TABLE III

Extract	Moisture, %	Initial Insol., %	Final Insol., %	Theoretical Value, %[a]
Cascara Aqueous	20	2.5	9.2	3.1
Cascara Aqueous	24	2.2	8.1	2.9
Krameria Aqueous	30	3.4	15.7	4.8
Valerian Aqueous	26	4.7	14.3	6.3
Valerian Aqueous	21	1.8	11.6	2.3

[a] Assuming no increase during drying.

matter content of the product. The small amount of information which is available is summarized in Table III.

SUMMARY

The infrared drying of pharmaceutical extracts has been investigated on the laboratory scale.

More than forty different samples were dried by this means, and the proportion of insoluble matter was determined in the undried and dried material in each case.

By comparing the figures thus obtained before and after drying, it is shown that in the majority of cases the deterioration which occurs during the drying process is slight.

This conclusion is further supported by the results of some additional experiments, carried out on four of the samples, in which the undried and dried extracts were each assayed for a particular active constituent.

None of the samples examined required more than fifty minutes' exposure to the infrared radiation; complete drying was invariably effected within that period.

The maximum temperature reached by the material being dried was 80°.

A Method for the Differentiation of Hydroxocobalamin from Cyanocobalamin Employing the Ascorbic Acid Reaction*

By J. A. CAMPBELL, J. M. McLAUGHLAN, and D. G. CHAPMAN†,‡

The fact that hydroxocobalamin is readily destroyed in solutions containing ascorbic acid while cyanocobalamin is relatively stable in such solutions has been used as the basis of a procedure to determine hydroxocobalamin in the presence of cyanocobalamin. The method is simple, and appears to be specific and reliable.

FOLLOWING earlier work (1, 2) on the reaction of vitamin B_{12} and ascorbic acid, Trenner, *et al.* (3), reported in 1950 that vitamin B_{12a} (hydroxocobalamin) was readily destroyed in solutions containing ascorbic acid, and that vitamin B_{12} (cyanocobalamin) was relatively stable under such conditions. The reaction appeared to be influenced by *p*H.

These data suggested a possible means of differentiating between hydroxocobalamin and cyanocobalamin, using a microbiological assay as the basis for the test. In 1950, Frost, *et al.* (4), reported experience with such a procedure where measurements were made of vitamin B_{12} potency before and after destruction of the vitamin B_{12b} (hydroxocobalamin) with sodium ascorbate. They also described other factors affecting the reaction, but no details of the method were published.

Independent work in this laboratory has led to the development of a somewhat similar method for the differential estimation of vitamins B_{12a} and B_{12b} in the presence of vitamin B_{12}. Vita-

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‡ After this work was completed, the authors were privileged, through the kindness of Dr. D. V. Frost, Abbott Laboratories, to see the draft of a paper outlining experience in their laboratory with this reaction. The data reported here confirm some of their work.

mins B_{12a} and B_{12b} are both hydroxocobalamins and are usually considered to be identical. In this case, they were obtained from different sources. In our laboratory, preliminary work with ascorbic acid confirmed the effect of *p*H on the reaction, and since the stability of vitamins B_{12a} and B_{12b} was greater at a low *p*H, it was decided to use buffers in subsequent investigations. The work reported here was carried out using a sodium acetate (Molar)-HCl buffer at *p*H 5.0.

PROCEDURE

The sample (10–50 μg.) in 1 to 2-cc. solution was added to a large test tube (with pouring lip) containing 150 mg. of ascorbic acid freshly dissolved in 2 ml. of buffer. The tube was then placed in a water bath at 70°. After thirty minutes, the solution was transferred to a volumetric flask and diluted for assay. Microbiological assays for vitamin B_{12} activity were carried out according to the U. S. P. method (5) modified by using a 6-point design on a log dose-log response basis.

RESULTS

This method was applied to solutions of crystalline vitamins B_{12}, B_{12a}, and B_{12b}. The data in Table I indicate that, under the conditions described, complete destruction of vitamin B_{12a} and B_{12b} did not occur until the temperature of the reaction mixture was raised to about 70°. Under the same conditions, vitamin B_{12} was not affected. This was confirmed by absorbancy measurements on the Beckman spectrophotometer at 548 mμ. Table II shows the results of assays of mixtures of vitamins B_{12} and B_{12b}. These mixtures were prepared by combining solutions of the crystalline vitamins in the proportions indicated and applying the ascorbic acid reaction. The recovery of vitamin B_{12} appeared to be unaffected by the amount of B_{12b} present. This constituted further evidence that no measurable amount of the vitamin B_{12b} was present after reaction with ascorbic acid. The precision and accuracy of the method was reasonably good.

TABLE I.—THE EFFECT OF TEMPERATURE ON THE ACTIVITY OF VITAMINS B_{12}, B_{12a}, AND B_{12b} TREATED WITH ASCORBIC ACID FOR THIRTY MINUTES

Temperature, °C	Microbiological Activity Remaining after Treatment, %		
	B_{12}	B_{12a}	B_{12b}
25	...	8.6	8.0
37	...	6.6	3.5
50	99.0	2.0	2.0
60	98.0	...	...
70	98.0	0.1	0.7
75	100.0	...	...

TABLE II.—THE VITAMIN B_{12} ACTIVITY OF MIXTURES OF B_{12} AND B_{12b} AFTER TREATMENT WITH ASCORBIC ACID

Vitamin Added		Vitamin B_{12} Found	
B_{12b}, μg.	B_{12}, μg.	μg.	%
3.8	13.8	14.3	104
5.2	13.8	14.1	102
7.6	9.2	9.0	98
10.4	9.2	9.3	101
11.2	4.6	4.8	104
15.6	4.6	4.5	98

The reliability of the method was also tested by assaying five commercial B_{12} concentrates (fermentation sources) microbiologically both before and after treatment with ascorbic acid and comparing the results with those found using the chemical method of Boxer and Rickards (6). That part of the vitamin B_{12} activity which reacted with ascorbic acid was arbitrarily designated B_{12b}. As shown in Table III, there is good agreement between the proposed microbiological method and the chemical method for the estimation of the vitamin B_{12} content of concentrates. Product B was dark brown in color, which may have prevented the light from penetrating the solution sufficiently to split off all the cyanide in the chemical method, thus giving a recovery too low to estimate. The results indicate that the vitamin B_{12b}/B_{12} ratio varies widely from one concentrate to another and may vary also between different lots of the same product. It appears also that concentrates containing a relatively high proportion of vitamin B_{12} more often meet labeled claim and therefore presumably are more stable preparations.

This procedure has been applied to liver extracts but, as reported by the Abbott workers, iron prevents the destruction of hydroxocobalamin by ascorbic acid. No satisfactory method of removing the iron has been found.

SUMMARY

A procedure has been developed for the differentiation of hydroxocobalamin from cyano-

TABLE III.—THE POTENCY OF VITAMIN B_{12} CONCENTRATES, μG./ML. FERMENTATIVE SOURCE

Company	Lot	Labeled Potency	B_{12} Total Microbiol.	B_{12} (Cyano) Microbiol.	B_{12} (Cyano) Chemical	B_{12b} (Hydroxo)[a]
A	1	20	17.7	2.6	2.6	15.1
B	1	10	6.9	3.4	...	3.5
C	1	30	19.2	12.3	10.5	6.9
	2	30	38.8	2.8	1.4	36.0
D	1	30	38.0	33.3	30.2	4.7
	2	30	35.0	34.8	35.2	0.2
E	1	30	22.0	6.1	6.7	15.9
	2	30	27.6	7.9	7.0	19.7

[a] The difference between microbiological determinations before and after treatment with ascorbic acid.

cobalamin which appears to be specific and reliable and the results compare favorably with those obtained by the chemical assay. To those laboratories which are not equipped for the chemical procedure of Boxer and Rickards, it offers a simple means of detecting the proportions of these vitamins in concentrates from fermentative sources. With liver extracts it has not been found to give effective separation.

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Experiments with Khellin

IV. A Convenient Method for the Preparation of Khellin and Some Observations on the Constituents of *Ammi visnaga* Fruits Grown in Minnesota*

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Ammi visnaga **plants were grown and the unripe fruits were extracted to yield khellin and visnagin. Khellol glucoside was not found in isolable amounts. A modified procedure for the extraction of khellin from *Ammi visnaga* fruits has been developed and appeared to give better yields than previous methods.**

THE FRUITS of *Ammi visnaga* L. contain the closely related furochromones, i. e., khellin,[1] visnagin, and khellol glucoside (1–3) of which khellin is the only constituent possessing a significant medicinal value (4). Khellin has recently been synthesized by Geissman (5) who, however, indicated that his synthetic procedure is not suitable for large-scale production. Therefore, the source of khellin is limited for the time being to the naturally occurring material. Although khellin is not difficult to obtain from the fruits of *Ammi visnaga*, the yields have not been outstanding and the procedures used are somewhat tedious. For this reason, the authors have developed a simplified extraction process which is more convenient and gives excellent yields.

Khellin is ordinarily prepared from *Ammi visnaga* fruits by two principal procedures: (*a*) the ether method, and (*b*) the alcohol method.

(*a*) The ether method (1, 6, 7) involves the extraction of the powdered fruits in a Soxhlet apparatus for about one hundred hours. The ether extract is then concentrated to a small volume and kept in a cool place. The crude mixture of khellin and visnagin (khellol glucoside is insoluble in ether) that separates is removed from the mother liquor by filtration and is freed of fatty substances by washing with ether. Khellin may then be purified by crystallization from boiling water, followed by repeated crystallization from an appropriate organic solvent. The purity and the yield of khellin depend largely on the number of crystallizations. The yields of pure material vary from 0.1 to 0.4 per cent.

(*b*) The alcohol method (8, 9) involves the extraction of the powdered fruits with either hot or cold alcohol, using one of the pharmaceutical processes, i. e., maceration, percolation, or continuous extraction. The alcohol concentration varies from 70 to 95 per cent. The alcoholic extract is concentrated to a small volume and kept in a cool place for a considerable period of time to allow the khellol glucoside to separate. The alcoholic mother liquor, after removal of the

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[1] For an excellent review of khellin and related products, see Huttrer, C. P., and Dale, E., *Chem. Rev.*, 48 543(1951).