The Vitamin B_{12}-Content of Human Fetal Tissues

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Deficiency of B_{12} during fetal life has been suggested to cause malformation or death of the fetus [1]. Moreover the fetal erythroblasts have been considered to be related to the megaloblasts occurring in anemias caused by B_{12} deficiency. It was therefore of interest to study the B_{12}-content of fetal tissues.

Method. 25—500 mg of tissue was homogenised; crude papain and sodium cyanide were added. The volume was made up to 25 ml with phosphate-citric acid buffer, pH 5.0. One hour's digestion at + 60°C was followed by heating at 100°C for 15 min. After filtration or centrifugation the B_{12} content of the extract was assayed microbiologically using *Euglena gracilis* z[2]. The recovery of added B_{12}-^{60}Co was nearly complete as determined by both radioactivity and microbiological measurements. Extracts of liver and muscle from a hog previously injected with B_{12}-^{60}Co contained close to 100 % of the radioactivity of the organ.

Material. Liver, spleen, kidney, adrenals, heart, lung, thymus, brain, spinal cord and muscle from 28 fetuses of 6—25 cm length were analysed. *Results*, See Table 1.

Conclusions. No changes in B_{12} concentration of fetal organs were observed over the period of life investigated except for brain and spinal cord in which the vitamin was found to decrease with age. The data on fetuses will be compared with those on prematures, children and adults.

Table 1. Mean and range of the vit. B_{12} content of human fetal tissues in mμg/g fresh weight.

Tissue	Fetal length 6—15 cm No. of observations		Fetal length 16—25 cm No. of observations	
Liver	17	360 (250—490)	6	340 (230—530)
Spleen	6	59 (48—79)	11	50 (37—72)
Kidney	14	140 (115—170)	7	130 (75—230)
Adrenals	15	180 (120—270)	7	180 (130—260)
Lung	2	77 (55—99)	2	87 (82—92)
Heart	17	45 (39—62)	8	41 (32—54)
Thymus			4	23 (14—30)
Brain	14	25 (13—38)	8	9,7 (5,3—15)
Spinal cord	14	63 (36—106)	11	36 (14—51)
Muscle	13	39 (23—44)	6	29 (21—35)

1. Thiersch, J. B. *Am. J. Obstet. Gynecol.* 63 (1952) 1298.
2. Ross, G. I. M. und Mollin, D. L. in Heinrich, H. C. *Vitamin B_{12} und Intrinsic Factor*, Ferdinand Enke Verlag, Stuttgart 1957, p. 437.

A Twin Microscope for the Perkin-Elmer Model 21 Infra-red Double-Beam Spectrophotometer

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In our laboratory a Perkin-Elmer 21 infra-red double-beam spectrophotometer is employed. As the need arose for a microscope attachment to it, we began constructing such a device in the autumn of 1956.

The instrument now completed consists of 2 parallel microscopes lying horizontally directly in the beams. Each microscope has 10 mm reflecting Schwarzschild aplanats for objective and condenser. A lamp was built into the monochromator of the spectrometer to project its entrance slit upon the sample. The latter is observed through a retractable viewer with a semi-reflecting right-angle prism.

The stage is vertical, it has right-angle slides and moves axially for focusing. It can also be swung upwards-outwards for attention.

The whole microscope attachment forms a unit. It is easily removed and replaced with accuracy, being secured in position by two pins.

It is, however, still our opinion that no satisfactory device for infra-red double-beam microspectrophotometry has yet been made [1,2]. In our microscope too much energy is lost at the entrance. Had this not been the case, an excessive heating of the sample would have been unavoidable.

The remedy should be to reverse the beam path by interchanging the energy source and the detector of the spectrometer. Thus the sample would be placed in a monochromatic beam causing little heat. We have, however, not been able to solve this problem practically. It seems a task for the makers.

1. *Cf.* Ford, M. A., Price, W. C., Seeds, W. E. and Wilkenson, G. R. *J. Opt. Soc. Am.* **48** (1958) 249, for a discussion of this subject.
2. *Cf.*, however, Ref. 1, p. 250.

The Nucleophilic Reactivity of Biological Thiols with Respect to Thiol-Disulphide Exchange Reactions *

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The biological significance of thiols can to a considerable extent be explained by the nucleophilic reactivity of their thiol group. It has generally been felt that in most of the nucleophilic exchange reactions of thiols the mercaptide ion is the active molecular species. Recently this mechanism has been proven in the case of thiol-disulphide exchange reactions [1,2]. At pH 7.4 the concentrations of the ionized forms of the physiological thiols are not inconsiderable as their pK values prove to be much lower than previously realized [3,4].

In the present investigation the relative nucleophilic reactivity of a series of biologically important thiols has been studied. The initial rate of interaction of the thiols (cysteine, glutathione, homocysteine, cysteamine, diethylcysteamine, and penicillamine) with cystine at pH 7.4 and 37°C was measured using ^{35}S-labeled cystine followed by paper electrophoretic separation of the reaction products [5]. The unexpected observation was made that the observed initial reaction rates (k_{SH}) were approximately equal in spite of the very large differences in the concentrations of the ionized thiols (pK varied from 7.7 to 9.75). The rate constants for the respective ionized thiols (k_{S^-}) were found to obey the Brönsted equation with the following parameters:

$$\log k_{S^-} = 0.73\ pK_{SH} - 1.88$$

The above k values (expressed in liter $\times$ moles^{-1} $\times$ min^{-1}) refer to the interaction of the thiols with the one of the sulphur atoms of cystine. Since cystine contains two symmetrical sulphur groups which are equivalent in these exchange reactions, the observed rates were actually twice the one given in the equation. The above presentation was preferred for thermodynamic reasons.

Several thiols tested did not obey the above equation but reacted at distinctly slower rates. This was the case with certain N-acyl derivatives (N-acetylcysteamine, aletheine, and coenzyme A). Compounds where the ionized thiol is part of a resonating system (thiocyanide, *o*-aminothiophenol, ergothionine, thiolhistidine, *etc.*) did not interact at all with cystine.

Thiol-disulphide exchange reactions play an important part in the enzymatic reduction of disulphides [6] and similar reactions may be envisaged to occur in the establishment and the splitting of disulphide cross linkages in proteins. The fact that a series of thiols obey the above equation strongly indicates that in such reactions the pK value of the attacking thiol is of minor significance with regard to the reaction rates. This finding may be interpreted to mean that with increasing pK_{SH} value the decrease in the concentration of the ionized thiol at a given pH is effectively counteracted by a concurrent increase in the nucleophilic reactivity of the ionized thiol.

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5. Eldjarn, L. and Pihl, A. *J. Am. Chem. Soc.* **79** (1957) 4589.
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