emulsion of methoxyflurane by intravenous injection into the marginal ear vein. The emulsion contained 10 mg. % of SLS. Anesthesia was maintained for 30 minutes and repeated five times. The average dose was 16 ml./Kg./hr.

Isolated Rabbit's Heart.—The modified Langendorf procedure was used (8).

Perfused Frogs' Hearts *In Situ.*—The Greene frog-heart perfusion was used.

RESULTS

Hemolysis.—The plasma of three of the dogs was free of any visible hemolysis, whereas the plasma of one dog that received 10 mg. % SLS in 5% glucose showed a trace of hemolysis that was found to be below 30 mg. % hemoglobin.

Electrocardiograms.—The EKG tracings were remarkably devoid of significant findings. We observed peaking of *T* in two cases and an inversion of the *T*-wave in one case. The EKG of the monkeys was not changed.

Blood Pressures.—Dosage levels of SLS up to 3.5 mg./Kg. caused a fall in mean arterial pressure of 7–21% of the norm. Levels of 7 mg./Kg. evoked a 36–40% fall in blood pressure. Electrocardiographic changes were not significant.

Acute Toxic Effects On Liver, Lungs, and Kidneys.—The pathological diagnosis of the six dogs are shown in Table I. The table shows clearly that SLS causes cloudy swelling and congestion in lungs, kidneys, and liver with a preponderance on hepatic damage where even focal necrosis was observed.

Isolated Rabbit's Heart.—SLS dissolved in the perfusion fluid (60 mcg.) had no effect upon the amplitude of contraction of the rabbit's heart in two experiments. Levels of 120 and 240 mcg. caused a moderate depression of contractility.

Levels of 30 and 60 mcg. caused irregular transient coronary flow responses. Levels of 120 and 240 mcg. caused diminution of 35 and 80%, respectively.

Perfused Frogs' Hearts *In Situ.*—In three hearts 1 mg. % of SLS had no effect on the rate or amplitude of contraction. A level of 10 mg. % reduced the rate and amplitude of contraction in two hearts and in the third heart caused cardiac stoppage.

Rabbits.—The results of repeated administration of SLS in emulsion to rabbits are detailed in Table II. Two of the animals died before the study was completed.

DISCUSSION

We administered 10 mg. % SLS in 5% glucose solution to ascertain whether the hepatic toxicity that was found for 10 mg. % SLS in an emulsion could be ascribed to one of the constituents of the emulsion.

The recurrence of renal and especially hepatic changes compel us to ascribe these changes to the action of SLS. However, even at these levels hemolysis was not encountered. We believe that SLS should not be used intravenously in man.

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Modification in Sample Preparation for the Microbiological Assay of Vitamin B_{12}

By ELKO M. STAPERT, HELEN CRAIN, and ROBERT NEEDHAM

A modification of the U.S.P. XVI method for the assay of vitamin B_{12} eliminates some negative bias encountered with some products and permits quantitative recovery of the vitamin. Soft-elastic and hard-filled capsules are blended in the buffered sodium metabisulfite solution before autoclaving. This procedure is particularly necessary to assay older capsules. Products containing reducing sugars are protected with potassium cyanide instead of sodium metabisulfite.

BLENDING WHOLE CAPSULES BEFORE ASSAY

THE MICROBIOLOGICAL METHOD for assaying products containing vitamin B_{12} is described in U.S.P. XVI (1). In this method the product is placed into a buffered solution of sodium metabisulfite and autoclaved for 10 minutes during which all convertible B_{12} is changed to the more stable sulfite form. In assaying many types of products, lower values were obtained with samples stored at room temperature for 18 and 24 months by U.S.P. XVI method than by the assay described in the U.S.P. XIV, third supplement (2).

When whole capsules are placed in the sodium metabisulfite solution, a period of time is required before the contents are in contact with the sodium metabisulfite. A modification in sample preparation described in this paper permits quantitative recovery of the vitamin in contrast to the U.S.P. method where some B_{12} may be destroyed.

Experimental Methods

Sample Preparation with KCN.—All operations and ingredients were as directed in the U.S.P. XVI assay method except the sample preparation.

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TABLE I.—PER CENT RECOVERY OF VITAMIN B_{12} FROM FRESH SOFT-ELASTIC CAPSULES WITH VARIOUS PREPARATION METHODS[a]

KCN Method	U.S.P. Method	U.S.P. Method Modified
97.3	92.7	...
97.3	90.0	...
100.5	93.1	...
101.6	87.5	104.2
101.4	88.9	103.5
...	91.4	97.3
...	90.0	105.0
...	92.8	102.9
...	90.0	102.9
...	93.6	98.7
...	92.7	98.7
...	90.0	98.7
...	94.1	100.5
...	93.1	99.9

[a] All volumes were 300 ml. Per cent recoveries are based on theoretical values.

TABLE II.—PER CENT RECOVERY OF VITAMIN B_{12} FROM SOFT-ELASTIC CAPSULES STORED AT ROOM TEMPERATURE[a]

KCN Method	U.S.P. Method		U.S.P. Method Modified
18 Mo.	18 Mo.	24 Mo.	24 Mo.
98	82	79.5	99.7
100.5	87.7	79	101.2
96	84	81	103.5
92	79	...	...

[a] All volumes were 300 ml. Per cent recoveries are based on theoretical values.

Capsules were blended for 2 minutes in 300 ml. of a buffer solution in which 100 mg. of potassium cyanide replaced the sodium metabisulfite. The samples were made to volume and diluted to the desired concentration immediately. The autoclaving step was eliminated.

U.S.P. Assay Method.—All operations were carried out as specified in the U.S.P.

Sample Preparation by U.S.P. Method Modified.—The U.S.P. procedure was carried out as specified except that capsules were blended 2 minutes in a Waring Blendor containing the required amount of sodium metabisulfite solution. Hard-filled capsules were opened and dropped into the sodium metabisulfite solution before autoclaving. This method is similar to the one published after our studies were initiated (3).

Results and Discussion

The assay results obtained with the three methods of sample preparations as applied to both soft-elastic and hard-filled capsules containing 2.2 or 5.5 mcg. vitamin B_{12} per capsule are shown in Tables I, II, and III. The tables include results with freshly made capsules as well as those stored at room temperature. Soft-elastic capsules which had been stored at room temperature for 18 or 24 months showed greater differences than freshly prepared capsules. This was probably due to the gradual conversion of cyanocobalamin to the hydroxo form by reducing substances present in the product. The hydroxo form is less stable to heat and may be destroyed during autoclaving before the capsules are dissolved. Full recoveries were obtained when the capsule fill was immediately in contact with the potassium cyanide or sodium metabisulfite.

Summary

It is apparent from results in the tables that total B_{12} potency can be measured in more products using the modification described than by the U.S.P. XVI method. It is suggested that all products be dissolved in the sodium metabisulfite solution prior to autoclaving.

INACTIVATION OF B_{12} BY REDUCING SUGARS

Results obtained by the U.S.P. microbiological assay for B_{12} have been compared with results using a Co^{60} radioactive trace method. Products containing reducing sugars gave lower values with the microbiological method than with the tracer method; this difference was great enough to interest us in investigating the cause for the lower values. Experiments were set up to determine the effect of various sugars on vitamin B_{12} and its protection by sodium metabisulfite and potassium cyanide in the U.S.P. assay procedure.

Experimental

Crystalline vitamin B_{12} was assayed according to the U.S.P. procedure in the presence of various reducing and nonreducing sugars. Intrinsic factor concentrate N.F. was treated in a similar manner.

Crystalline vitamin B_{12} plus lactose was prepared for assay according to the U.S.P. method, except that 100 mg. of potassium cyanide was added instead of the sodium metabisulfite. The KCN was added to the buffer solution used in preparing the sodium metabisulfite solution to keep the mixture at the proper pH value. Another modification was the elimination of the autoclaving step as a control for determining whether the sugars might have some toxic effect on the organism. Only reducing sugars were used in this modification.

Results and Discussion

The results recorded in Table IV show that the

TABLE III.—PER CENT RECOVERY OF VITAMIN B_{12} FROM HARD-FILLED CAPSULES, NEW STOCK[a]

KCN Method	U.S.P. Method	U.S.P. Method Modified
106	77.8	104
106	89	104
105.2	86	106

[a] All volumes were 300 ml. Per cent recoveries are based on theoretical values.

TABLE IV.—EFFECT OF SUGARS ON VITAMIN B_{12} IN THE U.S.P. MICROBIOLOGICAL ASSAY[a]

Sugars, 2 Gm. + 50 mcg. B_{12}	Assay Method	Rec., %[b]
None	U.S.P.	100
Dextrose	U.S.P.	61.2
Galactose	U.S.P.	61.7
Mannose	U.S.P.	72.6
Arabinose	U.S.P.	61.3
Lactose	U.S.P.	65.7
Lactose	U.S.P.[c]	101.5
Sucrose	U.S.P.	100.3
Mannitol	U.S.P.	96.4
None	U.S.P. No $Na_2S_2O_5$ or KCN	84.0–82.7–90.8

[a] All volumes were 300 ml. [b] Per cent recoveries are based on theoretical values. [c] KCN replacing $Na_2S_2O_5$.

TABLE V.—EFFECT OF LACTOSE VITAMIN B_{12} AND B_{12} WITH INTRINSIC FACTOR CONCENTRATE (IFC) IN THE U.S.P. MICROBIOLOGICAL ASSAY[a]

B_{12}, Mcg.	Lactose	Rec., %[b]	Mcg. B_{12} as IFC	Lactose	Rec., %[b]
50	0	100.8–000	50	0	96–00
50	1	78.5–79	50	1	83.5–86.5
50	2	65.5–68.7	50	2	80.5–80.3
50	3	59.0–70.0	50	3	80.4–75.0
50	5	59.0–66.6	50	5	57.3–71.5

[a] All volumes were 300 ml. [b] Per cent recoveries are based on theoretical values.

TABLE VI.—EFFECT OF SUGARS ON VITAMIN B_{12} BY A MODIFIED ASSAY METHOD[a]

B_{12}, Mcg.	Sugars, 2 Gm.	Rec., %[b]
50	Lactose	99
50	Dextrose	100
50	Galactose	100.3
50	Mannose	100.4

[a] Samples made up without sodium metabisulfite and not autoclaved. [b] Per cent recoveries are based on theoretical values.

five reducing sugars affect the recovery of vitamin B_{12} to approximately the same amount. Full recovery of the B_{12} could be obtained in the presence of lactose when protected by potassium cyanide instead of sodium metabisulfite. Recoveries were complete in the presence of nonreducing sugars. It will also be noted that a sample of vitamin B_{12} protected by neither potassium cyanide nor sodium metabisulfite but autoclaved in the presence of water only gave a much higher value than the samples treated with reducing sugars and protected with sodium metabisulfite. This is further evidence that reducing sugars destroy vitamin B_{12} in the autoclaving step. The results indicating instability of vitamin B_{12} in the presence of reducing sugars confirm the report by Barr, Kohn, and Tice (4).

Table V shows the effect of increasing amounts of lactose in the assay of B_{12} and B_{12} with intrinsic factor concentrate. As indicated in the table, there is progressively more destruction of the vitamin with increasing amounts of lactose. This would probably be true with all reducing sugars. It appears that there may be some protection of the vitamin when B_{12} is combined with intrinsic factor concentrate, but the destruction is still progressive with increasing amounts of lactose.

Table VI shows the effect of reducing sugars when the samples are not subjected to the autoclaving step and not protected with sodium metabisulfite. This was a control to determine whether these sugars might have some affect on the growth of the test organism. As can be seen in the table, full recovery of the vitamin is obtained.

Some studies were made concerning the effect of dextrose in the medium during incubation of *Lactobacillus leichmanii* in the vitamin B_{12} assay. Since dextrose tends to reduce B_{12}, it should show its effect during the incubation period. In this experiment, which is shown in Fig. 1, a 15% greater growth was obtained with the sucrose medium. The authors' interpretation is that less vitamin is available to the test organism and therefore a depressed growth response is obtained. This may not affect assay precision since presumably all standard and test samples would be affected alike by the presence of dextrose in the medium.

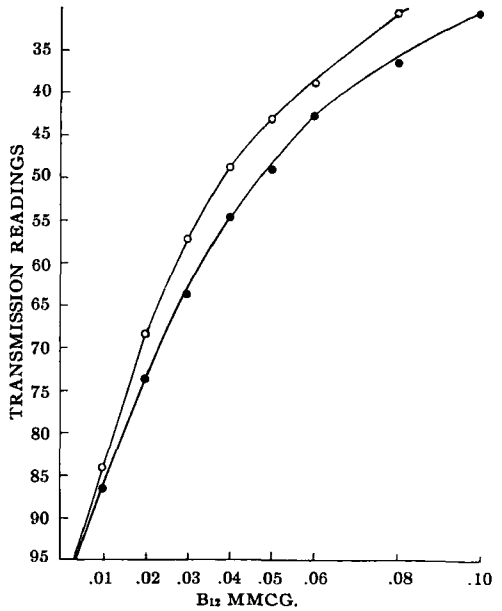

Fig. 1.—Effect of dextrose on vitamin B_{12} during incubation. ○, 40 Gm. sucrose per L. of medium; ●, 40 Gm. dextrose per L. of medium.

Summary

The U.S.P. XVI microbiological assay for vitamin B_{12} has been modified by replacing the sodium metabisulfite with potassium cyanide. This modification permits better recovery of vitamin B_{12} on products containing reducing sugars. The data indicate that potassium cyanide gives better protection in the presence of lactose during autoclaving than sodium metabisulfite.

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