

### Excretion and Hepatic Storage of 5,6-Dimethylbenziminazolyl Cobamide Coenzyme labelled with Cobalt-58

BARKER *et al.*<sup>1,2</sup> recently isolated a number of vitamin B<sub>12</sub> derivatives from bacteria and demonstrated that they act as coenzymes in the conversion of glutamate to  $\beta$ -methylaspartate. Since the 5,6-dimethylbenziminazolyl cobamide coenzyme (DCC) was found to occur in rabbit liver<sup>3</sup>, and to act as a co-factor in a rat enzyme system<sup>3</sup>, it is reasonable to assume that the coenzymes are the forms in which vitamin B<sub>12</sub> exists in the tissues and that the 'extrinsic factor' may largely consist of DCC and similar compounds. Since we have previously been engaged in research on the turnover of vitamin B<sub>12</sub> (refs. 4 and 5), we thought it of interest to perform similar work on the turnover of radioactive DCC in man.

Four healthy student nurses who volunteered for the experiment were given an intragluteal injection of DCC labelled with cobalt-58. The preparation was kindly provided by the Squibb Institute for Medical Research, New Brunswick, New Jersey, and had a specific activity of about 1  $\mu$ c. per  $\mu$ gm. The radioactive DCC was injected soon after its arrival from abroad and protected from light in order to minimize breakdown.

Three of the test subjects received a dose of 1  $\mu$ gm. and one 0.75  $\mu$ gm. in 1 ml. of physiological saline. The test subjects collected their excreta in one-day portions for 39 days (except on days off), and later faeces only during the seventieth to seventy-ninth day following the injection. The samples of faeces were incinerated at 600–800° C. in porcelain cups in a coal furnace in the boiler room of the hospital. Recovery experiments demonstrated that no loss of radioactivity occurred during this procedure. The urine samples were concentrated by evaporation. The ashes and the concentrates were counted in a well-type scintillation counter together with appropriate standards, and the radioactivity was expressed as  $\mu$ gm. of the injected DCC.

Radioactivity counts were also taken over the liver area under standard conditions, once a day for the first 39 days, and once on the sixty-fifth day. The counts were corrected for radioactive decay.

The results of the excretion work are given in Table 1. It is seen that during the first few days after the injection the urinary excretion was slightly elevated, but later on the excretion of radioactivity was remarkably constant. From the fifth day on, urinary and faecal excretions were about equal. The fraction of the total-body radioactivity lost per day was about 0.3 per cent or slightly less. This value corresponds to a biological half-life of about one year.

From the first day following the injection, relatively strong radioactivity was measured over the

liver area, the counts ranging from 246 to 828 c.p.m. above the background ( $\sim$ 410 c.p.m.). Maximum counts were observed within the first 12 days. During the 65-day period when liver counts were taken, there was no clear-cut decrease in hepatic radioactivity. Thus the hepatic biological half-life must be long.

On the whole, the results obtained with radioactive DCC are very similar to those observed after administration of radioactive vitamin B<sub>12</sub><sup>4,5-8</sup>. When radioactive DCC is injected there is possibly slightly less 'spill-over' of radioactivity into the urine in the days immediately following the injection. During later periods the excretion-rates and the total-body biological half-lives of the two compounds are very similar.

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## HÆMATOLOGY

### Occurrence of Heparin in the Blood of Fish and its Prevention of the Clotting of Fish Plasma

THE property of the blood of fish to clot rapidly provides a means of protection from fatal hemorrhage such as may follow injuries<sup>1</sup>. Sometimes in the same fish the blood clotting-time is known to differ markedly at two different phases of its life-cycle. In the pre-spawning phase when the fish is fully laden with reserves, blood clotting is almost instantaneous, whereas during the period of physiological stress which generally follows spawning, clotting is slow and prolonged. Such variations in the coagulation-time have been attributed to some unknown factors which may involve a change in the chemistry of the blood of fishes<sup>2</sup>.

Table 1. MEAN DAILY EXCRETION OF RADIOACTIVITY

The radioactivity is expressed as  $\mu$ gm. of the injected preparation. F, faeces; U, urine; %, sum of faecal and urinary excretions expressed as percentage of the radioactivity estimated to be contained in the body at the time of excretion.

Case Injected dose	A 1 $\mu$ gm.			B 1 $\mu$ gm.			C 1 $\mu$ gm.			D 0.75 $\mu$ gm.		
	F	U	%	F	U	%	F	U	%	F	U	%
1st-4th day	0.4	6.2	0.68	2.2	5.1	0.75	0.3	5.9	0.64	0.6	3.7	0.59
5th-39th day	1.5	1.1	0.28	1.0	1.4	0.26	1.0	1.4	0.26	1.1	1.1	0.32
70th-79th day	1.3	1.0*	0.29	1.2	1.7*	0.38	—	—	—	0.7	0.7*	0.24

\* Because quantitative collection of urine for long periods is difficult, the subjects collected only faeces during this period. The urinary excretion was estimated on the assumption that the U:F ratio was the same as during the preceding collection period.