated plasma lipid levels.

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From time to time we have similarly noted disparities on comparison of nitrogen values and electrophoretic concentrations in certain abnormal human plasmas. In some of these plasmas, elevated lipid levels were found. Extraction of plasma lipids was therefore carried out, and the results of a number of such extractions are included in this report.

Methods

Samples of dog plasma were obtained during the course of low protein feeding experiments previously mentioned. Human plasma samples were obtained from patients on the medical and surgical services of the Strong Memorial and Rochester Municipal Hospitals.[1]

All samples were collected in flasks containing dried potassium oxalate in a concentration of 250 mg. per 100 ml. of whole blood. After standing for one hour at room temperature, samples were centrifuged for 35 minutes at 3500 R.P.M. and plasma collected by siphoning. Plasma was stored at 2° C. when not immediately analyzed. Aliquots analyzed before extraction are in the following tables listed as *native plasma*, aliquots analyzed after extraction as *extracted plasma*.

Total plasma protein concentrations were determined by macro-Kjeldahl analysis and for native plasma, corrected for non-protein nitrogen content as estimated by the method of Greenwald (7). Sample determinations of non-protein nitrogen in extracted plasma indicated its complete removal by the extraction process, so that no such correction was necessary. Plasma cholesterol and total lipid of native plasma were determined according to Bloor, (3–5). Extracted lipid was estimated by the same methods on samples of the pooled solvents after concentration to suitable volume.

Electrophoresis was carried out for 3 hours at 1° C. in the standard 11 ml. single section Tiselius cell with a potential gradient of 6.8 ± 0.1 volts per cm. All samples were diluted 1:1 with veronal buffer of pH 8.5 and ionic strength 0.1 and were dialyzed against 2 liters of the same buffer. Details of dialysis, electrophoresis, and the recording and measurement of the patterns have been fully described in recent reports (17, 18).

The extraction procedure used was developed from a method originally described by Hardy and Gardiner (9), and later modified by Blix (1). 10 ml. of the oxalated plasma was cooled to 2° C. and precipitated by adding it dropwise to 80 ml. of absolute ethyl alcohol previously cooled in a dry ice–acetone bath to a temperature of −10° to −15° C. This precipitation and all subsequent manipulations were carried out in a 110 ml. straight walled centrifuge tube. All operations, including centrifugation, were performed in a cold room at 1° C. to avoid sudden changes in temperature. The precipitated mixture was allowed to stand 5 minutes in the dry ice–acetone bath, then centrifuged at 3500 R.P.M. for 3 or 4 minutes. By using a little cold acetone in the centrifuge tube carrier, it was possible to prevent the temperature of the precipitated material from rising above 0° C. The supernatant fluid was decanted from the packed precipitate which was quickly resuspended by stirring in 80 ml. of fresh absolute alcohol, also at −10° to −15° C. The suspension was allowed to stand for 2 hours in the cold bath, and the supernatant fluid again decanted after centrifugation. The precipitate was then similarly treated with one more 80 ml. portion of alcohol, one 80 ml. portion of a 7:3 mixture of alcohol and ether, and finally two successive 80 ml. portions of dry, aldehyde-free ether. Each treatment was allowed to continue for 1 to 2 hours. Throughout the extractions the temperature was kept as close as possible to −15° C. by adding dry ice as required to the acetone bath. Care was exercised to expose the precipitate to atmospheric moisture for only short periods of time.

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Supernatant fluids from all extractions were pooled in a large flask and later analyzed for cholesterol and total lipid as described above. Following the last ether extraction, the precipitate was quickly spread over the lower wall of the centrifuge tube with a glass rod and dried over fresh sulfuric acid in a vacuum desiccator at room temperature by means of an oil pump. The resulting white cake could be easily ground to a fluffy powder which dissolved slowly at room temperature in 9 to 10 ml. of normal saline solution to yield a clear, limpid solution. The powdered material could be kept for several days exposed to the atmosphere with no apparent loss of solubility. Occasionally on reconstitution, a small, undissolved pellicle remained. Analyzed after repeated washings with normal saline, several such residues contained only 1 to 3 mg. of nitrogen. Analysis of samples reconstituted and carefully adjusted at 2° C. to the original volume of 10 ml., as well as nitrogen analysis of the pooled supernate usually indicated a loss of 1 to 5 per cent of the initial plasma protein into the extraction fluid. In practice, therefore, the dried material was simply dissolved in approximately 9.5 ml. of saline at room temperature and the protein content checked by Kjeldahl determination. We have assumed that the loss during extraction was a mechanical one involving all electrophoretic components proportionately. On this assumption, the electrophoretic areas of the extracted samples in Tables 1-*a* and 2-*a* are corrected for the differences in total protein nitrogen found before and after extraction. While it is not impossible that selective denaturation of certain components might have occurred during extraction, there is no reason to believe that this should have rendered them more susceptible to mechanical loss during extraction.

EXPERIMENTAL OBSERVATIONS

Figs. 1 to 9 represent reproductions of electrophoretic patterns from three samples of dog plasma and six samples of human plasma before and after extraction.

The protein content of these native and extracted samples and of six other samples of dog plasma similarly treated is indicated in Tables 1 and 2. The extent to which plasma lipids were removed by extraction is indicated for each sample in the same tables. It is apparent that while cholesterol extraction is generally quite complete, phospholipid is less thoroughly removed. In sera extracted with cold acetone, Blix (1) has found the larger amount of the removed lipid to consist of lecithin, while cephalin was less thoroughly extracted. Fractionation of the extracted phospholipid was not attempted in the present experiments. It might be surmised, however, that the acidic phosphatides, cephalin and phosphatidyl serine, would comprise the bulk of the material not removed by cold neutral alcohol.

In Fig. 1 are shown ascending boundaries of the plasma of a normal dog (dog 43-427). The sample represented in Fig. 2 was obtained from a dog on a high protein diet during recovery from hypoproteinemia produced by low protein feeding (18). At the time of sampling, total protein of the plasma had returned to normal concentrations but there was a considerable elevation of plasma lipid levels (Table 1, sample 4). The plasma whose patterns are shown in Fig. 3 was obtained from a dog (43-381) during low protein feeding. At the time of sampling, there was marked hypoproteinemia and plasma lipids were elevated (Table 1, sample 7).

In all of these dog samples the most conspicuous alteration produced by extraction involves the alpha globulin components. In the electrophoretic

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patterns of normal dog plasma, three distinct peaks are usually present between the albumin and beta globulin components (Fig. 1). In hypoproteinemic

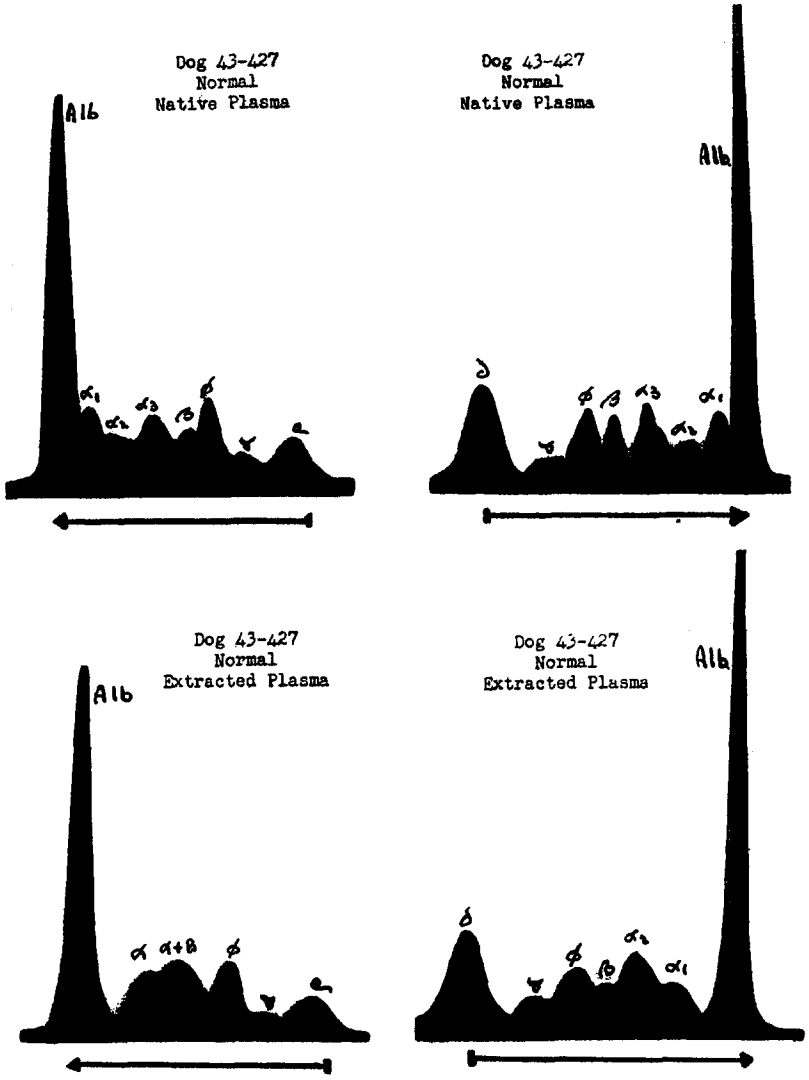

FIG. 1. Electrophoretic patterns of normal dog plasma before and after extraction of plasma lipids.

dogs, such peaks are frequently four in number, the fourth peak often poorly separated from the beta component (Figs. 2 and 3). In previous reports we have referred to these peaks as alpha 1, 2, 3, and 4, and suggested that the alpha 4 peak might, if one preferred, be designated beta 1 and the next slower

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component beta 2. In these extracted plasmas of hyperlipemic dogs, the alpha 1, alpha 2, and alpha 3 components regularly merge into a single peak (Figs.

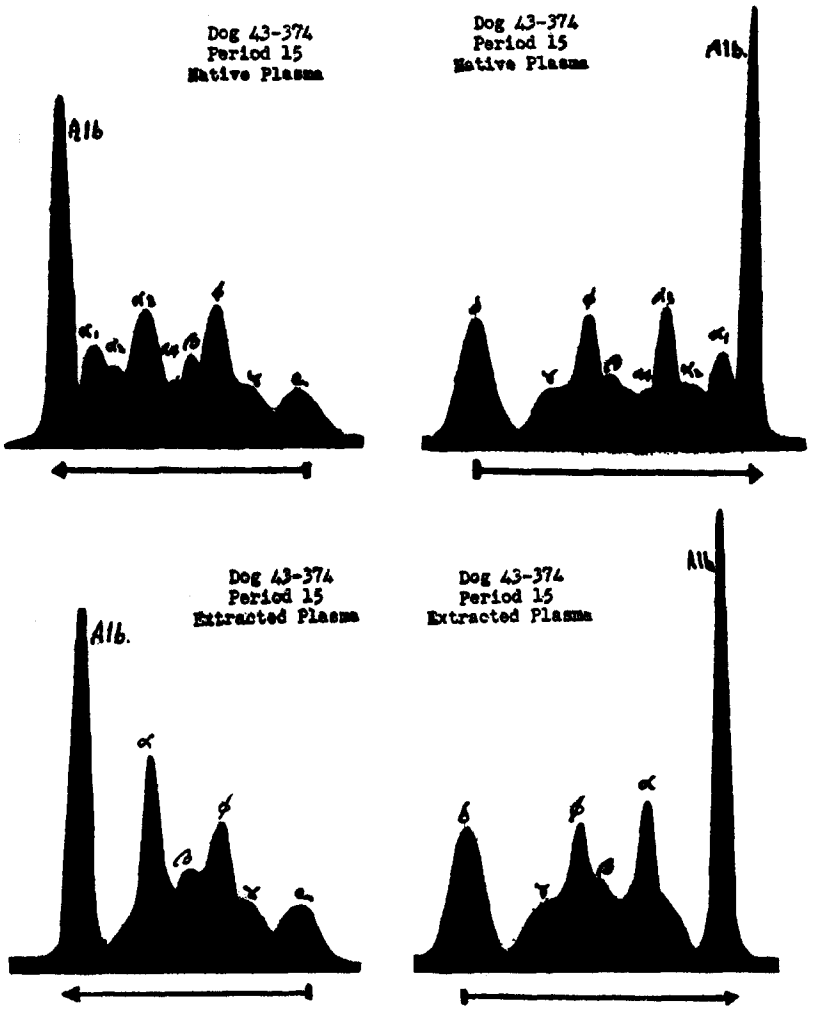

FIG. 2. Electrophoretic patterns of hyperlipemic dog plasma before and after extraction of plasma lipids.

2 and 3). In normal dogs the alpha 1 and 2 peaks are merged, and the alpha 3 peak apparently merges with the beta component (Fig. 1).

These changes might be interpreted to mean that the differential mobilities of these components in native plasma are due to their lipid content, and that a more homogeneous phase is formed simply by the removal of lipids during extraction. A different explanation is possible, however, namely that in-

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cipient denaturation without loss of solubility has altered the mobilities of the absent alpha globulin components, resulting in their inclusion in adjacent peaks. Finally, it appears possible that following the removal of lipids, certain

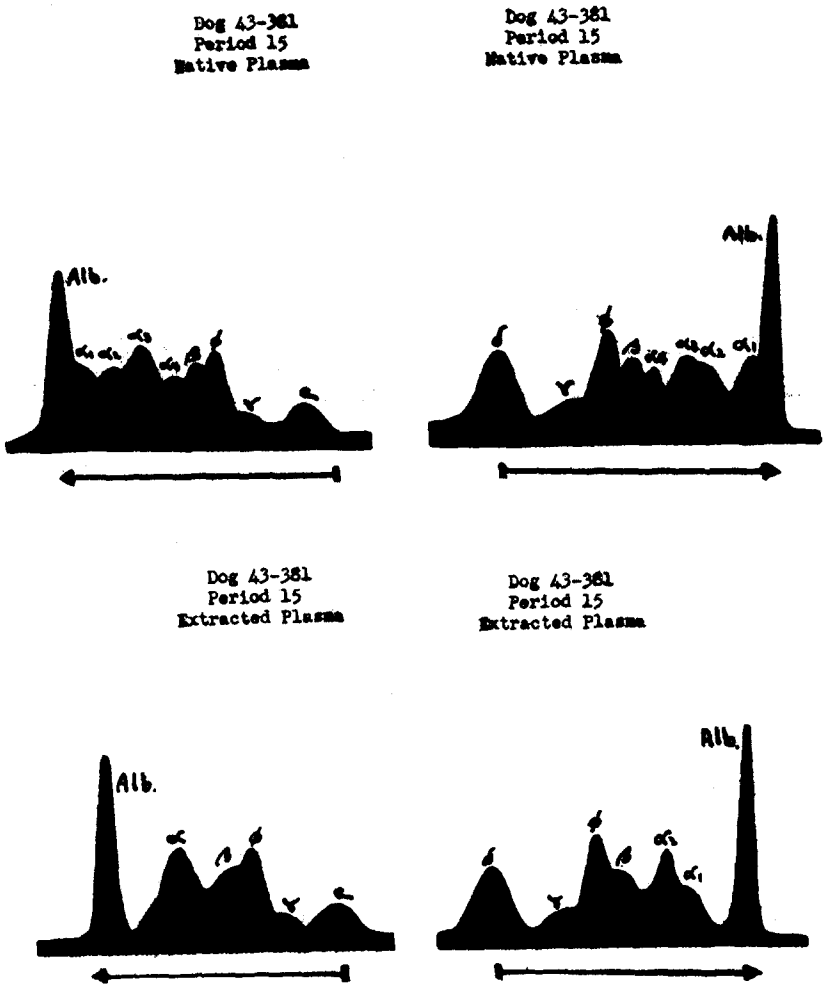

FIG. 3. Electrophoretic patterns before and after extraction of lipids from plasma of hypoproteinemic, hyperlipemic dog.

of the alpha globulin components, with or without incipient denaturation, form complexes with, or are adsorbed on other alpha globulins, or in the case of the alpha 4 globulin, on the beta component. Against an explanation assuming incipient denaturation is the fact that comparable changes in the alpha globulin peaks are not observed in human plasma similarly extracted.

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Moreover, while the mobilities of components other than the alpha globulins in extracted dog plasma were not measured, in several human samples in

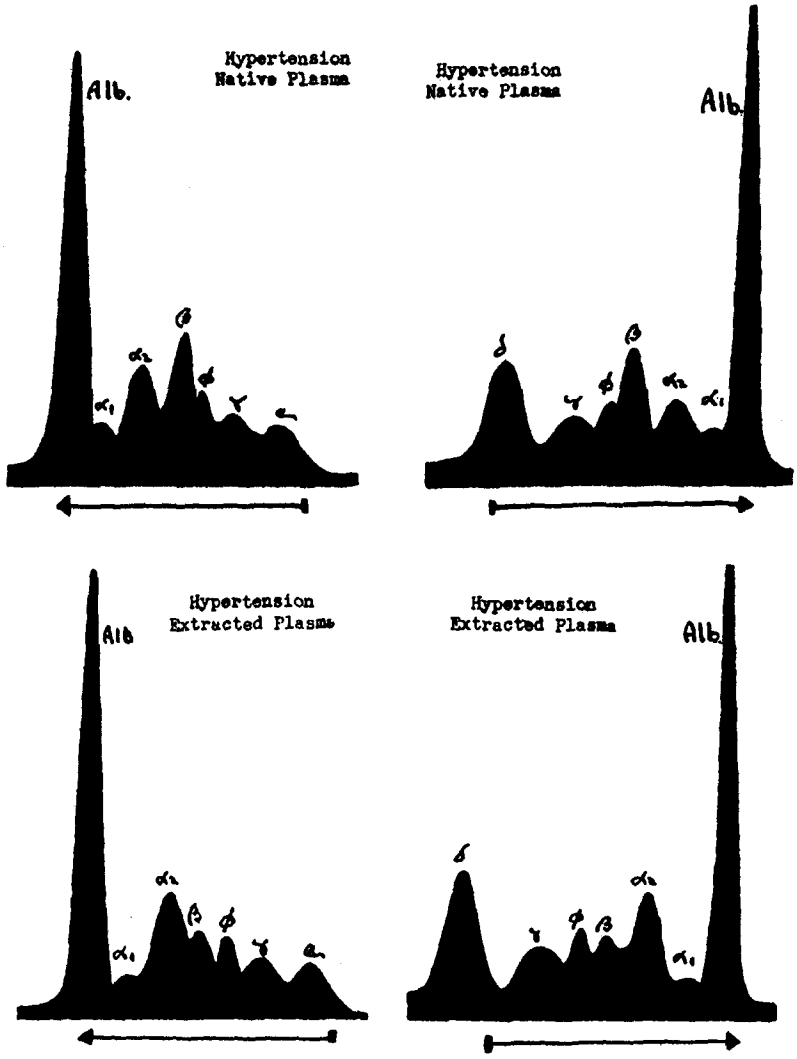

FIG. 4. Electrophoretic patterns before and after extraction of lipids from hyperlipemic human plasma in case of hypertension (patient M. B.).

which such measurements were made, no change in mobility was detected. Perhaps in support of the view that the alpha globulins of native plasma differ only by virtue of their lipid content is the observation that alpha 4 peaks are usually absent in normal dog plasma and quite regularly present in hyperlipemic samples.

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Less apparent than the alterations in number of the alpha globulin peaks are changes in the total electrophoretic area and the areas of individual peaks following extraction of these samples. Comparative measurement of the

TABLE 1

Alcohol-Ether Extraction of Lipids from Plasma of Normal and Hyperlipemic Dogs

Sample No.	Dog	Plasma	Total protein Kjeldahl	Plasma cholesterol	Total plasma lipid	Extract cholesterol	Extract total lipid
			gm. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1	43-427	Native	5.94	203	483	—	—
	Normal	Extracted	5.78	—	—	190	439
2	43-380	Native	5.94	363	1125	—	—
	Period 13*	Extracted	5.76	—	—	336	1035
3	43-380	Native	5.85	279	873	—	—
	Period 16*	Extracted	5.76	—	—	269	794
4	43-374	Native	6.67	390	1137	—	—
	Period 15*	Extracted	6.70	—	—	385	1025
5	43-374	Native	6.40	446	1229	—	—
	Period 17*	Extracted	6.34	—	—	401	1047
6	43-381	Native	4.32	283	777	—	—
	Period 14‡	Extracted	4.46	—	—	252	712
7	43-381	Native	4.23	319	924	—	—
	Period 15‡	Extracted	4.02	—	—	223	635
8	43-327	Native	4.64	238	964	—	—
	Period 8‡	Extracted	4.89	—	—	231	626
9	43-327	Native	5.52	203	563	—	—
	Period 13§	Extracted	5.51	—	—	211	513

* Hyperlipemia during recovery from chronic protein depletion by low protein feeding (18).

‡ Hyperlipemia during low protein feeding (18).

§ During intoxication produced by hemolytic reaction (18).

percentile changes in area following extraction is beset with a number of rather large errors, some of which cannot be satisfactorily estimated. The chief of these probably arises during dilution and dialysis of the samples, where a relatively small volume error may be of great importance when the alteration of area due to extraction of lipid material is small. A further large possible error is encountered in the resolution and planimetry measurement of the

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TABLE 1-*a*

Effect of Extraction of Plasma Lipids on the Areas of Electrophoretic Patterns in Normal and Hyperlipemic Dogs

Sample	Dog	Plasma*	Electrophoretic areas in planimeter units‡						
			Total	Albumin	α	β	ϕ	γ	
1	43-427	Native	33.1	16.9	5.5	6.0	2.7	2.0	Fig. 1
	Normal	Extracted	31.6	16.4	4.2	5.3	3.7	2.0	
		Difference	−4.5	−3.0	−23.6	−11.6	+37.0	0.0	
2	43-380	Native	36.1	9.6	13.9	5.4	3.9	3.3	
	Period 13	Extracted	30.2	8.6	9.9	4.4	5.2	2.2	
		Difference	−16.3	−10.4	−28.8	−18.5	+33.4	−32.4	
3	43-380	Native	36.9	14.1	11.6	4.7	3.9	2.6	
	Period 16	Extracted	31.3	12.4	9.0	4.4	3.3	2.2	
		Difference	−15.2	−12.0	−22.4	−6.4	−15.4	−15.4	
4	43-374	Native	38.0	12.8	13.2	3.1	4.5	4.4	Fig. 2
	Period 15	Extracted	33.3	12.2	19.9	2.8	4.4	4.2	
		Difference	−12.4	−4.7	−25.0	−9.7	−2.2	−4.5	
5	43-374	Native	38.2	12.8	13.0	3.4	3.3	5.7	
	Period 17	Extracted	34.1	12.2	10.2	2.9	4.6	4.0	
		Difference	−10.7	−4.7	−24.6	−14.7	+39.4	−29.8	
6	43-381	Native	27.4	7.7	10.9	4.1	2.8	1.9	
	Period 14	Extracted	22.8	6.7	7.9	3.8	2.8	1.6	
		Difference	−16.8	−13.0	−27.5	−7.3	0.0	−15.8	
7	43-381	Native	26.5	6.3	11.2	4.1	2.3	2.3	Fig. 3
	Period 15	Extracted	23.4	6.5	7.5	3.7	3.5	2.2	
		Difference	−11.7	+3.2	−33.0	−9.8	+52.3	−4.3	
8	43-327	Native	28.9	8.5	10.4	3.6	3.4	3.0	
	Period 8	Extracted	24.7	7.5	7.6	3.1	3.3	3.2	
		Difference	−14.5	−11.8	−27.0	−13.9	−2.9	+6.7	
9	43-437	Native	26.0	6.9	7.1	5.0	3.1	3.9	
	Period 13	Extracted	24.2	6.9	5.5	4.5	3.7	3.6	
		Difference	−6.9	0.0	−22.6	−10.0	+19.4	−7.7	

* Differences are expressed as per cent of the area of the native sample.

‡ Electrophoretic areas of extracted samples are corrected for the differences in protein nitrogen content between native and extracted samples indicated in Table 1.

individual peak areas. Under ideal circumstances, such measurement is probably accurate within 2 to 4 per cent (17, 15). In the present instance,

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the inaccuracy is considerably greater. This arises from the fact that following extraction the asymmetry of the peaks, as well as their slopes and the heights of their junctions with adjacent components is markedly altered. There is thus great difficulty in resolving the limits of the individual peaks in comparable fashion in the native and extracted samples. The further possibility of error due to incipient denaturation with alteration of the specific refractive increments we cannot at present evaluate.

TABLE 2

Alcohol-Ether Extraction of Lipids from Abnormal Human Plasmas

Patient	Diagnosis	Plasma	Total protein Kjeldahl	Plasma cholesterol	Total plasma lipid	Extract cholesterol	Extract total lipid
			gm. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
M. B.	Hypertension	Native	6.59	251	835	—	—
		Extracted	6.51	—	—	238	787
J. M.	Obstructive jaundice with cholangitis	Native	7.06	858	2181	—	—
		Extracted	6.82	—	—	794	1947
G. W.	Xanthoma tuberosum	Native	6.85	510	1227	—	—
		Extracted	6.69	—	—	464	970
C. M.	Multiple myeloma	Native	7.11	174	473	—	—
		Extracted	7.15	—	—	76	276
K. U.	Nephrosis	Native	4.90	420	1543	—	—
		Extracted	4.75	—	—	424	1256
R. T.	Cirrhosis	Native	6.42	103	371	—	—
		Extracted	6.54	—	—	99	348

With these considerations in mind, the data of Table 1-*a* are presented. The possible magnitude of the errors mentioned is perhaps indicated in sample 7, in which there is a 3 per cent increase in the area of the albumin peak following extraction. It is obvious that such an absolute error applying in the measurement of the smaller components of the patterns and thus magnified several fold on a percentage basis would be fully as large as the probable maximum change due to lipid extraction. We believe, however, that for the present purpose these data are instructive if their interpretation is properly limited to certain changes which appear validated by magnitude and consistency.

In general it appears that the total electrophoretic area may be reduced by

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as much as 10 to 15 per cent by the extraction of a large part of the plasma lipids in hyperlipemic dogs. This is in agreement with the magnitude of the discrepancies between nitrogen content and electrophoretic concentrations

TABLE 2-*a*

Effect of Extraction of Plasma Lipids on the Areas of Electrophoretic Patterns of Abnormal Human Plasmas

Patient	Diagnosis	Plasma*	Electrophoretic areas in planimeter units‡						
			Total	Albumin	α	β	ϕ	γ	
M. B.	Hypertension	Native	39.2	18.8	7.8	6.8	1.9	3.9	Fig. 4
		Extracted	36.8	19.0	7.8	4.0	2.2	3.8	
		Difference	−6.1	+1.1	0.0	−41.1	+15.8	−2.6	
J. M.	Obstructive jaundice with cholangitis	Native	45.8	11.1	9.8	9.6	6.1	9.2	Fig. 5
		Extracted	33.4	10.1	8.4	4.7	5.5	4.7	
		Difference	−27.0	−9.0	−14.3	−51.1	−9.8	−48.9	
G. W.	Xanthoma tuberosum	Native	39.6	17.0	9.1	6.8	2.8	3.9	Fig. 6
		Extracted	35.7	16.7	8.3	4.7	2.5	3.5	
		Difference	−9.8	−1.7	−8.8	−30.9	−10.7	−10.2	
C. M.	Multiple myeloma	Native	39.7	13.7	6.6	4.3	2.4	12.7	Fig. 7
		Extracted	38.8	13.7	5.2	2.6	9.2	8.1	
		Difference	−2.3	0.0	−21.2	−39.5	+283.0	−36.2	
K. U.	Nephrosis	Native	31.2	6.5	8.7	11.3	3.3	1.4	Fig. 8
		Extracted	23.2	5.7	7.3	2.5	6.1	1.6	
		Difference	−25.8	−12.3	−16.1	−78.0	+85.0	+14.3	
R. T.	Cirrhosis	Native	38.2	14.9	6.9	4.0	6.4	6.0	Fig. 9
		Extracted	36.3	13.8	6.9	3.5	7.0	5.1	
		Difference	−5.0	−7.4	0.0	−12.5	+9.4	−15.0	

* Differences are expressed as per cent of the area of the native sample.

‡ Electrophoretic areas of extracted samples are corrected for the differences in protein nitrogen content between native and extracted samples indicated in Table 2.

previously reported in such dogs (18). In the presence of normal lipid levels, the reduction is less marked (compare samples 1, 2, and 9, Table 1-*a*).

Both the greatest relative and the greatest absolute loss of area regularly occur in the alpha globulin peaks, and may amount to 20 or 30 per cent of the original area of these components. Reduction in area of the beta globulins is also quite consistent and averages about 10 per cent of the original area. It should be mentioned that in those instance in which, following extraction,

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the alpha 4 component has apparently merged with the beta globulin, it is measured with the beta globulin in the pattern of the native plasma.

It will be noted that in five samples the area of the fibrinogen peak was increased by extraction. The absolute change in planimeter area of this component is small, and in some instances the increase can be accounted for by possible errors in separating the areas of the beta and gamma globulins from the fibrinogen peak (samples 5 and 9). In other instances, however, the increase in the fibrinogen peak is too large to be explained on this basis. Thus, in sample 7, inclusion with the fibrinogen component of the entire area lost by the beta and gamma peaks does not account for the increase in fibrinogen area. More striking instances of such increases in fibrinogen peaks will be referred to subsequently in connection with several samples of human plasma.

Alterations in gamma globulin area in these dog plasmas are too small for satisfactory appraisal.

In Figs. 4 to 9 inclusive and Tables 2 and 2-*a* are shown the results of similar extraction procedures applied to a number of human plasma samples.

The plasma of Fig. 4 was obtained from a 50 year old white woman with moderately severe essential hypertension. For 5 weeks before the sample was obtained, the patient had eaten an experimental diet low in protein. Plasma protein concentration was slightly reduced and plasma lipids somewhat elevated (patient M. B., Table 2). There are minor abnormalities in the electrophoretic pattern of the native plasma—the albumin peak is slightly low and the alpha 2 and beta globulin components are both increased. Extraction reduces the total electrophoretic area to a small extent, the entire change being accounted for by a 40 per cent decrease in the beta component, while other peaks are practically unaltered (Table 2-*a*). It will be noted in this and subsequent patterns of human plasma that there is no reduction in the number of alpha globulin peaks comparable to that regularly found on extraction of dog plasma.

The plasma sample represented in Fig. 5 was obtained from a severely jaundiced 72 year old white male (J. M.). A diagnosis of biliary obstruction and severe acute and chronic cholangitis due to fibrosis of the common bile duct was established by exploratory laparotomy and biopsy of the liver. Plasma lipids were markedly elevated (Table 2).

The pattern of the native plasma is of considerable interest. The albumin peak is low and all globulin components, particularly the beta, fibrinogen, and gamma peaks, are elevated. It will be noted that the gamma peak of the descending boundary is marked by a spike ordinarily identified with the beta globulin component in normal human electrophoretic patterns—the beta disturbance or anomaly. A possible rôle of lipid materials in the production of the beta disturbance has been considered (6). The usual beta disturbance is absent in this pattern. The region of the descending gamma

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boundary during electrophoresis of this sample was marked by a turbidity similar to that usually seen in the region of the beta disturbance.

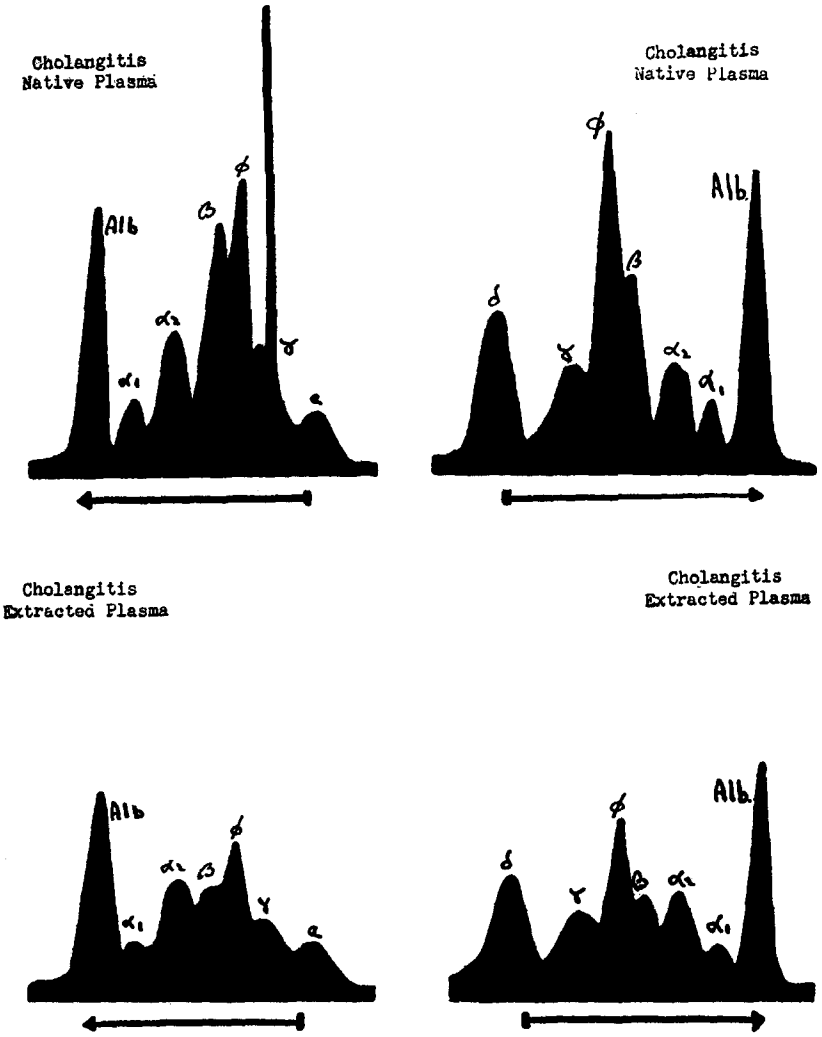

FIG. 5. Electrophoretic patterns before and after extraction of lipids from human plasma in case of obstructive jaundice with cholangitis (patient J. M.).

Marked alterations are apparent following extraction (Fig. 5). The spike disappears from the gamma peak, as does the beta peak in other extracted plasmas (compare Figs. 6, 7, and 8). Total electrophoretic area is reduced by more than 25 per cent (Table 2-*a*). While the reduction involves all com-

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ponents, the largest loss of area occurs in the beta and gamma peaks, each of which is reduced by approximately one-half. It is obvious that a considerable error is probably involved in dividing the areas of these poorly separated globulin peaks for measurement.

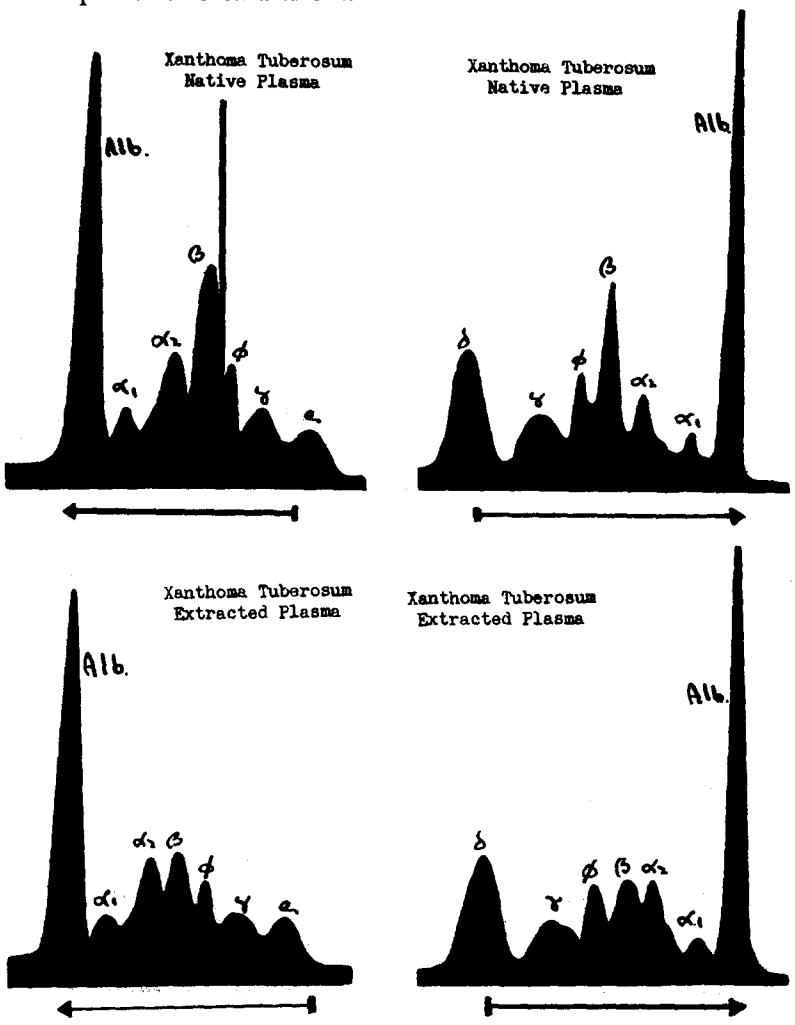

FIG. 6. Electrophoretic patterns before and after extraction of lipids from human plasma in case of xanthoma tuberosum (patient G. W.).

The patterns reproduced in Fig. 6 are of the plasma of a 40 year old white woman in whom a diagnosis of xanthoma tuberosum had been established clinically. In association with the elevated plasma lipids in this patient (G. W., Tables 2 and 2-*a*), the beta globulin peak is markedly elevated. Ex-

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traction reduces this peak by approximately one-third, resulting in an essentially normal electrophoretic pattern.

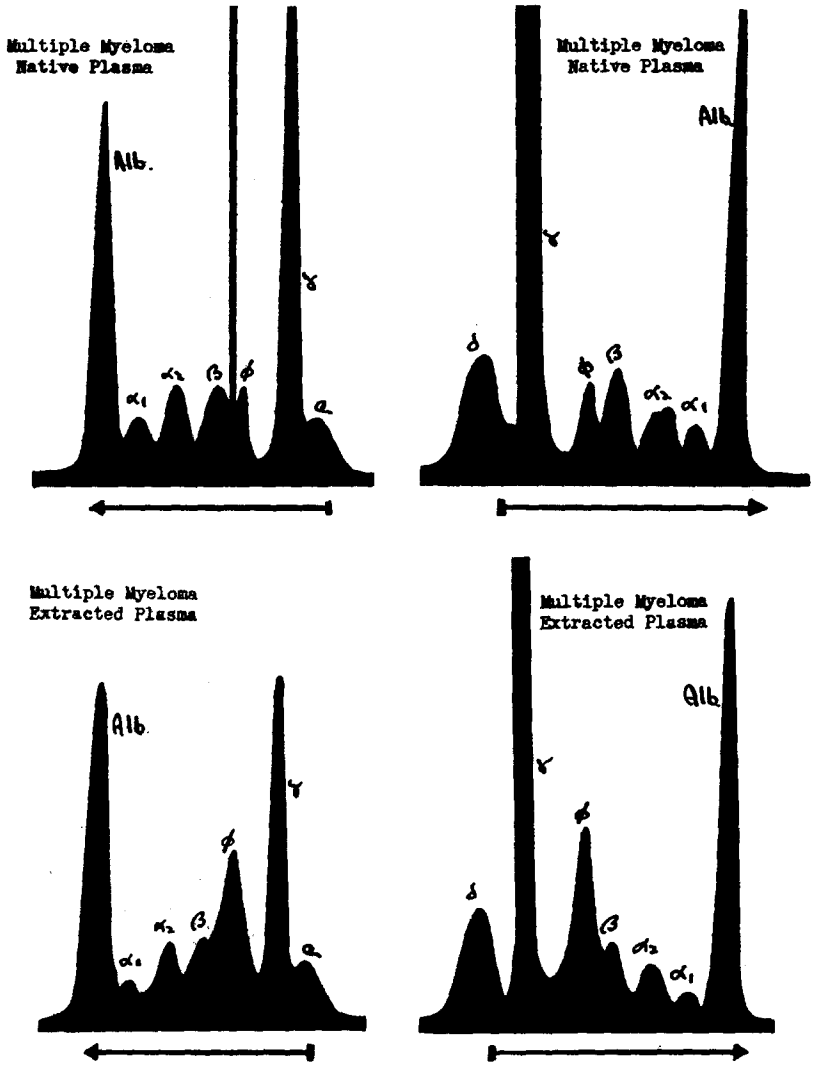

FIG. 7. Electrophoretic patterns before and after extraction of lipids from human plasma in case of multiple myeloma (patient C. M.).

Patient C. M., Fig. 7, was a 76 year old woman in whom a clinical diagnosis of multiple myeloma was confirmed by autopsy. There was extensive bone involvement. The striking abnormality of the pattern of the native plasma is a large peak with the mobility of gamma globulin. Similar patterns have

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been previously described in a number of cases of multiple myeloma (8), and are usually not associated with Bence-Jones proteinuria, which was absent in this patient. The area of this abnormal peak could not be accurately de-

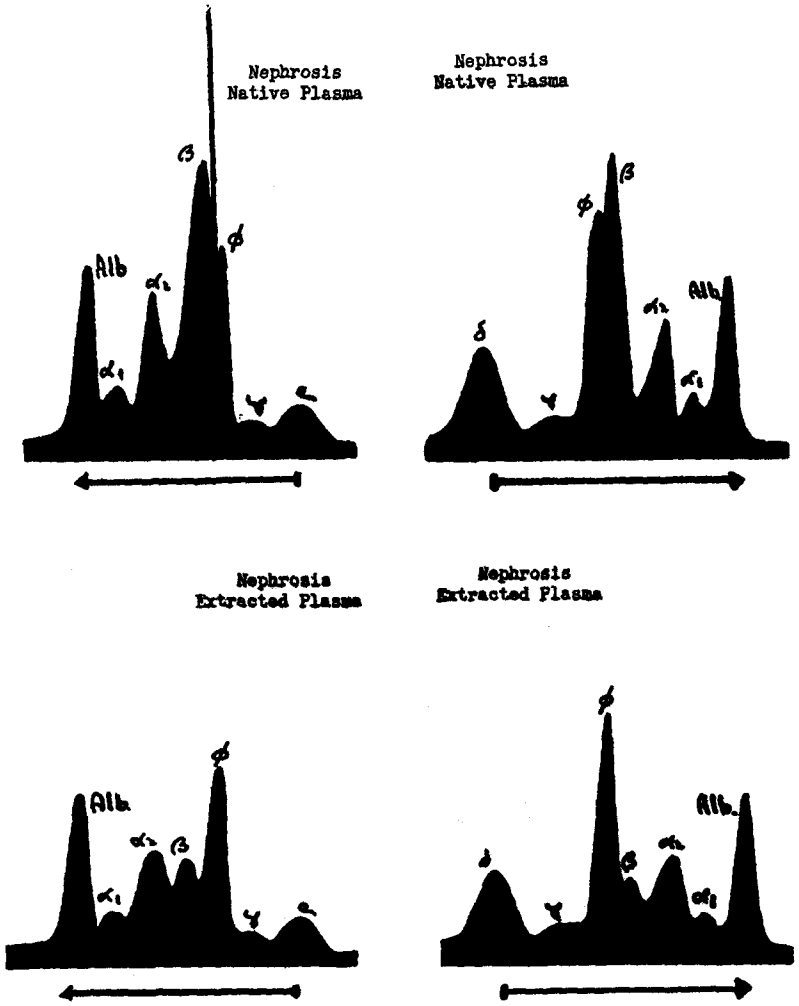

FIG. 8. Electrophoretic patterns before and after extraction of lipids from human plasma in case of nephrosis (patient K. U.).

termined, since it extended beyond the limits of the photographic plate in both boundaries. Estimates of its area in the descending boundaries, however, (Table 2-*a*), indicate that it was reduced one-third or more by extraction.

It will be noted that less than 50 per cent of the plasma cholesterol and only about 60 per cent of the rather low total plasma lipid were removed in extracting this sample (Table 2). We have no adequate explanation for this

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since the extraction was carried out in the same manner as that of other samples.

In spite of the considerable reduction in the area of the abnormal gamma component of this pattern and some reduction of alpha and beta peaks, the

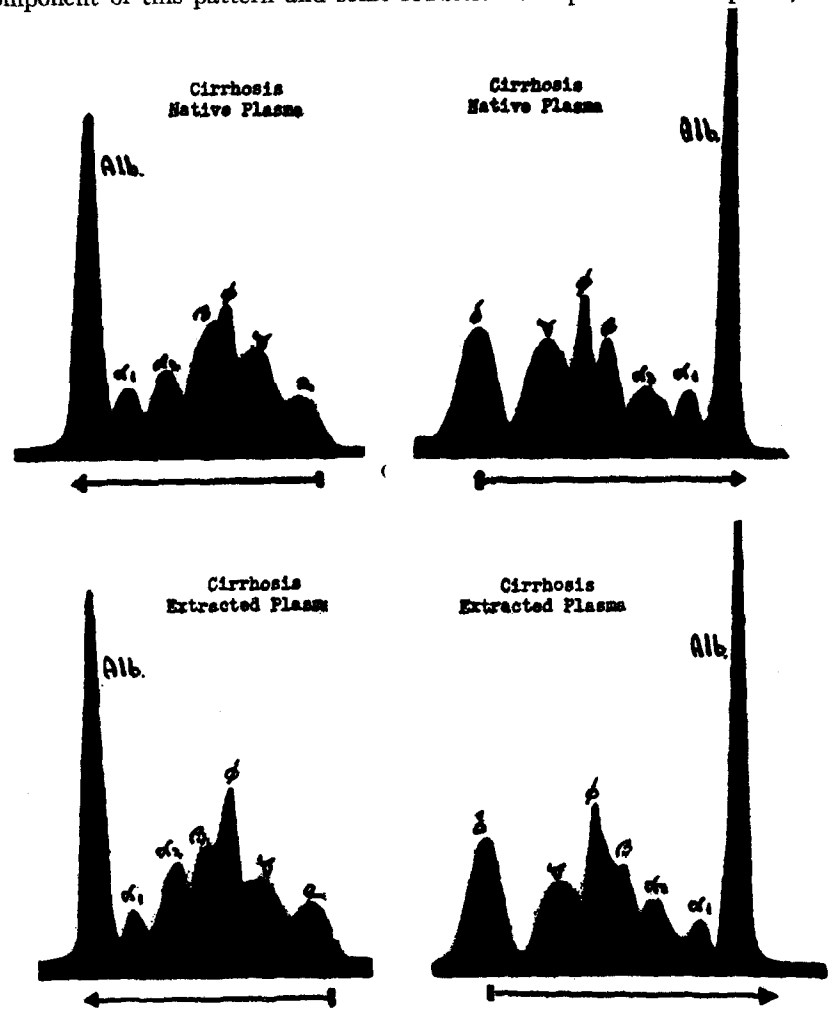

FIG. 9. Electrophoretic patterns before and after extraction of lipids from human plasma in case of cirrhosis (patient R. T.).

total electrophoretic area is practically unchanged following extraction (Table 2-*a*). This results from a threefold increase in the area of the fibrinogen peak. Lesser increases in this peak have been alluded to in extracted dog plasmas and occur in one other human plasma (patient K. U., Table 2-*a*). As in the case of the fusion of alpha globulin peaks of dog plasma, such increases may involve alterations of the mobility of proteins other than fibrinogen and their con-

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sequent inclusion in the fibrinogen peak. The alteration could conceivably result simply from the removal of lipid, or may involve some degree of denaturation of the components. On the other hand, it is possible that such increases are due to denaturation of fibrinogen itself, with alteration of the specific refractive increment. Such denaturation, if it occurred, was incomplete, since there was no marked loss of solubility and since these extracted plasmas formed firm clots on the addition of calcium salts.

The plasma of Fig. 8 is from a case of nephrosis in a 63 year old white woman (K. U.). The pattern of the native sample is similar to others described (10) in nephrosis and is characterized chiefly by a low albumin and a markedly elevated beta globulin peak. Extraction results in a striking decrease in the area of this beta component and lesser changes in other components (Table 2-*a*). Similar changes in nephrotic sera following ether extraction have been reported by Longsworth and others (10, 11). The increase of fibrinogen in this extracted sample, unlike that in the previous figure, may be in large part related to inaccuracy in the measurement of this component in the native plasma pattern in which the beta globulin and fibrinogen peaks are poorly separated.

The sample of Fig. 9 is from a case of cirrhosis in a 39 year old man, (R. T.), diagnosis being established by liver biopsy. Plasma lipids were low in this patient (Table 2), and alterations following extraction are not striking. It can be noted that the abnormally high gamma peak in this plasma is reduced much less than that in the case of cholangitis with markedly elevated plasma lipids.

DISCUSSION

These data demonstrate that increases previously reported to occur (18) in the alpha globulin components of the plasma of hypoproteinemic dogs are due largely to elevated plasma lipid levels. In all instances in which lipids are extracted from such plasmas, the elevated alpha globulin areas and the total globulin areas return to essentially normal values, while the lowered albumin areas are little altered. These observations confirm the suggestion (18) that all electrophoretic globulin components of the plasma respond in a similar fashion during long continued low protein feeding and are selectively maintained in spite of severe albumin depletion.

Studies reported by Moore and others are of interest in connection with alterations noted here in the number of alpha globulin components of dog plasma following extraction. These authors have shown in the sera of hypophysectomized (13) and thyroidectomized (14) rats the regular appearance of an alpha globulin component usually absent in the sera of normal rats. In view of the well known influence of the thyroid on plasma lipids, it would be interesting to determine lipid levels and to study the electrophoretic patterns

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of extracted serum or plasma of such rats. If associated with increased blood lipid levels, the appearance of this alpha component may be analogous to the appearance of the alpha 4 component in the plasma of hyperlipemic dogs. It has been mentioned that the occurrence of this peak offers some evidence that differential electrophoretic mobilities may at times depend simply on lipoprotein complex formation.

The data indicate clearly that the amount of lipid associated with a given electrophoretic component is not fixed, but may vary within wide limits. In the lack of fractional analyses of the phospholipids in the experiments reported here, it appears futile to speculate on the somewhat different distribution of lipoid materials among the electrophoretic components in dog and human plasma. The difference is probably not related simply to variations in cholesterol:phospholipid ratios since these are quite similar in the dog and human plasmas studied. Similarly, the explanation of the high lipid content of the gamma globulin in several abnormal human plasmas (Figs. 5 and 7) must probably await further study. One is tempted to consider the possibility that in the plasma of Fig. 5 increased lipids are associated with the gamma component because of "saturation" of the beta globulins. The failure to find similarly elevated gamma peaks in other plasmas in which the beta components appear to contain as much or almost as much lipid (Figs. 4, 7, and 8) argues against this view.

The nature of the association between serum lipids and proteins has been reviewed by Chargaff (6). The presence of some form of lipoprotein complex has been suggested to explain the lack of turbidity of normal serum and plasma (16). It would appear probable that various types of linkage are involved. It is known, for example, (12) that little lipid material can be extracted by shaking serum with cold ether. Freezing in the presence of ether, however, results in the removal of a larger amount of serum lipids (12). Extraction with cold alcohol in the procedure reported here, or with cold acetone (1), removes the major portion of the cholesterol and a large proportion of the phospholipids. Other lipids, however, can apparently be freed only by denaturation of their protein components. It is of interest to note in this connection the persistence of the beta anomaly in the ether-extracted sera reported by Longsworth (10) and its regular disappearance in the extracted plasmas reported here.

SUMMARY

Electrophoretic patterns of human and dog plasma are markedly altered by the extraction of a large part of the plasma lipids. Total electrophoretic areas, relative areas of individual electrophoretic components, and electrophoretic albumin:globulin ratios undergo change.

For human plasma, such extractions confirm previous observations that a

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particularly rich lipid content characterizes beta globulin. Abnormally large beta peaks regularly occur in the presence of elevated plasma lipids. Marked increases in gamma globulin, however, are also found to be due in large part to elevated plasma lipid levels in certain abnormal human plasmas.

The greatest relative amount of lipid in dog plasma, in contrast to human plasma, is associated not with the beta globulin, but with components usually designated as alpha globulins. Not only the areas, but the configuration and the number of alpha globulin peaks in dog plasma are altered by the extraction of plasma lipids. The results demonstrate that increased alpha globulin areas which occur in the plasma of hypoproteinemic dogs are due in large part to elevated plasma lipid levels.

BIBLIOGRAPHY

1. Blix, G., *J. Biol. Chem.*, 1941, **137,** 495.
2. Blix, G., Tiselius, A., and Svensson, H., *J. Biol. Chem.*, 1941, **137,** 485.
3. Bloor, W. R., *J. Biol. Chem.*, 1914, **17,** 377.
4. Bloor, W. R., *J. Biol. Chem.*, 1928, **77,** 53.
5. Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, **52,** 191.
6. Chargaff, E., in Anson, M. L., and Edsall, J. T., Advances in protein chemistry, New York, Academic Press, Inc., 1944, **1,** 16.
7. Greenwald, I., *J. Biol. Chem.*, 1915, **21,** 61.
8. Gutman, A. B., Moore, D. H., Gutman, E. B., McClellan, V., and Kabat, E. A., *J. Clin. Inv.*, 1941, **20,** 765.
9. Hardy, W. B., and Gardiner, S., *J. Physiol.*, 1910, **40,** 68.
10. Longsworth, L. G., and MacInnes, D. A., *J. Exp. Med.*, 1940, **71,** 77.
11. Longsworth, L. G., Shedlovsky, T., and MacInnes, D. A., *J. Exp. Med.*, 1939, **70,** 399.
12. MacFarlane, A. S., *Nature*, 1942, **149,** 439.
13. Moore, D. H., Levin, L., and Leathem, J. H., *J. Biol. Chem.*, 1944, **153,** 349.
14. Moore, D. H., Levin, L., and Smelser, G. K., *J. Biol. Chem.*, 1945, **157,** 723.
15. Moore, D. H., and Lynn, J., *J. Biol. Chem.*, 1941, **141,** 819.
16. Sörenson, S. P. L., *Kolloid-Z.*, 1930, **53,** 306.
17. Zeldis, L. J., and Alling, E. L., *J. Exp. Med.*, 1945, **81,** 515.
18. Zeldis, L. J., Alling, E. L., McCoord, A. B., and Kulka, J. P., *J. Exp. Med.*, 1945, **82,** 157.

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