STUDIES ON ISOLATED CELL COMPONENTS

XIV. AN ELECTROPHORETIC ANALYSIS OF THE SOLUBLE PROTEINS OF RAT LIVER, KIDNEY, BRAIN, AND TESTIS

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CHANGES occurring during differentiation probably include the appearance of new proteins specific to an organ or tissue, changes in the proportions of proteins common to all tissues, and disappearance of proteins characteristic of the egg, sperm, or early embryo. Comparable protein preparations from several organs of the same animal were therefore examined in a search for evidence of common proteins and to determine whether organ-specific proteins are demonstrable electrophoretically. It might also be possible to determine which proteins constitute the fundamental machinery of the cell (and might be expected to occur in all tissues of the same animal) and which are involved in the thermal instability of soluble tissue proteins previously discussed [8].

In the present study, the soluble or supernatant proteins obtained by ultracentrifugation of breis from perfused liver, kidney, testis, and brain were examined electrophoretically at pH 7.5.

Previous electrophoretic analyses of soluble tissue proteins have yielded results that are difficult to compare because of differences in the preparation of the protein extracts and the experimental treatment of the animals, the analytical methods used, and the pH and ionic strength of the extracts. Paper-strip electrophoresis has been used to analyze soluble proteins from liver [2, 3, 12–15, 18, 19, 22, 28, 29, 34, 36, 41], brain [14, 23, 28, 31, 37, 39, 40, 42, 43], kidney [14, 28, 34], spleen [18, 19], lymphatic tissue [22], and nerve [32]. Demling *et al.* also used this method to analyze mucosa, pancreas, uterus, testis, lung, and muscle [14]. Starch gel or agar electrophoresis has been used in the analysis of liver [9, 33, 34, 36, 57, 59], brain [39], and kidney [33]. Moving-boundary electrophoresis has been used only in the analysis of rat, rabbit, dog, bovine, and human liver [5, 10, 11, 16, 18, 19, 21, 24–26, 29, 30, 38, 45, 47, 55], brain [17, 35], red blood cells [56], spleen

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[18, 19], and lymphoid tissue [1, 44, 56]; in most of these analyses phosphate buffer, pH 7.5, or veronal buffer, pH 8.6, was used. Variations in the results obtained are considerable, depending on the methods used. Sorof and Cohen, for example, found large differences in the electrophoretic pattern of supernatant proteins of liver, depending on whether the tissue had been ground in a Waring Blendor or in a coaxial homogenizer [49].

In this study, marked and characteristic interorgan differences in electrophoretic patterns are reported.

EXPERIMENTAL

Male Sprague–Dawley rats were perfused with cold Locke's solution through the dorsal aorta, as previously described [4, 6] for the preparation of soluble rat liver proteins (also termed supernatant or soluble-phase proteins). This technique also resulted in perfusion of kidneys and testes; brains were perfused in ether-anesthetized animals by back-perfusion through the dorsal aorta. The organs were rapidly weighed in tared beakers of cold Locke's solution and suspended in 0.25 *M* sucrose. The volume was adjusted so that the final volume of sucrose plus tissue was equal in milliliters to three times the tissue weight in grams for liver and testis, and twice the tissue weight in kidney and brain. After homogenization in a coaxial hand homogenizer, the brei was centrifuged at 4500 × *g* for 15 minutes in a No. 855 head in the International PR-1 centrifuge at 0°C. The floating lipid layer was removed and the

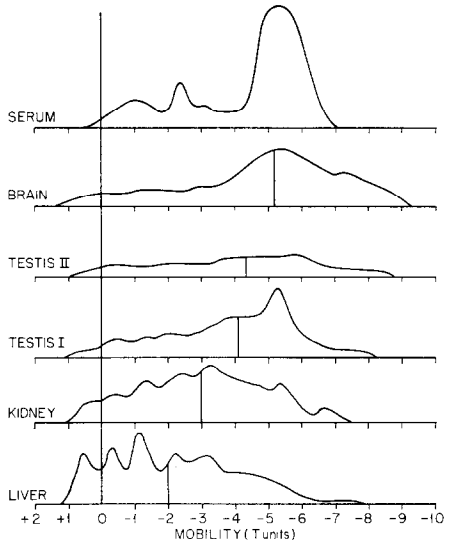

Fig. 1.—Electrophoretic analysis of soluble proteins of rat liver, kidney, brain, and testis (with and without removal of tubular fluid). Rat serum proteins are included for comparison.

turbid supernatant centrifuged for 1 hour at 104,000 × *g* (40,000 rpm) in the Spinco No. 40 rotor in a Model L centrifuge. The small lipid layer was again removed after deceleration and the clear supernatant protein solution removed with a bulb pipette. Approximately 20 ml of supernatant solution was dialyzed in No. 8 Visking dialysis tubing against several changes of cold Miller–Golder buffer (pH 7.5, 0.1 ionic strength), for at least 24 hours with the buffer agitated by a magnetic stirrer. The last 12 hours of the dialysis were carried out in 2 liters of the electrophoresis buffer, which was later used to fill the right limb of the cell and the buffer vessels. All electrophoretic analyses were done in 11-ml quartz cells in the Spinco Model H at 0.9°C. A current of 16 mA, a voltage of ~ 130 volts, and a field strength of 2.4 volts/cm were used in each instance for an average of 4 hours. The Philpot–Svenson cylindrical lens system with either a single knife edge or a phase plate was used. Patterns were enlarged on graph paper by using adjusted magnifications that allowed mobilities to be read directly. Mobilities were measured from the photographed starting boundary, not from the salt boundary.

Initially the method of Hoxter *et al.* [27], for dividing Schlieren diagrams into Gaussian peaks was used. Although this method is adaptable to less complex diagrams, it was not adequate for an unambiguous analysis of the tissue protein preparations studied here. Rather, the amount of material between certain mobilities, representing minima in the Schlieren patterns, was determined as well as the mobilities of maxima. In this way, data that may be used for interorgan or experimental-vs.-normal studies were obtained without implying that any certain number of proteins was present.

RESULTS

Representative patterns from each organ are shown in Fig. 1. Because a peak corresponding in mobility to the rat serum albumin group [7] was seen in the first group of testis experiments, a more detailed study was made. Since the possibility exists that seminiferous tubule fluid may contain serum proteins, homogenization, which broke only a small fraction of the cells, was used. The cell suspension obtained was centrifuged, the cells were resuspended, and the suspension was homogenized with a close-fitting homogenizer until microscopic studies showed few unbroken cells. The soluble proteins obtained with this second method are shown in Fig. 1 as testis II. Comparison of testes I and II show a large albumin group contamination in testis I. Comparison of the patterns for liver and kidney give evidence for peaks having similar mobilities; whereas brain and testis show little relation to each other or to liver and kidney.

The mobilities of maxima observed in the four organs are given in Table I, and the mobilities of minima are listed in Table II. To facilitate comparative studies the percentages of total areas between fixed minima are given in Table III. As will be discussed, the ratio of fast- to slow-moving components

TABLE I. *Mobilities of maxima observed in soluble-phase patterns.*[1]

Run	Mobilities							
				Brain				
1796	0.15	1.44	3.28	5.29	7.90			
1801	0.41	1.50	3.23	5.60	7.60			
1802	0.51	1.44	2.90	5.03	7.21			
1806	0.20	1.32	3.00	5.60	7.50			
Av.	0.32	1.43	3.10	5.38	7.55			
				Testis I				
1786	0.38	1.20	1.92	3.80	5.24	—		
1785	0.37	1.10	1.88	3.90	5.26	7.40		
1784	0.37	1.08	1.89	3.85	5.30	7.58		
1773	0.27	1.02	2.02	4.10	5.57	—		
1769	0.40	1.26	1.92	3.95	5.39	7.70		
1766	0.39	—	2.20	4.00	5.51	7.74		
1756	0.28	1.20	1.89	3.72	5.09	7.70		
Av.	0.35	1.14	1.96	3.90	5.34	7.52		
				Testis II				
1916	0.49	1.80	4.60		5.90	7.00		
1897	0.55	2.00	—	5.30	—	7.00		
Av.	0.52	1.90				7.00		
				Kidney				
1789	+0.43	0.30	1.18	2.30	3.09	5.20	6.39	
1788	+0.40	0.29	1.13	2.35	3.16	5.32	6.68	
1787	+0.35	0.29	1.19	2.32	3.10	5.22	6.59	
1770	+0.39	0.33	1.26	2.37	3.20	5.29	6.49	
1767	+0.51	0.28	1.24	2.40	3.21	5.39	6.52	
Av.	+0.42	0.30	1.21	2.35	3.15	5.30	6.53	
				Liver				
1755	+0.49	0.29	1.07	2.02	2.99	3.71	4.39	6.70
1758	+0.59	0.21	1.17	2.00	3.02	—	4.52	7.00
1762	+0.55	0.32	1.11	2.10	3.00	3.78	4.30	6.88
1763	+0.61	0.28	1.07	2.09	3.00	3.89	—	7.18
1768	+0.60	0.24	1.09	2.16	3.08	3.94	4.52	7.17
1780	+0.63	0.19	1.02	2.06	2.94	—	4.45	7.18
1778	+0.66	0.14	1.21	2.13	3.13	—	4.60	7.35
Av.	+0.59	0.24	1.11	2.08	3.02	3.83	4.46	7.07

[1] All mobilities are negative unless otherwise indicated.

TABLE II. *Mobilities of minima in soluble-phase patterns.*

Run	Mobilities						
			Brain				
1796	+0.44	2.62	3.54	7.56			
1801	+0.64	2.56	3.45	7.15			
1802	+0.78	2.41	—	6.75			
1806	+0.82	2.60	3.32	7.15			
Av.	+0.67	2.55	3.44	7.15			
			Testis I				
1786	0.80	1.44	2.48	4.75			
1785	0.80	1.48	2.51	4.80			
1784	0.80	1.43	2.46	4.75			
1773	1.02	1.60	2.84	5.05			
1769	0.78	1.48	2.65	4.95			
1766	0.95	—	2.70	5.05			
1756	0.77	1.47	2.59	4.60			
Av.	0.85	1.48	2.62	4.85			
			Testis II				
1916	1.20	3.00	5.40	6.60	7.90		
1897	1.25	3.00	—	6.66	7.90		
Av.	1.22	3.00		6.63	7.90		
			Kidney				
1789	+0.17	0.66	1.56	2.66	4.78	6.07	3.63
1788	+0.27	0.63	1.61	2.68	4.92	6.28	3.81
1787	+0.11	0.64	1.56	2.66	4.79	6.21	3.95
1770	+0.17	0.69	1.65	2.78	4.93	6.30	3.89
1767	+0.20	0.70	1.72	2.78	4.90	6.25	3.78
Av.	+0.18	0.66	1.62	2.71	4.86	6.22	3.81
			Liver				
1778	+0.24	0.54	1.70	2.64	3.84	6.83	
1780	+0.22	0.56	1.63	2.53	3.81	6.87	
1768	+0.20	0.66	1.68	2.56	3.70	6.83	
1763	+0.13	0.64	1.66	2.52	3.65	6.89	
1762	+0.12	0.71	1.65	2.59	3.61	6.55	
1758	+0.12	0.62	1.55	2.54	3.57	6.55	
1755	+0.05	0.64	1.68	2.58	3.58	6.50	
Av.	+0.16	0.62	1.65	2.57	3.68	6.72	

TABLE III. *Percentage areas between fixed minima in soluble-phase Schlieren patterns.*

Brain

Run	Mobilities of fixed minima								
	0.0	0.67	1.90	2.55	3.44	4.50	6.0	7.15	8.5
	Percentage areas								
1806	2.53	3.12	7.43	4.31	6.24	11.59	29.42	17.83	14.26	3.27
1802	2.05	3.18	9.20	4.32	7.29	13.02	24.56	16.21	15.43	4.74
1801	2.59	2.84	8.72	4.46	6.64	10.70	23.63	16.68	16.13	7.61
1796	3.78	3.64	9.23	4.48	6.99	10.35	23.92	15.10	14.69	7.83
Av.	2.74	3.20	8.64	4.39	6.76	11.42	25.38	16.46	15.13	5.86

Testis I

	Mobilities of fixed minima							
	0.0	0.85	1.48	2.6	4.85	5.85	7.0	
	Percentage areas							
1786	2.09	5.07	4.02	10.58	39.79	24.89	9.54	4.02
1785	2.73	5.59	4.50	10.92	37.93	25.23	9.14	3.96
1784	3.39	6.07	5.08	11.44	35.31	25.85	8.76	4.10
1773	4.15	9.27	4.15	9.27	30.35	25.88	12.14	4.79
1769	2.10	5.52	4.95	11.43	35.43	23.05	10.10	7.43
1766	2.01	4.27	3.77	10.80	36.18	26.38	11.56	5.03
1756	3.62	6.91	5.42	10.85	36.28	21.38	9.89	5.64
Av.	2.87	6.10	4.56	10.76	35.90	24.67	10.20	5.00

Testis II

	Mobilities of fixed minima					
	0.0	1.22	3.00	1.64	7.90	
	Percentage areas					
1916	4.0	11.7	16.0	56.3	8.7	3.3
1897	4.1	10.9	14.1	56.6	10.1	5.1
Av.	4.0	10.8	15.0	56.4	9.4	4.2

(Table III, cont.)

Kidney

	Mobilities of fixed minima							
	+0.18	0.66	1.62	2.71	3.81	4.86	6.22	
Run	Percentage areas							
1767	4.39	8.23	13.42	18.61	22.51	14.71	15.15	3.03
1770	4.41	7.72	11.76	19.12	21.69	15.81	15.07	4.41
1787	4.56	6.85	12.66	18.67	22.20	15.35	15.35	4.36
1788	5.52	7.98	13.06	18.72	22.06	15.24	13.64	3.77
1789	5.30	8.48	13.18	18.79	21.36	15.61	13.48	3.79
Av.	4.84	7.85	12.82	18.78	21.96	15.34	14.54	3.87

Liver

	Mobilities of fixed minima							
	+0.16	0.62	1.65	2.57	3.68	5.0	6.72	
	Percentage areas							
1778	13.3	12.6	21.6	13.4	16.3	14.4	7.6	0.8
1780	11.9	12.9	19.9	15.5	17.7	15.2	5.8	1.8
1768	13.2	13.3	19.8	14.9	17.6	14.2	6.4	0.7
1763	12.8	13.9	20.0	14.4	17.0	14.5	6.2	1.1
1762	11.3	14.6	18.8	14.8	17.8	15.2	6.7	0.7
1758	14.4	15.0	20.0	14.3	15.7	13.4	6.5	0.8
1755	10.6	13.2	18.6	14.9	17.6	16.2	6.8	1.9
Av.	12.6	13.6	19.8	14.6	17.1	14.7	6.6	1.1

may be important in relation to cell function. The mobilities of points dividing the area into two equal parts were also determined. The mobilities or T values obtained were -2.0 for liver, -3.0 for kidney, -4.1 for testis I, -4.3 for testis II, and -5.2 for brain.

DISCUSSION

Soluble proteins from the liver, kidney, testis, and brain, studied electrophoretically at pH 7.5, exhibit both similarities and marked differences. The results provide specific evidence for the widely accepted view that structural alterations in cells during differentiation are accompanied by qualitative and quantitative changes in the relative amounts and possibly the species of soluble proteins as well.

The results reported are difficult to compare with previous studies, which have often involved one or more of the following: (a) methods of breaking cells that may damage subcellular particles, (b) incubation of breis after preparation, (c) centrifugation that did not completely sediment microsomes, (d) lyophilization, (e) dialysis against distilled water, (f) extraction with fat solvents, (g) use of different buffer systems, or (h) use of zonal techniques where interaction with the supporting medium may occur. For these reasons, detailed comparison of our results with previous studies, except for the work of Sofor and co-workers [47–54], is not feasible. The practice of applying to tissue proteins the designations (α, β, γ) used for serum proteins is deceptive, since it suggests other similarities that have not been demonstrated. Electrophoretic peaks are therefore designated here according to their mobility (T) as previously described [7].

Ideally, Schlieren diagrams should be resolvable into a series of Gaussian curves. In practice, however, unamiguous results may be obtained only when the mixture analyzed consists of a few electrophoretically distinct components present in comparable quantities and there is little interaction in solution. Even when an apparently unambiguous analysis can be made, some of the curves often are wider or narrower than might be expected from the diffusion coefficients of the individual proteins (after allowing for boundary effects). Thus a long, low, broad peak, such as would be obtained from the electrophoresis of a dialyzable substance with a low molecular weight, cannot be interpreted as being produced by a single protein species in a dialyzed mixture. If these restrictions are understood, however, analysis by the method of Hoxter *et al.* [27] is often useful if it can be done with reproducible results.

Our procedure involved locating the apparent minima in all patterns from a particular organ and finding an average value for each in terms of mobility units. The apparent mobility of different portions of the pattern can be determined to about 1 per cent accuracy. Vertical lines were then drawn at these average mobility values, and the distributions of areas among the regions formed were determined. In regions where the minima were far apart, an arbitrary division was often made to facilitate detailed comparison.

Since the patterns were not selected to give a low variability but were from all properly completed runs, the table of areas gives some idea of the amount of variation to be expected in an electrophoretic study of a series of normal organs, and provides a basis for comparison of electrophoretic data from experimental animals.

The five major peaks of liver may correspond to five peaks of kidney,

which has two additional well-defined ones. The two lower mobility components of liver and kidney might correspond also to the lower ones of testis and brain, but it is easy to confuse peaks of low mobility and low resolution. A peak with a mobility somewhat over -5 appears in kidney, testis, and brain but not as a distinguishable peak in liver. This is the approximate mobility of rat serum albumin, and results obtained by first isolating whole cells of the testis and then homogenizing them suggest that some of this material may be albumin contained in the testis tubules as a serum ultrafiltrate. Analysis by immunological methods has demonstrated less albumin in liver than in many other organs [20].

Although most of the mobility range is shared by all four organs studied, there are some qualitative differences. A peak of low positive mobility is present in liver and kidney, but there is little evidence for such a component in testis and brain. This indicates that a definite part of the protein of liver and kidney has an isoelectric point more basic than 7.5 and thus has a net positive charge at physiological pH. In addition, about 6 per cent of the brain material has a mobility of -8.5 or more, whereas less than 1 per cent from other organs has such a high mobility. Experimental results suggesting that the nuclear volume is related to the level of basic cytoplasmic protein will be presented in a subsequent paper.

It is evident that the quantitative distribution of material along the mobility scale differs greatly from organ to organ. This is most simply indicated by the position of the "median". On this basis, liver, kidney, testis, and brain form a series of increasing mobility. This indicates a marked difference in average charge of proteins in these organs.

The most striking feature of the majority of the patterns is the tendency for an even spacing of peaks. The five major peaks of liver are separated, fortuiotusly, by increments of almost exactly one mobility unit. A Gaussian distribution of electrophoretic mobilities would be expected from analysis of a mixture having a certain average amino acid composition and containing a large number of different proteins, each in low concentration. The presence of distinct peaks therefore suggests that either (a) extensive complexing occurs in solution giving rise to aggregates with certain distinct average mobilities, (b) a few proteins are present in fairly high concentration, or (c) families of proteins with similar mobilities are present. The latter could arise by mutation and evolution from distinct ancestral proteins (or more precisely, ancestral templates). It is also possible that charge increments of more than unity are favored for structural reasons. This could occur among proteins composed of two or more identical subunits. Addition of one charged group

to a subunit would increase the total number of charged groups by a number equal to the number of subunits. Experiments aimed at distinguishing between these possibilities will be presented in subsequent papers.

SUMMARY

Detailed analysis of the electrophoretic patterns of the soluble proteins of rat liver, kidney, brain, and testis in pH 7.5, 0.1 ionic strength buffer reveal characteristic differences and similarities. The large differences in average mobility are indicated by the median mobilities, which were −2.0 for liver, −3.0 for kidney, −4.1 for testis, and −5.2 for brain. Implications of the presence of peaks in such complex mixtures are discussed.

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