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B₁₂ Vitamins (Cobalamins)

2. NEUTRAL, BASIC AND ACIDIC COBALAMINS

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Since 1949 we have been studying the reactions of the B₁₂ vitamins with sulphur dioxide, with cyanide, and with other reagents. Publication was delayed, and independent work in the same field has meanwhile been published (Fricke, Lanius, DeRose, Lapidus & Frost, 1950; Veer, Edelhausen, Wijmenga & Lens, 1950; Brink, Kuehl & Folkers, 1950; Kaczka, Wolf, Kuehl & Folkers, 1950; Beaven, Holiday, Johnson, Ellis & Petrow, 1950; Conn, Norman & Wartman, 1951; Kaczka *et al.* 1950; Kaczka, Wolf, Kuehl & Folkers, 1951).

It has become clear that the B₁₂ vitamins readily form adducts with a variety of reagents in a similar manner to the haem pigments. In the light of our own and other investigations, we can now offer a more general interpretation of these phenomena than has previously been given.

It appears that there are three classes of adducts: neutral, basic and acidic. Their nature can be illustrated by the partial structures (I), (II), (III) and

(IV) (see Buhs, Newstead & Trenner, 1951). In vitamin B₁₂ itself (Cyanocobalamin, I) the trivalent cobalt atom is linked to three neutral groups (0 0 0) and to three negative groups (— — CN[−]),

making the molecule as a whole neutral. The cyanide ion can be replaced by others with strong co-ordinating tendencies, to yield other neutral cobalamins; thus nitrite and thiocyanate give respectively nitritocobalamin (vitamin B_{12c}) and thiocyanatocobalamin. The compounds in this series are comparatively stable and can readily be obtained crystalline.

Photolysis or treatment with hydrogen and a catalyst or with sulphurous acid or certain other reducing agents, displaces the cyanide ion from vitamin B₁₂ to yield vitamin B_{12b} (vitamin B_{12a} appears to be a dehydration product: see Kaczka, Wolf & Folkers, 1949; Kaczka, Denkwalter, Holland & Folkers, 1951; Cooley *et al.* 1951*a*). Vitamin B_{12b} appears to be an equilibrium mixture of hydroxocobalamin and aquocobalamin (II), the latter being the favoured form except in alkaline solution. As in vitamin B_{12b} the negative ion is replaced by the neutral molecule H₂O, the molecule as a whole acquires a positive charge, and accordingly vitamin B_{12b} titrates as a base (Kaczka, Wolf *et al.* 1950, 1951; Lester Smith *et al.* 1951; Cooley *et al.* 1951*b*).

We find that the linkage with strong acids such as sulphuric is substantially ionic in character. Accordingly, these compounds are better named as salts—aquocobalamin sulphate, etc.—rather than as sulphatocobalamin, etc. (Lester Smith, 1952).

Another member of this class of basic B₁₂ vitamins is the substance called cobalichrome by Cooley *et al.* (1951*b*), in which NH₃ replaces the H₂O in aquocobalamin. The unstable adduct with elemental iodine also belongs presumably to this category.

Adducts belonging to the acidic class arise from the addition of two acidic ions to the cobalamin residue. It has been suggested that, where both groups are CN^- , the second displaces the benzimidazole N-3 from co-ordination with the cobalt (Beaven *et al.* 1950). This is indicated in (III) by omission of one of the O groups. In any event, this second acidic group confers a net negative charge on the molecule, which accordingly behaves as a weak acid (Lester Smith *et al.* 1951). The CN^- groups can be replaced by two other suitable groups, which need not be the same, so that mixed double adducts

techniques employed, namely measurements of partition coefficients and of absorption spectra, and radioactive tracer studies.

Partition data

Partition coefficients for a range of cobalamins between benzyl alcohol and dilute aqueous buffer solutions are presented in Table 1. Comparison with the values given by Kaczka, Wolf *et al.* (1950, 1951) is not possible because they used a different solvent mixture and also because they did not specify pH values, which are sometimes important.

Table 1. *Partition coefficients of various cobalamins and the effect of pH on partition*

(Partition coefficients: $\frac{\text{Concn. in benzyl alcohol}}{\text{Concn. in water}}$.)													
pH	...	...	...	...	9.5	8.5	8	6.5	5.8	5	4	3	2
Vitamin B ₁₂ (cyanocobalamin)					0.8	—	—	0.8	—	—	0.8	—	0.7
Dicyanocobalamin					0.75	—	0.9	—	—	1.9	—	2.9	—
Vitamin B _{12b} (aquocobalamin)					0.4	—	—	—	—	—	0.045	—	0.04
Vitamin B _{12c} (nitritocobalamin)					0.6	—	—	—	—	1.2	1.3	—	—
Thiocyanatocobalamin					0.5	—	0.65	0.85	—	—	—	0.7	—
Thiocyanatocobalamin + trace KCNS					—	—	—	—	1.15	—	1.7	—	2.5
Thiocyanatocobalamin in 5% NH ₄ CNS					—	0.25	0.4	0.8	—	—	2.1	—	12.5
Vitamin B ₁₂ + trace KCNS					0.65	—	0.65	—	0.85	—	0.95	—	1.4
Vitamin B ₁₂ in 5% NH ₄ CNS					—	—	0.2	0.3	—	—	0.5	—	3.5
Vitamin B _{12b} + I ₂					—	—	—	2	—	6.5	—	—	3
Vitamin B ₁₂ + I ₂					—	—	—	0.7	—	1.15	—	1.2	—
Sulphitocobalamin										Too low to measure			

can exist. The acidic cobalamins are much less stable than the neutral and basic ones. It is almost impossible to isolate them in crystalline form because conversion to the double adduct is only complete in presence of excess of the ion concerned. The behaviour of the sulphite adduct clearly places it in this class (IV); evidently the bivalent sulphite ion has the same effect as the two univalent ions present in the double adducts.

The existence of these compounds can only be inferred from the properties of their solutions. Conn *et al.* (1951) have provided polarographic evidence for the uptake of additional cyanide by vitamin B₁₂. The striking colour change to purple and the corresponding considerable changes in the absorption spectrum provide further evidence. The changes in partition coefficients between water and suitable solvents caused by addition of cyanide afford further proof of combination, while the variation in partition coefficient with pH indicates the acidic character of the complex. Adducting agents other than cyanide cause similar, though sometimes less marked, changes in the behaviour of the original cobalamin.

RESULTS

The experimental results that have contributed to our understanding of the numerous cobalamins can conveniently be grouped together under the various

Neutral cobalamins. As may be seen from Table 1 the partition coefficient for vitamin B₁₂ is independent of pH and that of two other neutral cobalamins is nearly so. The concentrations in the two phases are similar for these compounds, whereas vitamin B_{12b} strongly favours the aqueous phase, especially at acid pH values.

Iodocobalamin. On adding a trace of iodine to a neutral or acid solution of vitamin B_{12b}, most of the colour passed into benzyl alcohol; it passed back into the aqueous phase on shaking with a little thiosulphate or isopropenyl acetate—substances that react readily with iodine. Some iodine was taken up on gradual addition to an aqueous solution of vitamin B_{12b}; however, when the amount reached about half an atom of iodine per molecule, tests for iodine became positive (starch test and extraction by carbon tetrachloride). Combination with iodine is evidently loose and reversible: it was not possible to prepare a pure crystalline specimen of the adduct. The compound should presumably be called 'iodocobalamin', although in the nomenclature proposed by Kaczka and his colleagues this would signify the different compound of vitamin B_{12b} with iodide ion, which we regard as aquocobalamin iodide.

Dithiocyanatocobalamin. The partition behaviour of the acidic adducts is best typified by that of the dithiocyanate adduct. Vitamin B_{12b} combines with 1 molecule of thiocyanate to yield the stable crystallizable thiocyanatocobalamin. Addition of excess

thiocyanate, however, causes changes in the spectrum (see below) and the partition coefficient: distribution into benzyl alcohol is markedly depressed in alkaline and enhanced in acid solution.

Dicyanocobalamin. The purple cyanide adduct behaves differently in acid solutions because these complexes can only persist in presence of excess of the ion concerned, and hydrocyanic acid is very feebly ionized. If an alkaline solution of the adduct is acidified and shaken immediately and briefly with benzyl alcohol, the purple compound is readily extracted (distribution coefficient $K = 2.9$ at pH 3). On standing for a few minutes, however, the purple colour changes to red in both layers, and if the mixture is then shaken again, much of the colour passes back into the aqueous layer ($K = 0.8$ for the resulting vitamin B₁₂).

Further evidence for the acidic character of these adducts is provided by the formation of insoluble salts on adding soluble salts of suitable metals. Examples are the purple silver and zinc salts of dicyanocobalamin and the red silver salt of dithiocyanatocobalamin.

Thiocyanatocyanocobalamin. The addition of an excess of thiocyanate to vitamin B₁₂ solutions greatly assisted extraction from acid and hindered extraction from alkaline solutions, suggesting the formation of a mixed adduct (Table 1). The values of the partition coefficients, the absorption spectrum and tracer studies all combined to show that these results were not due merely to displacement of the cyanide ion by two thiocyanate ions, although this did occur to some extent.

Interconvertibility of cobalamins

Vitamin B_{12b} reacts very quickly with sulphite to give the adduct. Reaction with vitamin B₁₂ itself is slow near neutrality, requiring about 1 day for completion, but is more rapid in acid solution. On aeration or evaporation of a solution of sulphitocobalamin, conversion to vitamin B_{12b} occurs, with loss of sulphur dioxide or oxidation to sulphate. Vitamin B_{12c} (nitritocobalamin) is also slowly converted into sulphitocobalamin on treatment with sulphite. Conversely, sulphitocobalamin is converted into nitritocobalamin by treatment with excess of nitrite. All the cobalamins are converted into vitamin B₁₂ itself on treatment with cyanide.

Absorption spectra

On the addition of 1 equivalent of various acid ions to vitamin B_{12b} which is in the form of aquocobalamin there is little or no change in the principal ultraviolet or visible absorption maxima, except with cyanide to yield vitamin B₁₂ itself. Vitamin B₁₂ is the only one of these compounds to have a bright ruby-red colour in the crystalline state: the others are all very dark red in colour.

The addition to vitamin B_{12b} of two cyanide or thiocyanate ions or of a single sulphite ion does cause spectral shifts, but again the effect is largest with cyanide. Dithiocyanatocobalamin has an absorption maximum in the ultraviolet at 257 m μ . and a flat maximum in the visible at 510–540 m μ . Thiocyanatocyanocobalamin has the principal maxima at the same wavelengths as vitamin B₁₂. Dicyanocobalamin has maxima at 368 and 580 m μ .; sulphitocobalamin at 364 and 515 m μ .

Tracer experiments

Radioactive isotopes of cobalt, phosphorus, carbon, sulphur and sodium have all been used in the course of our studies of the structure and stability of the cobalamins.

[¹⁴CN]Cyanocobalamin. We have prepared [¹⁴CN]cyanocobalamin (Boxer, Rickards, Rosenblum & Woodbury, 1951) by treatment of vitamin B_{12b} in strong aqueous solution with K¹⁴CN and the subsequent addition of acetone to induce crystallization; the specific activity (0.73 μ c./mg.) agreed with the calculation for one cyanide group per molecule. Alternatively, vitamin B₁₂ itself could equally well be used, since facile exchange between —CN[−] and ¹⁴CN[−] occurs in alkaline solution.

Dicyanocobalamin. Potassium cyanide in excess (or, better, ethylamine cyanide, which is soluble in acetone) was added to strong aqueous solutions of vitamin B₁₂, followed by excess acetone. A purple microcrystalline powder was slowly deposited, sometimes mixed with larger crystals of vitamin B₁₂. From radioactive cyanide and vitamin B_{12b} we were never able to obtain a preparation having a specific activity corresponding with two cyano groups per molecule: the best appeared to contain about equal parts of di- and mono-cyanocobalamin.

The purple compound was readily extracted from alkaline aqueous solutions with phenol-benzene mixtures or benzyl alcohol, and we attempted to determine its cyanide content by radioactivity measurements on these extracts. This approach failed because inorganic cyanide was also extracted and could not be removed fully by two alkali washes.

²⁴Na experiments. In a similar fashion we tried to confirm the acidic character of this and other adducts by extracting into organic solvents their salts with radioactive ²⁴Na. These experiments were also frustrated by extraction of sodium cyanide into benzyl alcohol and of sodium sulphite into phenol. However, after making allowance for the rather large 'blank', it did appear that the sulphite adduct forms a salt with one sodium ion. We could also show clearly, with the help of ²⁴Na, that nitritocobalamin (vitamin B_{12c}) forms no such salt.

Aquocobalamin sulphate. Vitamin B_{12b} was freed from acid ions by passing its aqueous solution

through a column of a strongly basic ion-exchange resin, or more simply by shaking a phenolic solution with aqueous sodium hydroxide. A strong aqueous solution of the vitamin B_{12} was acidified with $H_2^{35}SO_4$, and acetone was added to cause crystallization. The specific activity of the product showed that it contained 1 equivalent of sulphuric acid, i.e. $\frac{1}{2}H_2^{35}SO_4$, per molecule. The linkage

Exchange experiments

The results given in Table 2 show that at pH 10 exchange between the cyano group of vitamin B_{12} and potassium cyanide was in 1 hr. almost complete in dilute solution and complete in stronger solutions. At pH 7 exchange was much slower and still slower at pH 3.

Table 2. Exchange between vitamin B_{12} and cyanide using ^{14}C

Components		Concentrations (mg./ml.)		pH	Time	Exchange (%)
		Cobalamin	KCN			
$[^{14}CN]$ Cyanocobalamin	KCN	0.07	0.065	10	10 min.	60
$[^{14}CN]$ Cyanocobalamin	KCN	0.03	0.50	10	1 hr.	97
Cyanocobalamin	$K^{14}CN$	6.0	1.6	10	1 hr.	100
$[^{14}CN]$ Cyanocobalamin	KCN	0.063	0.063	7	1 hr.	30
$[^{14}CN]$ Cyanocobalamin	KCN	0.035	0.04	7	50 hr.	98
$[^{14}CN]$ Cyanocobalamin	KCN	0.063	0.063	3	1 hr. dark	5
$[^{14}CN]$ Cyanocobalamin	KCN	0.063	0.063	3	0.25 hr. light + 0.75 hr. dark	40
$[^{14}CN]$ Cyanocobalamin	KCN	0.063	0.063	3	0.5 hr. light + 0.5 hr. dark	77
$[^{14}CN]$ Cyanocobalamin	KCN	0.063	0.063	3	65 hr. dark	99
$[^{14}CN]$ Cyanocobalamin	KCN	0.035	0.04	3	50 hr. dark	65
Cyanocobalamin	$K^{14}CN$	6.0	1.6	3	1 hr.	25

appeared to be ionic in character. The radioactive sulphate exchanged immediately with sodium sulphate in dilute neutral aqueous solution. It was also completely removed on shaking a phenolic solution of the aquocobalamin sulphate with dilute sodium hydroxide for a few seconds.

In contrast, the specific activity of vitamin B_{12} containing ^{14}CN was undiminished by this treatment, and exchange with neutral cyanide was slow (see later). However, tracer experiments confirm other evidence that the stability of the Co—CN bond is unique. Thiocyanatocobalamin, for example, behaved more like the sulphate salt: washing a phenolic solution with dilute sodium hydroxide removed 85 % of the radioactivity from $[^{35}S]$ thiocyanatocobalamin; exchange with dilute neutral thiocyanate took place to the extent of 45 % within half a minute.

Sulphitocobalamin. Attempts to prepare a pure specimen of sulphitocobalamin were unsuccessful, partly because of facile atmospheric oxidation to aquocobalamin sulphate, already noted by Kaczka, Wolf *et al.* (1951). Instability of the adduct also prevented efforts to determine its sulphite content radiometrically by partition methods.

$[^{35}S]$ Thiocyanatocobalamin. Thiocyanatocobalamin was prepared by the method of Buhs *et al.* (1951), but using $KCN^{35}S$. The specific activity agreed within experimental error with that calculated for one sulphur atom per molecule. Solubility of thiocyanate in benzyl alcohol prevented us from establishing, by tracer methods, the existence of dithiocyanatocobalamin, inferred from partition and spectroscopic evidence.

Only 5 % exchange occurred in 1 hr. at pH 3 in the dark, but exchange was much greater if the solution was exposed to the light of a 500 W. bulb for part of the time. This effect of light was to be expected from the observation of Veer *et al.* (1950) that light causes conversion of vitamin B_{12} into vitamin B_{12b} .

The rapid exchange in alkaline solution does not imply that the Co—CN bond is ionic in character or that it is broken even momentarily, because exchange almost certainly occurs via the labile dicyanocobalamin; this implies that the two CN groups are equivalent. This exchange mechanism would operate much less effectively in neutral solution and scarcely at all at acid pH, because dicyanocobalamin is only stable in alkaline solutions.

It was of interest to determine whether the cyano group of vitamin B_{12} could be exchanged for other acid groups, such as thiocyanate. The results were similar, whether we used ordinary vitamin B_{12} with $KCN^{35}S$ or $[^{14}CN]$ cyanocobalamin with ordinary $KCNS$. Relatively high concentrations were used and a reaction time of only 10 min. Under these conditions, as Table 3 shows, about 30 % exchange occurred in acid or alkaline solutions, but little in neutral solution. It appears that equilibrium is reached rapidly, but that even an overwhelming excess of thiocyanate displaces only a small proportion of the co-ordinated cyanide, owing to the much stronger attachment of the latter to the cobalt. A similar experiment with $[^{14}CN]$ cyanocobalamin in 5 % (w/v) sodium nitrite at pH 10 showed only 5 % exchange in 10 min.

Table 3. *Exchange between vitamin B₁₂ and thiocyanate measured using ³⁵S*

Components		Concentrations (mg./ml.)		pH	Time	Exchange (%)
		Cobalamin	Salt			
Cyanocobalamin	KCN ³⁵ S	1.5	10	2.5	10 min. dark	30
Cyanocobalamin	KCN ³⁵ S	1.5	10	2.5	10 min. light	32
[¹⁴ CN]Cyanocobalamin	KCNS	0.3	50	7	10 min.	<5
[¹⁴ CN]Cyanocobalamin	KCNS	0.3	5	7	10 min.	<5
[¹⁴ CN]Cyanocobalamin	KCNS	0.3	0.5	7	10 min.	<5
[¹⁴ CN]Cyanocobalamin	NH ₄ CNS	0.25	50	7	10 min.	5
Cyanocobalamin	KCN ³⁵ S	1.5	10	8.5	10 min. dark	33
Cyanocobalamin	KCN ³⁵ S	1.5	10	8.5	10 min. light	29
Cyanocobalamin	KCN ³⁵ S	3	30	10	10 min.	26
[¹⁴ CN]Cyanocobalamin	KCNS	0.7	50	9.5	10 min. dark	26
[¹⁴ CN]Cyanocobalamin	NH ₄ CNS	0.25	0.5	8.5	10 min.	5
[¹⁴ CN]Cyanocobalamin	NH ₄ CNS	1.1	50	8	1 hr. dark	10
[³⁵ S]Thiocyanatocobalamin	NH ₄ CNS	0.15	1	7	Immediate	45
[³⁵ S]Thiocyanatocobalamin	NaOH	0.3	0.4	8.5	Immediate	85
[³⁵ S]Thiocyanatocobalamin	NaOH	0.2	0.4	8.5	Immediate	85

EXPERIMENTAL

Partition coefficients. Partition coefficients were measured at room temperature by shaking an aqueous solution of the cobalamin in dilute phosphate buffer with benzyl alcohol, centrifuging to separate the layers and then diluting measured volumes of the two layers with ethanol to suitable strengths for colorimetric assay. The intensity of colour was the same whether the solvent was water, aqueous ethanol or a mixture of benzyl alcohol and ethanol. The pH of the aqueous phase was checked before dilution. Volumes of the two phases were chosen such that the cobalamin was distributed between them in roughly equal amounts. About 2 mg. of the cobalamin in a total volume of about 10 ml. were used for each measurement.

Preparation of [¹⁴CN]cyanocobalamin. Vitamin B_{12b} (9.5 mg.), prepared by treatment of vitamin B₁₂ with H₂ in presence of platinum oxide catalyst (Kaczka *et al.* 1949) was dissolved in water (0.3 ml.) containing K¹⁴CN (0.5 mg.: 7.5 μ c.). After 1 hr. at room temperature 2 ml. water and 1 drop of glacial acetic acid were added, and the mixture was extracted with 0.5 ml. of benzene-phenol mixture (2:1). The solvent layer was washed with two portions of about 1 ml. of water, then enough acetone was added to precipitate all the coloured material. The precipitate was collected by centrifuging, washed with acetone, and dissolved in 1 ml. of 70% acetone. The solution was centrifuged to remove a trace of suspended matter, then further acetone was added to the point of incipient turbidity. The crystals that separated in a few hours were redissolved in a few drops of water and again crystallized by adding acetone. The yield was 5 mg. (0.73 μ c./mg.) of anhydrous vitamin B₁₂.

Preparation of [¹⁴CN]cyanocobalamin by exchange. Vitamin B₁₂ (8.5 mg.) was dissolved in 1 ml. of water containing K¹⁴CN (1.65 mg.: 25 μ c.). After 1 hr. at room temperature, 1 drop of glacial acetic acid was added and the solution was extracted with 1 ml. of benzene-phenol mixture (2:1). The solvent layer was washed with 2 ml. portions of water, 0.01 N-NaOH and water, successively, and then mixed with enough ether-acetone mixture (1:1) to precipitate nearly all the coloured material. This was centrifuged down, washed with acetone, crystallized and recrystallized from aqueous

acetone. The specific activity was 0.62 μ c./mg. anhydrous vitamin B₁₂, compared with the value of 0.61 μ c./mg. calculated on the basis of complete exchange.

Preparation of KCN³⁵S. Radioactive KCNS was prepared by fusing KCN (0.5 g.) with ³⁵S (0.25 g.: 20 μ c.). The melt was extracted with water and the KCN³⁵S purified by crystallizing and recrystallizing from water; this was difficult on the small scale owing to the high solubility of the salt. The yield was 350 mg. of material, 95% pure by titration against 0.1 N-AgNO₃. The activity was 3200 counts/min./mg. KCN³⁵S.

Cyanide exchange experiments. The cobalamin in dilute buffer solution at the desired pH was mixed with KCN solution (one or other component being labelled with ¹⁴C) in a total volume of 10 ml. The concentrations are listed in Table 2. After the desired period at room temperature, the solution was acidified with acetic acid (if alkaline) and the vitamin B₁₂ was extracted with 2 ml. of liquefied phenol. After being washed twice with water (5 ml.), the extract was shaken vigorously with a few drops of water and 10 vol. of ether. Nearly all the colour was thus transferred to the small volume of water, which was separated and diluted with acetone until just turbid (about 10 vol.). The resulting crystals were rinsed with acetone, then redissolved in water. The vitamin B₁₂ concentration was estimated colorimetrically, and the radioactivity as described below.

Thiocyanate exchange experiments. The experiments involved either loss of radioactivity from [¹⁴CN]cyanocobalamin on partial conversion to thiocyanatocobalamin, or gain of radioactivity in the same reaction between normal cyanocobalamin and KCN³⁵S. They were performed in the same manner as those involving cyanide exchange, except that the volume of the reaction mixture was 0.5–2 ml. at higher concentrations, as listed in Table 3. Table 3 also includes results for reactions between [³⁵S]thiocyanatocobalamin and KCNS or NaOH, for which the same experimental procedure was used.

Radioactivity measurements. The crystalline preparations were dissolved in a suitable volume of water (1.5–10 ml.). The cobalamin concentration was determined with the Spekker photoelectric colorimeter, either directly on this solution or on a dilution, as appropriate.

The radioactivity was measured with a thin end-window Geiger-Müller counter on 0.5 ml. of the solution dried on a nickel planchette. Loss of radioactive cyanide or thiocyanate was prevented by adding excess AgNO_3 before drying and by using a hotplate in place of the more usual infrared lamp, which could cause some photolytic decomposition.

SUMMARY

1. The structures and stabilities of a series of cobalamins have been studied, mainly by tracer techniques and by measurements of absorption spectra and partition coefficients.

2. It has been shown that, besides the neutral and basic classes of cobalamins, there is a third class

of acidic cobalamins. They contain two acidic ions, which need not be the same, or one divalent ion like sulphite. They are unstable and only persist in solutions containing excess of the co-ordinating ion.

3. Using radioactive carbon and sulphur it was shown that the cyano group in vitamin B_{12} exchanges with inorganic cyanide, rapidly in alkaline solution but very slowly in acid. In high concentrations of thiocyanate in acid or alkaline, but not in neutral solutions, the cyano group is partly displaced by thiocyanate.

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Studies on the Composition of Horse Oil

2. THE COMPONENT FATTY ACIDS OF LIPIDS FROM FATTY TISSUES, MUSCLE AND LIVER

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In part 1 of this series (Brooker & Shorland, 1950) it was shown that the fats of pasture-fed animals differed considerably from species to species in fatty acid composition. Whereas linolenic (octadecatrienoic) acid, the chief component of the pasture fatty acids, comprised about 17 % of the depot fat of horses, it was found only in traces in the fats of pasture-fed sheep and oxen. Horse fats differed further from those of sheep and oxen by having greater proportions of hexadecenoic acid and smaller amounts of stearic acid. These results have since been generally confirmed by Gupta & Hilditch

(1951). In addition, Bruce & Shorland (1951) have shown in a preliminary communication that, as observed for other animals (cf. Hilditch & Shorland, 1937), the liver phospholipins of the horse contained more stearic and highly unsaturated C_{20-22} acids, but less palmitic and hexadecenoic acid, than did the liver glycerides. It was found, however, that although the respective contents of total C_{18} unsaturated acids and of oleic acid of the phospholipin and glyceride fractions of the horse liver were similar, the phospholipin contained about three times as much octadecadienoic acid but only one-