results; they pointed out, however, that they were using much lower concentrations than Crismon.

It appears from these scattered and rather confused data that an investigation of these interference effects from the standpoint of the spectroscopist would be desirable.

SUMMARY

1. The construction and operation of a photoelectric flame photometer are described.

2. The use of the photometer for the determination of sodium and potassium in blood and urine is described.

3. The interference between various cations in the flame is discussed with reference to previous work on the subject.

We are indebted to Prof. E. J. King for his interest and encouragement, to Messrs C. Lordan and W. Weedon for constructing the photometer in use at the Postgraduate Medical School, to Messrs R. A. Brennan and J. Gray for much technical assistance, and to Miss S. Tompkins for drawing the figures.

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Vitamin A in Seals

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Specimens of livers of polar bears and bearded seals (*Erignathus barbatus*) collected during the 1939-40 expedition to north-east Greenland (Rodahl, 1943) were found by Rodahl & Moore (1943) to be very rich in vitamin A. During a further expedition to the sealing grounds off Newfoundland and Labrador, carried out under the auspices of the Royal Norwegian Government between March and May 1941, the vitamin A content of seal liver was further investigated. During this expedition the livers of hooded seals (*Cystophora cristata*) and Greenland seals (*Phoca groenlandica*) were weighed and assayed for vitamin A (considerable work on the extraction of vitamin A from this source was also carried out), whilst a limited

number of determinations was made on other tissues. The present paper deals also with the results of similar analyses by one of us of material from a single specimen of the Atlantic seal (*Halichoerus grypus*), obtained off the Pembrokeshire coast in October 1946, from two specimens of the common seal (*Phoca vitulina*) obtained in the Wash in August, 1947, and from the four specimens of *Cystophora cristata* collected in the pack-ice east of Greenland in July 1948.

EXPERIMENTAL

Vitamin A was determined colorimetrically, following the alkaline digestion procedure described by Davies (1933), except for the four 1948 specimens in which the determinations were made spectrographically. All specimens from the sealing grounds off Newfoundland and Labrador were dealt

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with in a laboratory on board ship, while those from Pembroke-shire and the Wash were transported to Aberystwyth in large vacuum-walled flasks packed with solid carbon dioxide.

Except in the case of baby seals, the stomachs of all specimens of the four species examined were empty of foodstuffs.

Hooded seals (Cystophora cristata)

A total of sixty livers of this species was examined during March and April 1941. The results are summarized in Table 1.

Table 1. *Livers of hooded seals*

(a) Liver weights (g.)				
Sex and age	Mean	Range		
Baby seals, about 7 days old	1494	1000-2100		
Females, fully developed	2929	2200-3750		
Males, fully developed	4136	3600-5000		
(b) Mean liver vitamin A reserves (i.u./g.)				
Specimen	No. of animals	March 1941	April 1941	Combined samples
Baby seals				
Foetus	1	120	—	—
1 hr. after birth	1	150	—	—
7 days (approx.)	1	300	—	—
14 days (approx.)	4	1500	—	—
21 days (approx.)	9	—	300	—
Young seals				
1 year (approx.)	2	—	—	2400
2 years (approx.)	2	—	—	1200
Fully developed				
Males above 4 years	26	5180	4320	4934
Females above 4 years	14	1370	2742	2110

Of the total of twenty-six adult male hooded seals, eighteen were examined in March and eight in April, while a total of seven adult females were examined in March, and the same number in April.

RESULTS

There is some evidence to indicate that the vitamin A reserve varies at the different breeding grounds. A further investigation of more material should therefore be carried out at other breeding grounds and at other periods of the year.

In this connexion it may be of interest to note that spectrographical examination of samples of liver from four hooded seals caught in the pack-ice east of Greenland, latitude 74° N., in July 1948, showed lower vitamin A reserves than in the same species caught at the Newfoundland sealing grounds. The results are given in Table 2.

The oil content of the livers of hooded seals up to the age of 3 weeks is usually about 9%. After this age the oil content remains fairly constant at approximately 2.4%. The results of the vitamin A analyses of the liver oil of this species have been combined with the essentially similar results for Greenland seals, and are summarized in Table 6.

Table 2. *Vitamin A content of livers of hooded seals caught in the pack-ice east of Greenland, July 1948*

Sex	Age	Fat in liver (%)	Vitamin A in liver (i.u./g.)
Male	4 months	5.2	450
Female	4 months	3.6	350
Male	16 months	4.3	300
Male	4 years	4.1	450

Greenland seals (Phoca groenlandica)

A total of 145 livers of Greenland seals was examined between March and May 1941. The combined results are shown in Table 3.

Table 3. *Livers of Greenland seals*

(a) Liver weights (g.)			
Sex and age	No. of animals	Mean	Range
Baby seals			
Newborn	1	361	—
8-10 days	1	814	—
Young seals			
Males, 1 year	10	1142	769-1600
Females, 1 year	14	1050	900-1200
Fully developed seals			
Males	17	1783	1600-2250
Females	28	1681	850-2180

(b) Mean liver vitamin A contents (i.u./g.)			
Sex and age	No. of animals	Mean	Range
Baby seals			
Foetus	1	240	—
8-10 days	7	340	120-600
28 days approx.	10	3000	—
Young seals			
Males, 1 year	24	1620	600-4500
Females, 1 year	23	1560	600-1800
Females, 3 years	10	5000	800-7500
Fully developed seals			
Males above 4 years	47	6570	3000-12000
Females above 4 years	23	2542	600-4500

With some livers separate determinations were made on portions from the right and left quadrate and caudate lobes, but in all instances an even distribution throughout the liver was found.

The possibility of a correlation between liver vitamin A reserve and physiological condition at one time of the year was tested on a series of thirty-six animals caught on 19 and 26 April 1941. In each case a record was made of the approximate age, sex, thickness of subcutaneous fat (blubber) and any abnormalities. In all cases it was noted that the stomach was empty. The results in Table 4 show that no obvious correlation emerged.

Table 4. *Vitamin A content of livers from Greenland seals with reference to physiological conditions*

Sex	Approx. age (years)	Thickness of blubber (cm.)	Liver wt. (kg.)	Oil (%)	Vitamin A content		
					Liver (i.u./g.)	Oil (i.u./g.)	Total in whole liver (i.u. $\times 10^{-6}$)
19 April 1941							
Male	>4	2.5	1.9	3.0	750	25,000	1.425
Male	>4	3.5	1.9	2.0	3,000	150,000	5.700
Male	>4	3.3	1.8	2.8	3,000	104,300	5.400
Male	>4	2.3	1.8	2.8	1,800	64,300	3.240
Male	>4	3.5	—	2.4	750	31,250	—
Male	>4	3.4	—	2.0	300	15,000	—
Male	>4	2.3	—	2.0	1,500	75,000	—
Male	>4	3.5	2.1	5.0	12,000	240,000	25.200
Male	4	3.8	2.25	5.0	15,000	300,000	33.750
Male	4	3.5	1.5	4.0	240	6,000	0.360
Male	4	3.5	2.25	3.6	1,200	33,500	2.700
Female	4	4.2	0.85	4.0	2,400	60,000	2.040
Female	4	4.3	1.85	3.0	1,500	50,000	2.775
Female	4	3.8	—	3.0	900	30,000	—
Male	1	1.8	1.25	4.4	900	20,450	1.125
Male	1	3.1	1.25	2.4	1,200	50,000	1.500
Male	1	2.1	1.6	3.0	240	8,000	0.384
Male	1	2.5	—	4.0	750	18,750	—
Female	1	2.5	1.0	2.0	1,500	75,000	1.500
26 April 1941							
Male	5	3.2	1.75	2.0	1,500	75,000	2.625
Male	5	3.3	1.9	2.0	12,000	600,000	22.800
Male	5	4.3	1.75	3.0	1,500	60,000	2.625
Male	5	4.0	1.7	4.0	2,400	60,000	4.080
Male	5	2.8	1.6	2.0	1,200	60,000	1.920
Female	5	3.5	1.75	3.0	3,000	100,000	5.250
Female	5	3.8	1.65	2.0	750	37,500	1.237
Female	5	4.5	1.5	3.0	1,200	40,000	1.800
Female	5	3.0	2.0	4.0	1,200	38,000	2.400
Female	5	4.1	2.0	4.4	2,400	54,545	4.800
Male	2	3.0	1.25	3.6	300	8,333	0.375
Male	2	2.8	1.35	3.0	1,200	40,000	1.620
Male	3	4.8	1.45	3.6	900	25,000	1.305
Female	1	2.8	1.0	3.6	2,400	66,666	2.400
Female	1	2.3	1.35	3.0	1,500	50,000	2.025
Male	1	4.4	1.1	3.0	3,000	100,000	3.300
Male	1	3.0	1.2	2.0	600	30,000	0.720
Mean results							
Male	5	3.2	1.82	2.7	3,208	151,588	7.975
Male	4	3.3	2.0	4.2	5,480	113,166	12.147
Male	1	2.4	1.09	3.4	1,165	39,023	1.487
Female	5	3.8	1.78	3.3	1,710	52,309	3.098
Female	4	4.1	1.35	3.3	1,600	55,000	2.408
Female	1	3.3	1.1	2.3	1,700	68,333	1.840

The fat contents of the livers showed a distribution in relation to age essentially similar to that of hooded seals, and are summarized in Table 5.

Table 5. *Age and sex in relation to the fat content of the liver of Greenland seals*

Sex	Age	Mean fat (%)	Range (%)
Female	4 years	3.2	3.0-4.5
Male	4 years	2.8	2.0-5.0
Male	2 years	3.6	—
Male	1 year	3.4	2.4-4.4
Female	1 year	2.3	2.0-3.0
Female	8-10 days	10.0	—

The results of vitamin A analyses on the liver fat of both species are combined in Table 6 which indicates that there was in most instances an inverse relationship between the amount of oil and its vitamin A potency.

The distribution of vitamin A in the organs of a single specimen of Greenland seal was studied, with the results shown in Table 7. The vitamin A reserve in the liver of this individual was only moderate and below the mean value for comparable specimens. Thus only small amounts were detected in the retina, kidney, lung and subcutaneous fat. The pancreas gave a positive reaction, but the con-

centration of vitamin A was too low to permit an accurate determination. The figures are in agreement with unpublished findings on organs of bearded seals (*Erignathus barbatus*) collected during the 1939-40 expedition to north-east Greenland.

Table 6. *Fat content of Greenland seal and hooded seal livers in relation to vitamin A potency*

Group no.	No. of animals	Oil (g./100 g.)	Mean vitamin A content	
			Liver (i.u./g.)	Oil (i.u./g.)
1	20	2.0-2.4	3,675	134,687
2	3	2.5-2.9	2,000	104,300
3	10	3.0-3.4	1,459	51,444
4	4	3.5-3.9	1,200	53,374
5	7	4.0-4.4	1,470	38,500
6	8	5.0-5.4	13,500	270,000
7	8	9.0-9.4	300	33,300

Table 7. *Distribution of vitamin A in organs of a normal Greenland seal killed 4 May 1941*

Organ	i.u./g.
Liver	2400
Kidney	6
Lung	3
Spleen	0
Pancreas	Trace
Testes	0
Muscle	0
Cerebellum	0
Cerebrum	0
Heart	0
Thyroid	0
Epididymis	0
Subcutaneous fat	12
Lymph gland	0
Corpus vitreum	0
Retina	4

The vitamin A content of a sample of milk collected from a female with a baby in its second week of life was 10 i.u./g.

Atlantic (grey) seal (Halichoerus grypus)

A single specimen weighing 59.5 kg., and estimated to be 18 months old, was shot by a fisherman off the Pembrokeshire coast in October 1946. Blubber (mean thickness, 3.4 cm.) from the same specimen

Table 8. *Distribution of vitamin A in organs of an unmated Atlantic seal killed October 1946*

Organ	i.u./g.
Liver	1,550
Liver oil	38,750
Lung	2.5
Left kidney	14.5
Right kidney	17.0
Suprarenals	Nil
Spleen	0.45
Subcutaneous fat	3.58

The total vitamin A reserve of the liver was calculated to be 2.8×10^6 i.u.

was forwarded to Prof. T. P. Hilditch, Department of Industrial Chemistry, University of Liverpool, for a study of its composition (Hilditch & Pathak, 1947). The liver, which weighed 1.81 kg., contained 4% of fat. The vitamin A content of the different portions analysed is shown in Table 8.

Common seal (Phoca vitulina)

Vitamin A estimations were carried out on tissues of two of a total of four specimens recovered from the Wash on a field trip made in July 1947. These two animals were a very young female (estimated as being not more than 2-3 weeks of age, blubber thickness 2.5 cm.), and an immature male (estimated as not more than 4-6 weeks of age). The results are shown in Table 9.

Table 9. *Distribution of vitamin A in organs of two young common seals killed in July 1947*

Organ	i.u./g.	
	Very young female	Immature male
Liver	12.0	90.0
Liver oil (8%)	15.0	180.0
Lung	Nil	0.6
Kidney	Nil	0.9
Suprarenal	Nil	Nil
Spleen	Nil	Nil
Subcutaneous	Nil	Nil

SUMMARY

1. The weight, vitamin A content and fat content of the livers of sixty-four hooded seals (*Cystophora cristata*), 145 Greenland seals (*Phoca groenlandica*), one Atlantic seal (*Halichoerus grypus*), and two common seals (*Phoca vitulina*) were determined.

2. In *Cystophora cristata* and *Phoca groenlandica* from northern waters during the breeding season (March to late May) the concentration of vitamin A reached very high levels in mature specimens, up to 24,000 i.u./g. (mean 3,004 i.u./g.) in *Cystophora cristata*, and up to 15,000 i.u./g. (mean 3,441 i.u./g.) in *Phoca groenlandica*. The mean liver vitamin A content of four specimens of *Cystophora cristata* taken in the pack-ice east of Greenland in July 1948, was only 388 i.u./g.

3. The liver vitamin A content of the single specimen of *Halichoerus grypus* recovered from British waters fell within the range of the northern series, but those from the two young specimens of *Phoca vitulina* from the Wash were of much lower order.

4. There did not seem to be any correlation among mature specimens of any species between age, sex or thickness of the blubber and liver vitamin A.

5. The liver fat content in all species appeared to be high (8-10%) in very young specimens, falling to between 2 and 4% in mature animals.

6. Only relatively small amounts of vitamin A were found in the other tissues examined.

Our thanks are due to the Royal Norwegian Ministry of Commerce for financing the scientific expedition to the Newfoundland sealing grounds, and to the Dunn Nutritional

Laboratory, Medical Research Council and University of Cambridge, for leave of absence of one of us to participate in the expedition; to Dr L. Harrison Matthews for his encouragement and valuable co-operation in the collection of material; and to Prof. A. N. Worden and Dr Thomas Moore for their interest in this work.

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A Method for the Estimation of Micro Amounts of Amino Nitrogen and its Application to Paper Partition Chromatography

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Since the introduction of paper partition chromatography (Consden, Gordon & Martin, 1944) it has been possible to separate completely many amino-acids and peptides from complex mixtures. This has focused attention on the need for quantitative methods for estimation of micro amounts of these substances after their separation on paper chromatograms. The method of Pope & Stevens (1939) has proved of great use in the estimation of amino nitrogen at macro and semi-micro levels. Preliminary notes of its use in a modified form for determining micro amounts of amino-acids separated by single-dimensional paper chromatography have already been given (Woiwod, 1948*a, b*). The present paper gives fuller details of the method, together with some preliminary results.

In the method of Pope & Stevens (1939) amino and 'peptide amino' nitrogen are estimated by allowing a suspension of copper phosphate to react with the material being investigated at pH 9.3, and, after filtration, the copper in the soluble complexes formed is estimated iodometrically. The terms 'amino nitrogen' and 'peptide amino nitrogen' just mentioned are used in this paper in the following sense. The groupings with which the copper phosphate reacts in proteins and their breakdown products are the α -amino (or imino) group of free amino-acids and the free amino groups of peptides and proteins, i.e. any not involved in peptide linkage. The nitrogen of the reacting group in free amino-acids is designated α -amino nitrogen and the nitrogen of the free amino groups in peptides and proteins is designated here as 'peptide' amino nitrogen. Little is known about the reactions of copper with peptide

amino nitrogen. The limitation this imposes when interpreting results with partial digests of peptides and proteins is discussed later.

EXPERIMENTAL

The method of Pope & Stevens (1939) can be modified so that micro amounts of amino N can be estimated by using the diethyldithiocarbamate reaction (Callan & Henderson, 1929) to determine the Cu in the soluble copper complexes. The method consists of the following stages: (a) the amino-acid or peptide is allowed to react with a suspension of copper phosphate and the excess of the latter is then filtered off; (b) the Cu in a measured sample of the filtrate is determined by means of sodium diethyldithiocarbamate; (c) the Cu so determined is related to amino or peptide N, or weight of amino-acid or peptide, by means of a standard curve or use of a factor.

The ratio, F , of weight of α -amino N to weight of Cu reacting has been determined for a number of amino-acids by this method (Woiwod, 1948*b*). The copper phosphate suspension of Pope & Stevens (1939) was used in the preliminary work, but its use had several disadvantages at micro levels. These were: (a) a considerable deviation of the value of F from the theoretical value of 0.44 for complexes of the type A_2Cu was observed with most amino-acids, and the ratio was not constant from acid to acid; (b) the relationship between α -amino N and Cu was not linear; (c) the reagent did not keep well and needed to be freshly prepared for each set of determinations; (d) the reaction had a large temperature coefficient; and (e) the blank value given by the reagent was large.

By omitting the $Na_2B_4O_7$ and adding Na_2HPO_4 to the Cu reagent a linear relationship between amino nitrogen and copper over a considerable concentration range was obtained, and the value of F for many amino-acids was much nearer the theoretical value of 0.44. Thus it was possible to use this