Electrophoretic and Ultracentrifugal Studies of Rat Liver Lymph*

BETTY F. CLAUS, PHILIP P. COHEN, AND JESSE L. BOLLMAN

(Department of Physiological Chemistry, University of Wisconsin, Madison, Wis., and Section of Biochemistry, Mayo Clinic, Rochester, Minn.)

Initial studies by Sorof and Cohen (12) on the electrophoretic character of the soluble proteins from normal rat liver revealed that these proteins were strikingly different from those of normal rat serum. In an investigation of the soluble proteins of DAB-induced liver tumors and of various other tumors, a significant reduction in the slowly migrating electrophoretic components was demonstrated (13, 14). This was shown to be a characteristic of neoplasia rather than of the rapid growth phenomenon per se (10). Other investigators found that the electrophoretic nature of serum was not appreciably altered during carcinogenesis (4). A study of rat hepatic lymph was considered of interest, since it seemed probable that its electrophoretic and ultracentrifugal properties were intermediate between those of the soluble liver proteins and those of the serum proteins. A close similarity in the composition of normal rat hepatic lymph and plasma was suggested from the study of Li and Reinhardt (5), who studied rat cervical and thoracic duct lymph. A second objective in studying rat hepatic lymph was to note what change, if any, occurred in the lymph patterns during carcinogenesis and other liver changes. Some alterations in cisternal lymph—notably a relative increase in globulins—have been noted in carbon tetrachloride-treated rats (7).

MATERIALS AND METHODS

Male Sprague-Dawley rats were used in all the experiments. The hepatic lymphs and their corresponding serum samples from normal rats and from rats with damaged livers were obtained by methods of collection previously described (1, 2). The heparinized samples were frozen and maintained in that condition until preparation for analysis. Analyses were carried out after the samples had been stored in the frozen state for a period of 3–6 months. In all instances the thawed samples contained insoluble material characteristic of fibrin. The samples as analyzed were thus essentially lymph serum and blood serum rather than plasma. In view of this, the blood specimens will be referred to as serum.

In addition to the normal rats (fed a commercial chow diet) three groups with damaged livers were studied. Two animals (H95 and H96) were fed 2-acetylaminofluorene (2-AAF) at a level of 30 mg/day for 2 weeks and 10 mg/day for the remainder of 9 months before samples of lymph and blood were collected. These animals were on a diet of Friskies and water. The livers of these rats were markedly involved with hepatoma formation and were 25–50 per cent larger than normal and irregular owing to tumor formation. Microscopic examination revealed relatively little necrotic change but a considerable amount of fibrosis along with tumor formation.

A second group of rats (H101, H102, H189, H190) was exposed to the vapors of carbon tetrachloride for a period of 6 hours, 3 times a week for 5 months, while on a diet of Friskies. These animals had extensive liver damage, as evidenced by the gross nodular character of the liver and microscopically by the presence of numerous regenerative nodules surrounded by connective tissues. A minimal amount of acute necrotic changes was seen. The third group of animals was fed for a period of 6 months 0.06 per cent 4-dimethylaminoazobenzene (DAB) incorporated into the semisynthetic diet No. 3 of Miller *et al.* (6) with a riboflavin level of 1.0 μg/gm of diet. The resulting primary liver tumors in the rats so treated (H205, H206, H207, H209) represented one-half or more of the total liver mass.

Electrophoretic and ultracentrifugal analyses were carried out in the Laboratory of Physiological Chemistry, University of Wisconsin Medical School, Madison, Wisconsin. Since the volumes of hepatic lymph were relatively small, it was necessary to carry out electrophoresis in a micro cell having a 2-ml. capacity. Micro-electrophoretic and

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macro-electrophoretic patterns of human and of rat sera have been shown to be comparable in the mobilities and per cent composition of corresponding components (3). Protein concentrations of the micro-electrophoretic samples were kept at 2.0 per cent or slightly higher where possible. Samples were twice dialyzed against veronal buffer pH 8, 6, 0.1 μ for a period of 2–3 days. Micro-electrophoresis for 90 minutes at a potential gradient of 8.2 v/cm followed.

Ultracentrifugal analyses were made at room temperature in a Spinco Model E ultracentrifuge. One per cent concentrations of the lymph and serum were dialyzed overnight against 0.01 M sodium phosphate–0.15 M sodium chloride at pH 7.4. Analysis followed in the 12-mm. cell at 59,780 r.p.m.

RESULTS

The descending electrophoretic mobilities and per cent composition of the different samples of liver lymph (Table 1) and their corresponding sera (Table 2) are given, as are the centrifugal analyses (Tables 3 and 4). Calculation of electrophoretic mobilities and estimation of areas and of ultracentrifugal data followed procedures previously employed (11, 12). Anomalous behavior in the β region of normal sera H188, H191, and H192 (Table 2) tends to make the values for the β component somewhat high in this group. While it is possible that the increased values observed for the components in the β region were due to traces of fibrinogen, this is not supported by the ultracentrifugal analyses. Thus, it can be seen (Table 4) that there are no components with S^0_{20} values between the globulins and the fast components. The S^0_{20} value for fibrogen, as estimated with our ultracentrifuge, is 7.80 (9). The figure shows the striking similarities between hepatic lymph from normal and DAB-fed rats and the corresponding sera. In all groups the total protein content of the liver lymph as collected was less than that of the corresponding serum. Consistent in all groups was the

TABLE 1

ELECTROPHORETIC DATA: LIVER LYMPH

Animal	Protein concentration (per cent)	F	Albumin	α_1	α_2	β	γ
			Mobilities*				
H98 (Normal)	1.9		5.69	4.69	3.66	2.40	1.34
H99 (Normal)	1.2		5.71	4.79	3.63	2.53	1.42
H188 (Normal)	2.0		5.49	4.51	3.43	2.36	1.32
H191 (Normal)	2.1		5.46	4.53	3.61	2.38	1.33
H192 (Normal)	2.1		5.68	4.64	3.62	2.42	1.35
Av.			5.61	4.63	3.59	2.42	1.35
S.D.†			± 0.12	± 0.12	± 0.09	± 0.07	± 0.04
H95 (2-AAF)	2.2		5.94	5.10	3.90	2.70	1.77
H96 (2-AAF)	2.3		5.65	4.79	3.78	2.50	1.44
H101 (CCl_4)	1.7		5.72	4.99		2.54	1.51
H102 (CCl_4)	1.8		5.65	4.89	3.75	2.53	1.54
H190 (CCl_4)	2.0		5.50	4.61	3.66	2.42	1.29
H205 (DAB)	2.2		5.34	4.22	3.30	2.20	1.14
H206 (DAB)	2.1		5.95	4.93	3.98	2.68	
H207 (DAB)	2.0		5.51	4.66	3.52	2.42	1.52
H209 (DAB)	2.0		5.40	4.52	3.78	2.33	1.31
			Per cent composition				
H98 (Normal)	1.9		50.7	20.2	8.0	15.1	6.0
H99 (Normal)	1.2		48.6	23.6	4.2	16.4	7.2
H188 (Normal)	2.0		47.0	22.7	6.3	19.0	5.0
H191 (Normal)	2.1		44.1	1.19	10.5	17.7	8.6
H192 (Normal)	2.1		44.1	17.6	10.0	18.8	9.6
Av.			46.9	20.6	7.8	17.4	7.3
S.D.*			± 2.9	± 2.5	± 2.6	± 1.7	± 1.9
H95 (2-AAF)	2.2		42.3	19.3	12.4	22.2	3.7
H96 (2-AAF)	2.3		33.2	30.6	12.1	20.4	3.6
H101 (CCl_4)	1.7		34.3	17.4	15.5	22.8	10.1
H102 (CCl_4)	1.8		41.6	16.2	13.8	22.3	6.1
H190 (CCl_4)	2.0		46.2	12.0	11.7	25.2	5.0
H205 (DAB)	2.2	5.0	37.8	21.0	11.7	18.2	6.4
H206 (DAB)	2.1		41.3	26.4	15.0	15.2	2.2
H207 (DAB)	2.0		47.6	24.8	10.2	13.3	4.1
H209 (DAB)	2.0		43.1	24.8	13.8	14.2	4.2

* Mobilities × cm^2/volt sec × 10^{-5}.

† S.D. = $\sqrt{\frac{\Sigma (d^2)}{n-1}}$.

TABLE 2

ELECTROPHORETIC DATA: SERUM

ANIMAL	PROTEIN CONCENTRATION (PER CENT)	F	ALBUMIN	α_1	α_2	β	γ
			Mobilities*				
H98 (Normal)	2.3		5.61	4.71	3.69	2.36	1.11
H99 (Normal)	2.3		5.29	4.57	3.69	2.32	1.37
H188 (Normal)	2.0		5.24	4.46	3.60		0.94
H191 (Normal)	2.7		5.21	4.53	3.60		1.18
H192 (Normal)	2.3		5.46	4.67	3.79		1.09
Av.			5.36	4.59	3.67		1.14
S.D.†			± 0.17	± 0.11	± 0.08		± 0.16
H95 (2-AAF)	2.1		5.36	4.59	3.63	2.24	1.15
H96 (2-AAF)	2.1		5.29	4.65	3.54	2.25	1.26
H101 (CCl_4)	1.3		5.28	4.39	3.51	2.14	1.04
H102 (CCl_4)	2.9		5.26	4.49	3.60	2.16	1.15
H205 (DAB)	2.1		5.31	4.44	3.74	2.22	1.16
H206 (DAB)	2.4		5.08	4.29	3.32	2.04	0.86
H209 (DAB)	2.0		5.40	4.60	3.87	2.45	1.29
			Per cent composition				
H98 (Normal)	2.3		41.1	23.0	10.1	21.9	3.9
H99 (Normal)	2.3		50.2	15.4	11.3	17.7	5.5
H188 (Normal)	2.0		36.0	19.4	14.1	26.9	3.7
H191 (Normal)	2.7		32.8	22.8	13.0	27.3	4.2
H192 (Normal)	2.3		38.8	19.8	10.9	25.3	5.3
Av.			39.8	20.1	11.9	23.8	4.5
S.D.*			± 6.6	± 3.1	± 1.6	± 4.0	± 0.9
H95 (2-AAF)	2.1		36.6	18.2	15.0	26.7	3.5
H96 (2-AAF	2.1		30.1	29.2	18.2	20.2	2.3
H101 (CCl_4)	1.3		24.2	25.3	19.5	27.0	4.1
H102 (CCl_4)	2.9		32.2	22.2	16.8	25.6	3.4
H205 (DAB)	2.1	3.1	37.8	16.5	17.9	19.6	5.0
H206 (DAB)	2.4	4.9	36.2	22.8	14.9	18.8	2.4
H209 (DAB)	2.0		39.2	26.4	15.7	14.9	4.0

* Mobilities $\times$ cm²/volt sec $\times 10^{-5}$.

† S.D. $= \sqrt{\frac{\Sigma (d^2)}{n-1}}$,

TABLE 3

ULTRACENTRIFUGAL DATA: LIVER LYMPH

ANIMAL	FAST	II*	GLOBULINS	A-COMPONENT†
	S^0_{20}			
H188 (Normal)	17.9		6.3	4.0
H191 (Normal)	17.4		5.9	4.2
H192 (Normal)	17.5		6.1	4.2
H95 (2-AAF)	18.4		6.1	4.3
H96 (2-AAF)	17.1		6.5	4.2
H101 (CCl_4)	17.3		5.9	4.0
H102 (CCl_4)	18.9		6.4	4.2
H189 (CCl_4)	18.7		6.5	4.1
H190 (CCl_4)	17.9		6.3	4.0
H205 (DAB)	18.1		5.4	3.9
H206 (DAB)	16.8		5.3	3.8
H207 (DAB)	17.5		5.8	3.9
H209 (DAB)	17.1		5.6	3.8
	Per cent composition			
H188 (Normal)	3.7	3.2	21.4	71.8
H191 (Normal)	4.0	2.9	20.8	72.3
H192 (Normal)	4.0		23.7	72.3
H95 (2-AAF)	6.5	6.8	17.8	68.9
H96 (2-AAF)	10.6	9.6	20.3	59.4
H101 (CCl_4)	4.7		20.1	75.2
H102 (CCl_4)	5.1		15.7	79.2
H189 (CCl_4)	3.3		29.3	67.4
H190 (CCl_4)	4.5		21.5	74.0
H205 (DAB)	4.0		14.1	81.9
H206 (DAB)	7.3		12.7	80.0
H207 (DAB)	7.3		12.8	79.8
H209 (DAB)	9.7	5.0	14.3	71.0

* II = Material sedimenting between fast and globulins.

† The A-component is considered to contain albumin plus some globulins (8).

TABLE 4

ULTRACENTRIFUGAL DATA: SERUM

ANIMAL	FAST	II*	GLOBULINS	A-COMPONENT†
	S^0_{20}			
H98 (Normal)	16.8		6.5	3.8
H99 (Normal)	18.0		6.3	4.2
H188 (Normal)	16.6		6.8	3.8
H191 (Normal)	15.9		6.6	4.0
H192 (Normal)	15.8		6.5	4.2
H95 (2-AAF)	17.5		6.3	4.0
H96 (2-AAF)	16.9		7.2	4.3
H101 (CCl_4)	16.5		6.3	3.8
H102 (CCl_4)	16.1		6.5	3.7
H205 (DAB)	16.3		6.7	3.9
H206 (DAB)	16.0		6.6	3.7
H209 (DAB)	16.4		6.1	3.9
	Per cent composition			
H98 (Normal)	10.8	6.2	19.3	63.7
H99 (Normal)	11.5		19.3	69.2
H188 (Normal)	7.4		24.9	67.8
H191 (Normal)	7.5		26.8	65.6
H192 (Normal)	6.1		25.6	68.3
H95 (2-AAF)	11.1	4.7	18.0	66.2
H96 (2-AAF)	16.0	3.9	20.1	59.9
H101 (CCl_4)	15.7	4.9	18.8	60.6
H102 (CCl_4)	11.0	2.3	16.0	70.6
H205 (DAB)	7.3		18.6	74.1
H206 (DAB)	9.7		16.1	74.2
H209 (DAB)	14.8	3.1	13.0	69.1

* II = Material sedimenting between fast and globulins.

† The A-component is considered to contain albumin plus some globulins (8).

fact that the liver lymph contained a larger percentage of albumin than the corresponding serum. The liver lymph from normal and carbon tetrachloride-treated rats contained also a larger percentage of γ-globulin than did the corresponding serum.

COMMENT

Electrophoretic and ultracentrifugal data show that the protein composition of rat hepatic lymph is strikingly similar to that of serum. The four principal components of serum (albumin, α, β, and γ globulins) are present in the hepatic lymph. Data on a limited series of rats with neoplastic and cirrhotic liver changes reveal no striking alterations in the electrophoretic and ultracentrifugal patterns of rat liver lymph. The data in Table 3 indicate that hepatic lymph from rats with DAB-induced liver tumors has a lower globulin content than that from normal liver.

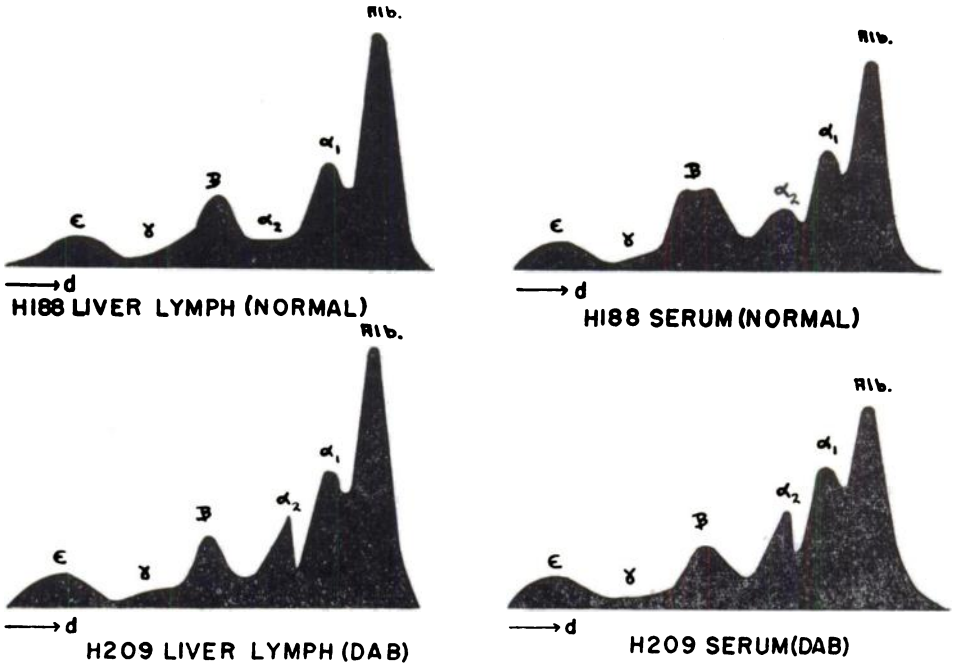

Chart 1.—Electrophoretic comparison of normal hepatic lymph and serum and of hepatic lymph and serum from rats fed DAB. Veronal buffer, pH 8.6, 0.1 μ. Microelectrophoresis time, 90 minutes. Protein concentration, 2 gm/100 ml.

SUMMARY

1. Electrophoretic and ultracentrifugal properties of normal rat hepatic lymph resemble closely the properties of normal rat serum.

2. In a limited number of rats with neoplastic and cirrhotic liver changes, no striking alterations in the hepatic lymph patterns were observed.

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