tained in this investigation. Rechenberg's values were calculated from the measurements of Feitler[11] made from around 90° to the boiling points of the compounds.

The vapor pressures of methyl amyl ketone have been measured by Park and Hofmann.[16] The agreement with the present work seems to be fair although their data are given in the form of a small logarithmic plot.

Acknowledgment.—We wish to express our gratitude to Dr. Paul Gross for numerous helpful suggestions made during the course of this investigation.

Summary

The vapor pressures of the xylenes, ethylbenzene, mesitylene, the chlorotoluenes, *o*-bromotoluene, *m*-bromotoluene, cyclohexane and methyl amyl ketone have been measured at temperatures ranging from about 4 to 75°.

The data have been fitted to suitable equations by the method of least squares.

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[CONTRIBUTION FROM THE DEPARTMENT OF ANATOMY AND INSTITUTE OF EXPERIMENTAL BIOLOGY, UNIVERSITY OF CALIFORNIA]

Studies on Pituitary Lactogenic Hormone. II. A Comparison of the Electrophoretic Behavior of the Lactogenic Hormone as Prepared from Beef and from Sheep Pituitaries

BY CHOH HAO LI, WILLIAM R. LYONS AND HERBERT M. EVANS

Introduction

Our preparations of pituitary lactogenic hormone have recently been shown to consist in each instance of a single protein, using both electrophoresis[1] and solubility[2] criteria. By the latter technique we have demonstrated that the hormone prepared from sheep pituitaries has a solubility which differs from that obtained from beef. Certain immunological methods had failed to distinguish one from the other.[3] The present report deals with an electrophoretic study of preparations obtained from beef pituitaries as well as further studies on sheep preparations. Sufficient data are now at hand to allow us to make a preliminary comparison of preparations made from the two species.

Experimental

The lactogenic hormone L 287 was prepared from beef pituitaries in essentially the same manner as has been published previously.[4] It has approximately 30 International Units per milligram.

The electrophoresis experiments were carried out with the Tiselius apparatus, with the technique described previously.[1] The migration of the boundary was observed by the schlieren method. The hydrogen ion concentration of the solution was measured with the glass electrode and its conductance with the usual Wheatstone bridge type of circuit and a Washburn conductivity cell. All experiments were conducted at 1.5°.

Results

In electrophoresis, Preparation L 287 showed a sharp boundary and migrated as a homogeneous substance. Figure 1 represents a typical schlieren photograph which was taken after exposing to the current for eighty minutes a 0.5% solution of the hormone in *p*H 4.19 acetate buffer. On scanning the whole field, there appeared no indications of a second boundary. The mobility of the preparation in different hydrogen ion concentrations is summarized in Table I. Each value[5] was calculated from at least three observations during an experiment. A typical series of readings is shown in Table II. In this experiment

Fig. 1.—Schlieren band of lactogenic hormone (beef). Exposure was made after passing current for 80 minutes.

(1) Li, Lyons and Evans, *Science*, **90**, 622 (1939); *J. Gen. Physiol.*, **23**, 433 (1940).

(2) Li, Lyons and Evans, *ibid.*, in press.

(3) Bischoff and Lyons, *Endocrinology*, **25**, 17 (1939).

(4) Lyons, *Cold Spring Harbor Symposia Quant. Biol.* **5**, 198 (1937).

(5) Shortly after we had finished our study using a mean value $(U_d + U_a)/2$ for the calculation of mobility, Longsworth and MacInnes [THIS JOURNAL, **62**, 705–711 (1940)] suggested that the descending boundaries yield correct values of the mobility. Since the concentration of the protein used in our experiments was below 0.5%, the mean value $(U_d + U_a)/2$ is only slightly higher than the correct value U_d. The conclusions drawn from our data therefore remain unchanged.

TABLE I

ELECTROPHORETIC MOBILITIES OF BEEF LACTOGENIC HORMONE AT 1.5° IN BUFFER SOLUTIONS OF VARYING *p*H AND CONSTANT IONIC STRENGTH (0.05)

Buffer	*p*H	Mobility in cm.² volt⁻¹ sec.⁻¹ 10^5
Borate	10.00	−11.20
Borate	8.25	− 8.74
Phosphate	7.53	− 6.80
Phosphate	7.15	− 5.54
Phosphate	6.70	− 4.55
Phosphate	6.12	− 3.44
Acetate	5.85	− 1.04
Acetate	5.47	+ 1.68
Acetate	4.19	+ 6.40
Chloride	2.25	+ 8.63

TABLE II

DATA FOR THE CALCULATION OF THE MOBILITY IN A TYPICAL TISELIUS "RUN" WITH BEEF LACTOGENIC HORMONE (L 287)

i 11.5 *Ma*, *p*H 4.19, λ 1.76 × 10^{-3}, concn. 0.5%; cross section area of the cell, 0.81; magnification factor, 1.43; migration anodic.

Δ*t*, min.	Migration of the boundary Δ*D*, min. De-scending	Δ*A*, min. As-cending	Δ*D*/Δ*t*	Δ*A*/Δ*t*	Δ*M*/Δ*t*	*U* × 10^5
15	..	3.40	...	0.226	0.226	6.69
15	3.5	3.15	0.234	.210	.222	6.56
15	3.0	3.65	.200	.243	.221	6.25
15	3.2	2.75	.214	.185	.200	5.91
15[a]	3.00	3.75	.200	.250	.225	6.66
15	3.00	3.50	.200	.234	.217	6.40
15	3.25	3.50	.216	.234	.225	6.65
					Av.	6.45 ± 0.26

[a] Current reversed at this time.

the current was reversed after the fourth reading. A study of Table II reveals that the rate of migration during each time interval is reasonably constant and that the ascending boundary migrates but little faster than the boundary moving into the protein. The latter fact is not surprising and has been explained recently by Longsworth and MacInnes.[6]

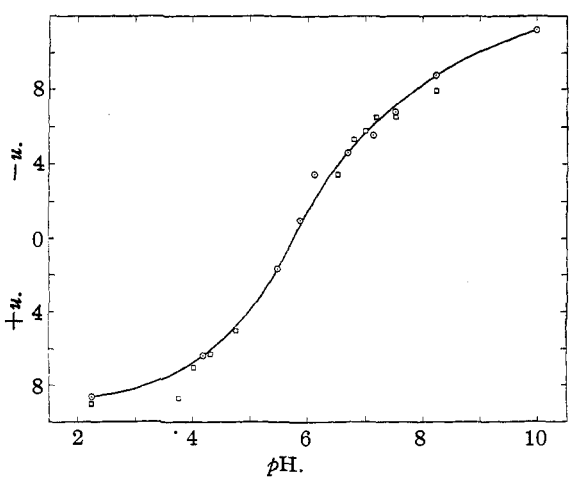

Fig. 2.—Mobility of pituitary lactogenic hormone of beef and sheep origin at different hydrogen ion concentrations: ⊙ = beef; □ = sheep.

In addition to this boundary anomaly, it was found in some experiments (particularly with phosphate buffer), that the descending boundary is not only diffused but also slow in its migration. Sometimes this boundary does not move. This cannot be explained by inequality of conductance and *p*H of the two sides of the boundary because in one such case we found that the protein had the same conductance and *p*H as the buffer. For the present, we are not able to offer an explanation of this phenomenon.[7]

Because of proximity to the isoelectric point, the experiment in buffers of hydrogen ion concentrations 6.12, 5.85 and 5.47 had to be made with very turbid solutions. Since the velocity of the boundary migration could not be measured accurately, only the initial velocity was used to calculate the mobility. In Fig. 2, the mobility is plotted against *p*H and the isoelectric point of beef lactogenic hormone is found to be 5.73.

For comparisons, a few experiments were made with a preparation derived from sheep pituitaries (*L 288*). These results together with those previously published[8] are summarized in Table III and plotted in Fig. 2.

TABLE III

ELECTROPHORETIC MOBILITIES OF SHEEP LACTOGENIC HORMONE AT 1.5–3° IN BUFFER SOLUTIONS OF VARYING *p*H AND IONIC STRENGTH 0.050–0.055

Buffer	*p*H	Mobility in cm.² volt⁻¹ sec.⁻¹ 10_5
Borate	8.25	−7.95
Phosphate	7.53	−6.65
Phosphate	7.20	−6.50
Phosphate	7.00	−5.75
Phosphate	6.80	−5.32
Acetate	6.50	−3.39
Acetate	4.75	+5.00
Acetate	4.30	+6.31
Acetate	4.00	+7.07
Acetate	3.75	+8.70
Chloride	2.25	+9.05

(6) Longsworth and MacInnes, *Chem. Rev.*, **24**, 271 (1939).

(7) It should be mentioned that this phenomenon has never occurred in acetate buffer. In this buffer, the descending boundary (diffused) does not in a single instance stand still although it always moves at a slower rate than the ascending boundary. The anomaly may therefore be due to the effect of phosphate ion on the protein.

(8) In Paper I of this series [*J. Gen. Physiol.*, **23**, 433 (1940)] we have compared the electrophoretic properties of our preparation with that reported by White, *et al.* [*J. Exptl. Med.*, **69**, 785 (1939)].

Discussion

As shown in Fig. 2, there is no difference in the electrophoretic mobility of lactogenic hormone prepared from either sheep or beef pituitary in the whole *p*H range studied. Both have an isoelectric point at *p*H 5.73. This merely indicates that the ratio of basic to acid groupings is identical in these proteins; it does not indicate that they necessarily have the same structural configurations. Their amino acid content may be very different and preliminary determinations of the tyrosine content do in fact indicate that the hormone derived from sheep pituitaries has less of this amino acid than that from beef. The fact that they can be distinguished, one from the other, by solubility studies suggests that the arrangement and number of polar and non-polar groups may be different.

Should our electrophoretic studies have shown that the mobilities of beef and sheep lactogenic hormone are different, we might justifiably have concluded that species specificity exists here. The fact that their electrophoretic mobilities are the same does not, however, allow one to conclude that there is no species specificity, since it has been shown[9] that proteins derived from different species may show the same electrophoretic behavior. It seems not improbable that slight differences in beef and sheep lactogenic hormone may be detected serologically with the help of the extremely delicate quantitative precipitin test used by Heidelberger[10] to differentiate thyroglobulins.

Summary

An attempt has been made to differentiate the lactogenic hormone prepared from sheep and beef pituitaries by electrophoresis experiments. The results show that they are not distinguishable by their electrophoretic behavior. They both have an isoelectric point at *p*H 5.73.

(9) Landsteiner, Longsworth and Van der Scheer, *Science*, **88**, 83 (1938).

(10) Heidelberger and Kendall, *J. Exptl. Med.*, **62**, 697 (1935).

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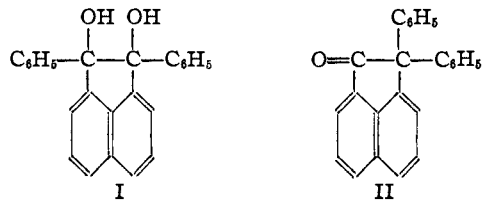
[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY AND THE BYERLY LABORATORY OF RADCLIFFE COLLEGE]

The Pinacol Rearrangement of *cis*- and *trans*-7,8-Diphenylacenaphthenediols-7,8

BY PAUL D. BARTLETT AND RONALD F. BROWN[1]

The fact[2] that both the *cis*- and *trans*-isomers of 7,8-diphenylacenaphthenediol-7,8 (I) undergo the pinacol rearrangement yielding the same product, 7,7-diphenylacenaphthenone (II), was the first apparent exception to the generalization[3,4] that in rearrangements of the pinacol type the migrating radical must displace the hydroxyl (or amino) group with Walden inversion.[5] This is a particularly interesting case, since this pinacol is free from the possibility of two reactions which, in the cyclic pinacols previously studied, compete with the migration of the substituents in the ring. Both ring contraction and simple dehydration are excluded by the nature of the five-membered ring of the acenaphthene system. We have therefore studied the rearrangements of these two isomers in some detail in order to gain information on the mechanism of the reaction. When our work was nearly completed, Criegee published some kinetic measurements on the same system which had been made several years previously.[6] We now report our work because the experiments performed were not entirely identical with those of Criegee and have led to theoretical conclusions of interest.



I II

In common with Criegee, we followed the rearrangements of the isomers by titration with lead tetraacetate. Our runs were carried out in acetic acid containing various concentrations of water

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(2) (a) Beschke, Beitler and Strum, *Ann.*, **369**, 184 (1909); (b) Wittig, Leo and Weimer, *Ber.*, **64**, 2405 (1931); (c) Bachmann and Chu, THIS JOURNAL, **58**, 1118 (1936).

(3) Bartlett and Pöckel, *ibid.*, **59**, 820 (1937).

(4) Bernstein and Whitmore, *ibid.*, **61**, 1324 (1939).

(5) A part of the evidence for this generalization has been criticized recently by Meerwein, *Ann.*, **542**, 123 (1939). This case will be reviewed in a forthcoming paper from this Laboratory.

(6) Criegee and Plate, *Ber.*, **72**, 178 (1939).