Table 2.

Treatment		Carotene content as per cent of the content of the fresh samples	
		23/3	25/4
		100(217)	100(259)
Acetone added immediately			
» » after 1 hour			63
» » » 20 hours		0	16
Acetone added after 20 hours	Ground with 0.5 % KCN		78
	» » 0.5 » Na ₂ S		91
	» » 0.5 » HgCl ₂		30
	» » 0.5 » NaF		0
	Vacuum desiccator, 20 mm Hg		29

0.5 % and the samples set aside for 20 hours before acetone was added. It will be seen from Table 2 that sodium fluoride had no effect and that mercury chloride was only slightly effective. A slight inhibition was also observed when the sample was stored in a vacuum desiccator at a pressure of approximately 20 mm Hg. On the other hand, both potassium cyanide and sodium sulphide were fairly effective. The inhibition obtained with sodium sulphide may be due to the anaerobic conditions set up by this substance. In the case of potassium cyanide, however, the effect must be due to the inhibition of an enzymatic reaction. It seems, therefore, to be safe to conclude, that an enzymatic reaction must be involved in the carotene breakdown in *Rhodymenia palmata*.

A similar inhibition of the carotene breakdown by means of potassium cyanide was observed in alfalfa by Mitchell and Hauge in 1946³. The enzyme system responsible for the carotene breakdown in alfalfa has later been found to be lipoxidase. The carotene is oxidised by some of the intermediate reaction products formed in the enzymatic oxidation of unsaturated fatty acids. Whether a similar reaction is responsible for the destruction of carotene in *Rhodymenia palmata* has not been established.

Acknowledgements. This work forms part of the research program of the *Norwegian Institute of Seaweed Research*. We are indebted to professor N. A. Sørensen for inspiring interest and helpful suggestions, and to Miss S. Svendsen for skilful assistance with the analyses.

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Received February 24, 1956.

Demonstration of Reichstein's Substance S in Dog Adrenal Vein Blood by Countercurrent Distribution

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The two major corticosteroids of dog adrenal vein blood, from a quantitative point of view, are cortisol and corticosterone, as demonstrated by several physical and chemical methods¹⁻⁴. Among other possible components, the presence of minor amounts of Reichstein's substance S (Δ^4 -pregnene-17 α ,21-diol-3,20-dione) seems to have considerable interest⁵ (cf. Eberlein *et al.*⁶).

Further evidence of its occurrence in dog adrenal vein blood is provided here by its demonstration after countercurrent distribution. A sample of 330 ml blood was collected from the left adrenal of a mongrel male dog weighing 18 kg during 60 minutes and extracted with ethyl acetate as previously outlined⁴. The common corticosteroid fraction was recovered from the polar phase after double distribution between *n*-hexane and 50 % ethanol in water and subjected to successive countercurrent distributions (Table 1).

Acta Chem. Scand. 10 (1956) No. 3

Table 1. Countercurrent distribution data of steroids in dog adrenal vein blood (24 transfers at 20°–21° C).

A: 20 % Ethanol + 80 % H₂O; 0.05 % (w/v) NaCl/ 50 % CHCl₃ + 50 % *n*-hexane
 B: 20 % Ethanol + 80 % H₂O; 0.05 % (w/v) NaCl/ 90 % CCl₄ + 10 % CHCl₃
 C: 50 % 2-Butanol + 50 % *n*-hexane/ H₂O
 D: 75 % 2-Butanol + 25 % *n*-hexane/ H₂O
 E: 20 % Ethanol + 80 % (w/v) H₂O; 0.05 % NaCl/ CCl₄
 F: 15 % 2-Butanol + 85 % *n*-hexane/ H₂O

Partition coefficients (<i>K</i>)				Quantities estimated by different methods *, μg					
	<i>K</i>	<i>Followed by</i>	<i>Steroid identified</i>	<i>UV</i>	<i>BT</i>	<i>PS</i>	<i>SA</i>	<i>An</i>	
A	0.33	B	Cortisol (<i>K</i> = 2.58)	573	(404)				
	2.13	C			720				
	8	D			(748)				
B**	?	E	Corticosterone (<i>K</i> = 0.82) Cortisone (<i>K</i> = 3.5)	+					
	0.79	F							133
	3.2								19
	12			—	(64)	+	+		
C	3.0		Cortisol (<i>K</i> = 2.9)	265					
D	0.11								
	1.17								
	24				(41)				
					(22)				
E	0.1			(25)	(12)			1.5	
	0.92		Substance S (<i>K</i> = 0.88)	77		28	45		
F	0.24		Corticosterone (<i>K</i> = 0.25)	68					

* Figures in brackets indicate cortisone equivalents. If no figure is stated, a positive reaction is denoted + and a negative reaction —.

UV: Specific extinction at 240 $m\mu$ in ethanol.

BT: Reduction of blue tetrazolium ¹².

PS: Porter-Silber reaction ⁷.

SA: Sulfuric acid chromogen at 280 $m\mu$ ⁸.

An: Anthrone reaction with specific extinction at 600 $m\mu$ ⁹.

** Ketonic fraction after Girard separation.

The identity with substance S is suggested by the following criteria:

1) identical partition coefficient within the limits of the method,

2) specific extinction at 240 $m\mu$ in ethanol (α,β -unsaturated ketone),

3) positive Porter-Silber reaction, carried out according to Wilson and Fairbanks ⁷ (dihydroxy acetone side chain at C₁₇),

4) pink sulfuric acid chromogen with the typical second peak in the region at about 550 $m\mu$, as well as the peak at 280–290 $m\mu$ indicative of a Δ^4 -3-keto configuration ⁸.

The final amount recovered was 28 μg . This is probably much less than the original amount owing to repeated losses during the

many different steps preceding the last determination. The higher values obtained by estimating the specific ultraviolet extinction at 240 $m\mu$ (77 μg) and the sulfuric acid chromogen at 280 $m\mu$ (45 μg) may be explained by the simultaneous occurrence of some Δ^4 -pregnene-21-ol-3,11,20-trione ($K = 0.75$). — The other component of System E ($K = 0.1$) may contain a very small amount of cortisone (Δ^4 -pregnene-21-ol-3,20-dione), having a specific extinction at 600 $m\mu$ with the anthrone reagent ⁹. At the same time a very sharp and high peak appeared at 418 $m\mu$, indicating the presence of a constituent which was not further characterized.

Besides cortisol, corticosterone and a small amount of cortisone, several other

components are demonstrated which have not yet been identified. The use of counter-current distribution for separating corticosteroids has previously been described¹⁰ but a more detailed discussion will appear elsewhere¹¹.

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Received March 9, 1956.

On the Hydrolysis of the Bi^{3+} Ion — a Preliminary Note

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In 1947, Granér and Sillén² studied the hydrolysis of bismuth(III) in 3 M NaClO_4 medium, measuring $[\text{H}^+]$ with quinhydrone and glass electrodes, and $[\text{Bi}^{3+}]$ with bismuth amalgam electrodes. A mechanism was proposed which seemed to agree well with their data, which covered the range $[\text{Bi}]_{\text{tot}} = 10$ to 50 mM.

One of us (Å.O.) has now undertaken a study with similar methods, but with total

Bi concentrations ranging from 0.1 to 50 mM, thus a factor of 1:500 instead of 1:5. The present data can be explained by assuming the presence of only two complexes, the mononuclear BiOH^{2+} and the hexanuclear $\text{Bi}_6(\text{OH})_{12}^{6+}$ (as usual, plus or minus an unknown amount of water) with the preliminary equilibrium constants:

This result agrees with the findings of Kraus, Holmberg and Johnson³ in so far as their "equilibrium ultracentrifugations suggest predominance of a low molecular weight polymer ($N = \text{ca. } 5 \text{ or } 6$)". In a more narrow concentration range Souchay and Peschanski⁴ concluded that there is only one complex present; they ascribed to it the formula, $\text{Bi}_4\text{O}_4^{4+}$.

The old data of Granér and Sillén were slightly distorted by the fact, then unknown, that quinone is a base and attracts protons at the highest acidities (Biedermann and Sillén⁵). After a correction for this effect, the present data are in general found to agree well with those of Granér and Sillén. This little deviation, however, sufficed (together with the narrowness of the $[\text{Bi}]_{\text{total}}$ range) to indicate an incorrect mechanism.

Attempts are now being made, in collaboration with Professor Cyrill Brosset, to study the structure of the complex by means of X-ray diffraction on aqueous solutions.

This work has been supported by Statens Tekniska Forskningsråd (Swedish Technical Research Council).

A detailed paper will be published elsewhere.

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Received March 19, 1956.

Acta Chem. Scand. **10** (1956) No. 3