Microbiologic Assay for the Thiamine Content of Blood of Various Species of Animals and Man*

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THERE ARE a number of reports in the literature concerning the thiamine content of the blood of various species of animals. Several methods of thiamine assay are involved, and there appears to be a wide range of values within a given species as well as among the various species. It is difficult to estimate how much of this difference in values is a result of the variety of methods employed in the determinations and how much is due to the actual difference in the thiamine content of the blood samples.

In this laboratory, samples of blood of a number of animals were assayed for thiamine by a modification of the *Lactobacillus fermenti 36* method advocated by Sarett and Cheldelin,[1] and also by a modification of a chemical method described by Friedemann and Kmieciak.[2]

This paper deals primarily with the adaptation of the Sarett and Cheldelin[1] thiamine assay to small quantities of blood and with a comparison of values received by the microbiologic and chemical methods on the same samples. In addition, values are given for the microbiologic estimation of thiamine on human blood samples obtained by finger puncture and on several other samples for which the quantity was not sufficient to permit analysis by both methods.

METHOD

Oxalated or citrated blood samples were pipetted directly into cold 5 per cent trichloroacetic acid (TCA) in the proportion of 22.5 ml TCA per 5 ml blood. After standing in the cold for one hour, they were centrifuged at high speed for one hour at 4° C. All of the protein-free supernatant was poured off and measured. If the volume did not equal exactly 25 ml for each 5 ml of blood, it was brought up to that amount with 5 per cent TCA. Since previous experimentation found no thiamine in repeated washings of the protein precipitate, it was assumed that each 25-ml portion of supernatant contained the thiamine of 5 ml blood. These samples were analyzed for thiamine microbiologically without enzyme digestion.

To 25 ml of supernatant, were added 10 ml sodium acetate-acetic acid buffer, pH 4.5, and a sufficient amount of distilled water to acquire the desired dilution of the samples in accordance with the estimated thiamine content. The samples were then autoclaved at 15 pounds pressure for 10 minutes to drive off the TCA, the pH was adjusted to 6.5, and the volumes were readjusted to compensate for the loss of water in autoclaving.

The method of Sarett and Cheldelin[1] was employed for the completion of the assay with the incorporation of the following modifications:

(1) Two suggestions of Fang and Butts[3] were carried out in the preparation of the basal medium. Thirty-two g of dextrose plus 8 g of maltose replaced 40 g of dextrose. The alkali-treated peptone solution used in the medium was boiled for five minutes during its preparation.

(2) A somewhat different medium was used for maintaining stock cultures. It was similar to the microassay culture agar de-

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scribed in the Difco Manual,[4] except that sorbitan mono-oleate was omitted and the medium was enriched with thiamine hydrochloride to the extent of 1 μg per ml.

(3) The modification of Cheldelin, Bennett, and Kornberg,[5] which consists of adding an excess of thiamine to the inoculum broth, was also adopted.

(4) The strength of the inoculum was increased. An 18-hour inoculum broth culture was centrifuged, the cells resuspended in 10 ml of 0.9 per cent saline, centrifuged again, and then resuspended in 10 ml saline. Ten drops (approximately 0.3 ml) of this suspension were added to 10 ml saline and each tube of the assay was inoculated with one drop of the resultant suspension. The procedure produced very low blanks and, in general, gave an almost linear response up to the level of 0.04 μg thiamine per tube. After this point the response leveled off, in most instances gradually, but occasionally quite abruptly.

RESULTS

As can be seen in Table I, there appears to be a great deal of variation in the thiamine content of the blood among species of animals. Horse blood, with a value of 1.3 to 1.8 μg per 100 ml contained the least thiamine, while hog blood, ranging from 10.4 to 18.0 μg per 100 ml contained the most. The values for the rest of the blood samples are well distributed between these figures.

Hog blood showed a wide range in thiamine content among individual animals; human blood showed little variation. There were not enough samples from the other animals to draw a conclusion as to the difference in thiamine content of the blood of individuals within a species. No attempt was made to obtain data on thiamine intake of either animal or human subjects. However, all the human subjects were presumably well-nourished individuals and the blood samples obtained by finger puncture (Table I) were drawn while the subjects were in the fasting state.

TABLE I

Comparison of Thiamine Values Obtained by Microbiologic and Chemical Assay on the Same Sample of Blood

Source of blood	Method of assay		Source of blood	Method of assay	
	Microbiologic	Chemical		Microbiologic	Chemical
	μg/100 ml	μg/100 ml		μg/100 ml	μg/100 ml
Bear cub	7.9	*	Human No. 10	5.1	4.7
Calf No. 1	9.0	5.2	Human No. 11	4.4	3.6
Calf No. 2	9.8	7.9	Human No. 12	4.5	4.4
Cow	7.5	8.0	Human No. 13†	4.6	*
Chicken	5.4	8.0	Human No. 14†	6.2	*
Deer	5.9	5.0	Human No. 15†	3.9	*
Hamster	14.4	*	Human No. 16†	3.8	*
Hog No. 1	18.0	19.2	Human No. 17†	3.7	*
Hog No. 2	14.2	17.6	Lamb	5.4	6.4
Hog No. 3	10.4	7.6	Lion cub	5.7	*
Horse No. 1	1.3	1.6	Mink No. 1‡	10.6	7.1
Horse No. 2	1.8	2.0	Mink No. 2‡	11.0	7.5
Human No. 1	3.1	3.3	Mink No. 3‡	8.4	5.0
Human No. 2	4.3	3.8	Monkey	3.4	*
Human No. 3	4.3	4.1	Rabbit	15.6	*
Human No. 4	4.2	4.3	Sheep No. 1	6.8	5.3
Human No. 5	5.2	4.8	Sheep No. 2	5.3	4.9
Human No. 6	4.2	4.0	Sheep (Mouflon)	8.9	5.6
Human No. 7	4.0	4.8	Turkey No. 1	5.4	6.4
Human No. 8	3.9	*	Turkey No. 2	4.8	6.8
Human No. 9	4.1	4.6			

* Sample not sufficient for chemical analysis.
† Samples obtained by finger puncture.
‡ Pooled sample from 4 animals.

DISCUSSION

In reviewing Table I for a comparison of thiamine values arrived at by two different methods, it can be seen that: (1) values on human blood agreed very closely; but (2) values for the blood of other species were not in such good agreement. They were neither consistently lower nor consistently higher by either method.

Albritton[6] has summarized some of the values given by other workers on the thiamine content of animal blood, including that of man. However, it is necessary to refer to the original publications to get information as to the particular method applied and as to the number of animals involved in each study.

Goodhart and Sinclair[7] used a manometric method which measures the CO_2 produced when a sample containing cocarboxylase is added to a suspension of alkaline-washed yeast and allowed to react with sodium pyruvate as a substrate. They reported values for human blood ranging from 4.5 to 12.0 μg per 100 ml.

Westenbrink *et al.*[8] modified this original manometric method somewhat and used it to determine the aneurin (thiamine) pyrophosphate (APP) content of the blood of a number of animals. Some of the values they record are shown in Table II.

TABLE II

The Thiamine Pyrophosphate Content in the Blood of Various Species According to Westenbrink et al.

Species	μg per 100 ml blood*
Calf	9.3 ± 1.3
Pig	19.4 ± 4.0
Sheep	7.3 ± 1.7
Horse	5.6
Man	11.2 ± 1.5

* From Westenbrink *et al.*[8]

Albritton also refers to the studies of Smits and Floryn[9] on human blood, for which they obtained values from 7 to 14 μg per 100 ml by the manometric method, and to Meiklejohn's work[10] adapting the *Phycomyces* growth technique to the analysis of blood. Meiklejohn reported values ranging from 6.5 to 14 μg per 100 ml for human blood and 6.5 to 9.8 μg/100 ml for sheep blood. Sinclair[11] questioned the specificity of the *Phycomyces* method and stated that "blood contains substances other than vitamin B_1 that affect the growth of the fungus" and indicated that the pyrimidine and thiazole moieties of thiamine can also be utilized by it. However, he felt that if the possible sources of error were considered and controlled as much as possible, it was still valuable for comparing the "apparent Vitamin B_1 in different samples of blood."

In addition to the works referred to by Albritton, there have been other studies involving the estimation of blood thiamine in the species of animals which were also studied in this laboratory.

Pence and associates[12] report the mean blood thiamine values for 13 pigs in the semifasting state as 17 μg per 100 ml and for 9 nonfasting pigs as 21 μg per 100 ml. They used a modification of the thiochrome technic of Hennessy and Lewis.

Also using a thiochrome technic Yamadori[13] obtained the following thiamine values for whole blood: horse, 6.38; cow, 6.36; hog, 9.9; human 6.95 μg per 100 ml.

Blanchaer and Cameron[14] report thiamine values ranging from 3.8 to 12.8 μg/100 ml for human blood samples assayed by the Sarett and Cheldelin method.

As far as blood thiamine values for man are concerned, those obtained in this laboratory by the microbiologic assay method are lower than most of the values cited. However, they agree well with values obtained by a macrochemical thiochrome assay on the same blood samples, and also with results reported from this laboratory by Dubé *et al.*,[15] who used a microchemical method. These workers found that the average fasting blood thiamine values ranged from 3.3 to 5.4 μg per 100 ml when the intake of thiamine was about 500 μg per 1000 calories. It is felt that the comparatively low thiamine values obtained in this laboratory can be attributed to the assay methods employed, rather than to differences in the nutritional status of the subjects involved.

Thiamine values obtained in this laboratory on the blood of various species of animals are in fair agreement in most instances with those recorded by other investigators. In comparing

values derived from microbiologic methods it must be borne in mind that blood is a complex substance, and the possibility that it contains factors other than the one being assayed which stimulate or depress the growth of certain micro-organisms must not be overlooked. The *Lactobacillus fermenti 36* culture used in this laboratory was not stimulated by the pyrimidine and thiazole moieties of thiamine. No growth was obtained when pyrimidine and thiazole were substituted either separately or together for thiamine hydrochloride in a series of tubes containing basal medium, and no additional stimulation was noted when they were added to tubes containing a known amount of thiamine. The organism, however, is able to utilize the pyrophosphate of thiamine.

It was found that saline suspensions of the organism contained considerable acid phosphatase activity, as confirmed by a modification of the method of Bessey, Lowry, and Brock[16] in which paranitrophenyl phosphate is used as a substrate. Since the organism itself produced an enzyme capable of splitting cocarboxylase, it seemed unnecessary to add an external enzyme (i.e., takadiastase) for this purpose. This was confirmed in this laboratory by setting up a series of samples and assessing the thiamine value both with and without enzyme digestion. Most of the values obtained using the two methods were either identical or checked each other within a reasonable range of experimental error.

In view of the many thiamine determinations made in this laboratory by a microbiologic assay procedure employing the use of *Lactobacillus fermenti 36*, and the generally good agreement with values achieved by other methods, it is felt that this is a reliable technique, with the additional advantage of requiring relatively small amounts of blood, i.e., 5 ml as compared with a minimum of 15 ml for the macrochemical method.

SUMMARY

A modification of the Sarett and Cheldelin method of thiamine assay by the use of *Lactobacillus fermenti 36* is outlined, and values are given for a number of blood samples assayed by this method as well as by a macrochemical method. The small amount of blood needed for a microbiologic assay is a distinct advantage over the macrochemical method usually employed. When the thiamine values obtained by the two methods are compared it is found that the values on human blood agreed very closely, but those for the blood of other species were not in such good agreement.

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E pluribus unum

"If one asks a variety of medical men what, in their opinion, are the disturbing tendencies in medicine, one will receive a variety of answers, but in general they will all reflect an awareness of the same underlying tendency. One will complain of the impossibility of keeping abreast of the flood of new medical knowledge. Another will deprecate the tendency to narrow specialisation. A third will complain of the increasing divorce between practice and research. Yet another will deplore the invasion of medicine by non-medical experts from the natural sciences; a fifth the shrinking number of medically qualified men in the pre-clinical fields. These apprehensions are all manifestations of the same need—the need to find a concept which enables us to organise the different branches of medical knowledge so as to preserve the intellectual unity of the whole."

—Sir H. Himsworth. *Brit. M. J.* 2: 217, 1955.