The U. S. P. method of assay was used for the determination of vitamin "A."

The vitamin "D" assay method consisted of the usual method of initiating the healing of induced rickets in albino rats. The rats were placed on the Steenbock and Black diet No. 2965 until rickets was well developed (about 21 days), then graded doses of the oil were administered for 8 days, the diet remaining otherwise unchanged. Two days after discontinuing the feeding of the oil the zones of epiphyseal cartilage of the hind leg bones were examined by means of X-ray photographs and by the so-called "line test" for the existence of a narrow and continuous line of calcification. The unit of vitamin "D" was taken as $^1/_8$ of the total weight of the oil required to produce the above-mentioned degree of calcification and the number of units per gram of oil was ascertained by dividing 1 gram by the weight of the unit. Rats which failed to gain in weight throughout the depletion and dosing periods and which consumed the ration poorly were discarded in calculating the results of the tests. The rats were protected from ultraviolet rays throughout the tests.

The U. S. P. physical and chemical requirements, while of minor importance in comparison with the vitamin potency and certain other desirable characteristics, were essentially met by the above oils: specific gravity ranged between 0.920 and 0.922 at 25° C. (U. S. P. requires 0.918–0.927); between 0.25 and 0.50 cc. tenth-normal Sodium Hydroxide was consumed in the test for free acid (U. S. P. allows up to 1 cc.); unsaponifiable matter ranged between 0.86 and 1.54% (U. S. P. allows up to 1.5%); saponification value ranged between 183 and 184 (U. S. P. requires between 180 and 190) and the Iodine value ranged between 172.5 and 183 (U. S. P. requires between 140 and 180).

RESEARCH LABORATORIES,
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CHONDRUS BLEACHED WITH SULPHUR DIOXIDE.*

BY CHARLES H. LAWALL AND JOS. W. E. HARRISSON.

About six months ago our attention was directed to the fact that a commercial product used as an ice cream thickener and stabilizer contained sulphites in an amount corresponding to about 1000 parts per million of SO_2. Upon following up the case, we were told by the chemists representing the manufacturers that "the sulphites found are a natural constituent of one of the ingredients in the same manner as benzoic acid is a natural constituent of cranberries."

This was startling news if true, for no such constituent as naturally occurring sulphites has ever been reported in a vegetable product so far as we could learn.

The commercial product referred to was claimed to be a mixture of mucilaginous principles obtained by extracting the plant materials and subsequently drying and grinding the extracted mucilages.

Later, we were given a list of the ingredients of this commercial preparation and by taking them one by one and testing them for sulphites by the official method of the A. O. A. C. (Association, Official Agricultural Chemists), all were eliminated but the chondrus, and a sample of which obtained from a wholesale

* Scientific Section, A. PH. A., Toronto meeting, 1932.

druggist showed several thousand parts per million of SO_2 when examined by the A. O. A. C. method.

Inasmuch as we had been referred to an article in the *Analyst* in which mention was made of the presence of ethereal sulphates in chondrus, we deemed the problem worth while investigating thoroughly and this paper is the result of such investigation.

Chondrus, also commonly known as *Carrageen,* was highly esteemed in Ireland for its medical virtues more than a century ago, and was vaunted as a specific by some users.

A Dublin physician named Todhunter introduced it into medical practice in Great Britain in 1831, and it soon thereafter attracted attention on the continent. Inasmuch as the principal supplies came from Ireland for a long time it soon came to be known as Irish Moss, although it is an alga and not a moss.

It was never admitted to the London Pharmacopœia or the British Pharmacopœia, but was official in the United States Pharmacopœia beginning with the 1840 edition and continued to be recognized up to and including the U. S. P. IX (1910).

It was officially recognized in the "Pharmacopœia Turinensis" (Turin) of 1833, the "Pharmacopœia noscomiorum civilium Argentinensum" (Strasburg) of 1840, and the "Pharmacopœia Badensis" (Heidelberg) of 1841. It has rejoiced in a great variety of names among which are the following:

Irish Moss, Carrageen, Hen's Dulse, Killen, Pigwrack, Pearl Moss; *Fucus crispus,* and *Lichen Hibernicus,* Russ.; *Carragaheen* and *Mousse Perlee,* F. Cod.; *Irlandisches Moss, Perlmoos* and *Knorpeltang,* Ger.; *Fugo corrageo,* F. It.; *Carragaheen* and *Musco marien Perlado,* Sp.; *Alga Perlada* and *Musgo Bianco,* Port.

The botanical origin and the Latin nomenclature have been almost as varied as the common names, as the following list will show: *Fucus crispus,* L.; *Fucus ceranoides,* Gmel.; *Fucus polymorphous,* Lmk.; *Chondrus crispus,* Lyngb.; *Ulva crispa,* de Cand.; *Sphaeroccoccus crispus,* Ag.; *Chondrus crispus,* Stack; *Chondrus crispus,* L. Lyngb. and the related species *Chondrus mammilosus* (Sic) Greville; *Gigartina mammilosa* (Sic), F. Agardh.; and *Gigartina mamillosa,* Goodenough and Woodward.

Chondrus is now official in the N. F. V (1926).

It is also official in the Danish Pharmacopœia (1907), the Swiss Pharmacopœia (1907), the French Codex (1908), The Nederland Pharmacopœia (1926), the German Pharmacopœia (1926) and the Belgian Pharmacopœia (1930).

It is not recognized in the pharmacopœias of Austria (1906), Great Britain (1932), Italy (1920), Japan (1922), Norway (1913), Spain (1905) or Sweden (1925).

Among the pharmacopœias mentioned above as recognizing chondrus officially, we found the following require the substance to be "sun-bleached" and give specific tests for evidence of sulphur-bleached moss.

Swiss Pharmacopœia IV—1907.—Requires "carrageen" to be "sun-bleached." Has test for limit of SO_2 with tenth-normal iodine added directly to aqueous extraction of the moss. This limit corresponds to a maximum of about 300 p. p. m. of SO_2.

Nederland Pharmacopœia V—1926.—Requires "carrageen" to be "sun-bleached," with similar test and limiting requirements to those described for the Swiss Pharmacopœia.

German Pharmacopœia VI—1926.—Requires "carrageen" to be "sun-bleached." The test for the limit of SO_2 is based upon the liberation of the gas from an aqueous extraction of

5 Gm. of the moss, the test being made with potassium iodate-starch test paper, the time limit for the reaction being 15 minutes.

That these tests are really intended to exclude the sulphur-bleached moss will be realized when the SO_2 figures for commercial samples are seen later.

Upon further searching the literature we found that Zörnig in his "Arzneidrogen" and Hager in his "Pharmaceutischen Praxis both call attention to the fact that the lighter colored varieties of chondrus are bleached with the fumes of burning sulphur and classify such sulphur-bleached varieties as adulterated. In "Tschirsch Pharmacognosie," mention is made of sulphur-bleaching and a reference is given to an article by Gueguen on Carrageen collection in Brittany. Dr. Hermann Thoms also refers to sulphur-bleached carrageen in his monumental work on Pharmacy.

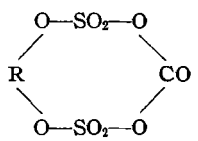
Dr. L. Van Itallie, the noted pharmaceutical authority of Leiden, Holland, to whom a letter was addressed asking for information concerning the situation, replied as follows:

"In the Dutch trade last year, chondrus indeed has been found bleached with sulphur dioxide, I believe that the bleached material is principally used in the textile industry."

The earliest reference we could find to the sulphur dioxide bleached moss was the announcement by Schack in 1886 that he had found SO_2 in a German specimen of carrageen.

Schack's conclusions were erroneous, however, in that he assumed, from the presence of sulphites in the moss, that the method then in common use for bleaching sponges with potassium permanganate followed by sodium thiosulphate had been employed.

All authorities who discuss the composition of chondrus mention the fact that the ash is rich in sulphates and in the article in the *Analyst*, previously referred to, there is mention of the fact that the sulphates are present in considerable proportion as an ethereal sulphur compound of the type structural formula as follows:



This compound upon treatment with hydrochloric acid liberates calcium sulphate but no mention is made in the article of sulphites being liberated.

The ethereal sulphates in chondrus had previously been reported by B. Russell-Wells, in 1922.

Indeed, in the light of many years' experience in testing animal and vegetable products for the presence of even minute amounts of SO_2 (less than 10 p. p. m. in some cases) and with the added evidence of the recorded facts, such as the recognition of the existence of sulphur-bleached moss in the several foreign pharmacopœias that have been quoted, and the different types of tests that are used to exclude the sulphur-bleached product, the chances that normal chondrus, unbleached or sun-bleached, liberates SO_2 when treated according to the directions of the official tests of the A. O. A. C. were rather remote.

However, in order that there might be no doubt as to the correctness of our original conclusions that added sulphites were present, we decided to make a

thorough investigation of samples from various sources, including some specimens of authentic origin which had been sun-bleached and not sulphur-bleached.

Commercial samples were first obtained from wholesale and importing firms. This survey of the material as sold on the market showed the following results:

	Volatile Sulphur Compound as Sulphur Dioxide.
No. 1—Sample of chondrus obtained from wholesale drug house (*Lab. No. 33469*)	1260 p. p. m.
No. 2—Samples of chondrus obtained from wholesale drug house (*Lab. No. 33641*)	920 p. p. m.
No. 3—Sample of chondrus obtained from wholesale drug house (*Lab. No. 33642*)	4040 p. p. m.
No. 4—Sample of chondrus obtained from wholesale drug house (*Lab. No. 33643*)	3480 p. p. m.
No. 5—Samples of chondrus marked "Technical" obtained from importing house (*Lab. No. 33705*)	4080 p. p. m.
No. 6—Samples of chondrus marked "medicinal" obtained from an importing house (*Lab. No. 33706*)	2520 p. p. m.

Correspondence with these sources of supply revealed the fact that all were of foreign origin, probably from France.

It was stated that very little chondrus is coming from New England at present, the reason being that it is of inferior appearance and sometimes contained sand and particles of shells.

One firm said

"It has been many years since we handled the domestic Irish moss."

Another replied

"We cannot give you very much assistance on the method of preparing Irish Moss. The bulk of our importations originate in France, from which source very little information has been obtained as to the process of bleaching."

"We are large users of Irish Moss. We purchase our supply in the open market according to our specifications. Some of our supply comes from Scituate, Mass., and some is imported from Great Britain and France. We are not familiar with the process of bleaching used abroad but we understand that the Scituate Moss is sun-bleached."

A number of firms promised to make tests and report later, but as several months have elapsed without any report from them we must assume that they are either not interested in the subject or trying to conceal the facts.

We were now ready to procure some authentic material.

Through the kindness of Dr. Heber W. Youngken, we received a sample of unbleached chondrus which he gathered himself from the rocks at Bass Point, Nahant.

Upon testing this sample for the presence of volatile sulphur compounds (SO_2) by the official method *negative* results were obtained.

Later, Dr. Youngken sent us three additional samples as follows:

	Volatile Sulphur Compound as Sulphur Dioxide.
No. 1—Moss collected June 30, 1932, from a layer which the gatherer had spread out on the beach to dry on June 28th (*Lab. No. 33902*)	None
No. 2—Moss which had been macerated in sea water and exposed on the beach for the second drying (*Lab. No. 33903*)	None
No. 3—The finished product, sun- and sea-water-bleached chondrus collected from the floor of the shed of a commercial shipper of the product (*Lab. No. 33904*)	None

Dr. Youngken also sent us the following interesting account of the collection and bleaching of chondrus as carried out at Scituate, Mass.

COLLECTION OF CHONDRUS AT SCITUATE.

The outstanding remarks gathered by the writer from one of the natives of Scituate, Mass., who has been collecting Irish Moss for the past 30 years, might be summed up as follows:

"Collection begins about the first of June. The collectors go out in rowboats with rakes. These rakes are similar to our common metal garden rakes, except that the teeth are a little closer together. The handle of the rake is about 16 feet in length, in order to allow for the depth of the water. Most of the collection is done at low tide. The moss is raked up from the rocks and carried ashore in the boats. It is spread out on the beach and allowed to dry and bleach for a week or so, depending upon weather conditions.

"The beach is a sand bar with a marsh on the back side which separates the beach from the mainland. The chondrus is carried up behind the cottages on the back side of the beach and placed in half hogsheads which are arranged around a hole dug in the ground which is connected with the back water of the marsh by a trench. When the tide comes in, the water flows up the trench and fills the shallow well. The collectors dip up this water and wet down the moss until it is soft, for about 20 minutes. The moss is then spread out again in the sun to bleach and dry. This process is repeated every day until the moss is bleached. The length of time required for the bleaching is from 10 days to 2 or 3 weeks, depending upon weather conditions. Showers at night tend to hurry the bleaching process and save the daily tubbing. Several days of rain, however, may bring out the mucilage and cause fermentation.

"The moss was formerly used in the brewing industry to give body to ale. Before prohibition, about 22,000 barrels of this material were shipped from Scituate each year. About 850 people were then connected with the industry. At the present time there are about a dozen collectors and the annual production at Scituate amounts to from 300 to 900 barrels a year. The moss brings about 6 cents a pound at the beach.

"Some Irish Moss is collected at a few other places by different collectors, but Scituate is the headquarters for the industry in the U. S. A.

"Chemical bleaching has never been used to any extent at Scituate. A Harvard professor suggested chemical bleaching a good many years ago, but it was only employed for a short time as an experiment. It was soon dropped on account of the expense and laws governing certain phases of the work which prohibited its practice."

We then learned that an Irish Moss collector named William J. Flynn, 3rd Cliff, Scituate, Mass., could supply first-hand information and specimens.

Upon writing to him the following interesting reply was received, which is a model of terseness and clarity:

"Received your letter. We gather the moss at low water. We go in rowboats and have a pole 12 feet long with an iron rake attached to it. We rake the moss off the rocks on the bottom in 4 or 5 feet of water. When we have the boats full we take it ashore.

"Then we carry it out of the boats and spread it on the beach (sand) to let the rain and sun bleach it. Then in about 6 or 7 days we take it up and put it in wooden casks. Then on warm sunny days we fill casks full of salt water—then after an hour we take it out of casks and spread it on the sand to bleach and dry. We do that for two days. Then we put it in sheds to keep it dry until we ship it. We do not use any sulphur to bleach it. I am enclosing a sample of bleached and unbleached."

Further correspondence led to his collecting and preparing four special samples especially for this investigation.

The results of the examination of these samples, with their accompanying designations were as follows:

	Sulphur Dioxide.
No. 1—Moss after four hours' drying (*Lab. No. 33772*)	None
No. 2—Moss after two days' sun-bleaching (*Lab. No. 33773*)	None
No. 3—Moss after six days' sun-bleaching (*Lab. No. 33774*)	None
No. 4—Moss in finished condition, ready for the market (*Lab. No. 33775*)	None

In the original brief submitted by the chemist supporting the view of the natural content of sulphites in the moss, stress was laid upon the fact (as tending to confirm the claim) that the rate of liberation of sulphur dioxide from the commercial product in question, was very gradual and required much longer heating than gelatin with which comparison in this respect was made.

The statement was essentially as follows:

"It is thus apparent that twenty minutes of steam distillation resulted in the recovery of 105 parts per million of sulphur dioxide from x—. This slow removal of sulphur dioxide from x— may be taken as clearly indicating that sulphur dioxide as such was not present in x—, and that the sulphur dioxide which is recovered by this method of analysis results from the decomposition by the non-volatile acid of naturally occurring sulphur in the original product, that is x—."

In drawing these conclusions, he has overlooked one important fact, that is, that a solution of gelatin in water, in the presence of mineral acid has a viscosity hardly greater than the water itself. An experienced chemist can rapidly boil such a solution without scorching, and the liberation of sulphur dioxide is almost immediate. x— in the same concentration, forms such a viscous mixture that indeed it is in some cases nearly impossible to pour the material. Liberation of SO_2 from such a viscous mass is slow, and gradually proceeds until the total quantity is obtained.

Furthermore, such a mixture cannot be boiled readily and if steam distillation is used, the material does not mix without mechanical aid, consequently the exposure to volatilization is reduced. It is, in our opinion, the nature of the product that causes the apparent gradual release of sulphur dioxide, the aqueous solution being so inherently viscous that the liberated sulphur dioxide is held tenaciously.

The evidence that we have been able to assemble up to this point is to our minds conclusive in the following respects:

Commercial chondrus, of foreign origin contains added sulphites derived from the process of "sulphur-bleaching" which is admittedly carried on abroad.

Such sulphite-containing chondrus when offered for sale for use in food products or for pharmaceutical purposes should be labeled with a declaration of the presence of added sulphites in some appropriate and intelligible manner.

Even when the sulphite is declared such chondrus is illegal for use in food products in Pennsylvania and other states where the state laws prohibit the presence of sulphites.

Natural chondrus unbleached or sun-bleached shows no evidence of the presence of "naturally occurring sulphites."

We might with propriety conclude our paper at this point, but there is another and economic angle to the situation.

A study of the early American literature on the subject reveals the fact that there was at one time a production of chondrus along the New England coast aggregating over 700,000 lbs. annually. In later years this output dropped to about one-third of this figure. This reduction in the American output would seem to be directly due to the fact that the sun-bleached American product was unfairly forced to compete with the "sulphur-bleached" foreign product, imported without any warning of its high sulphur content (and over 4000 parts per million is a high figure for a bleached product).

In brief the American market has been flooded with sulphur-bleached chondrus

which would have no legal status in several European countries except for "technical" uses, and this substandard material was imported and used for food purposes and as an ingredient in medicines to the economic disadvantage of the New England article.

The story of the early origin of the Irish Moss industry along the coast of New England is one of those forgotten epics that awaits the touch of a master hand to bring back the romance and glamour of post-colonial days when America was just beginning to find itself and develop new industries.

To quote from one of the early contributors (1866):

"In the town of Scituate, Plymouth Co., this business is carried on by natives of Ireland who are located upon the cliffs, at the base of which is a bold rocky beach where the moss is gathered in greater quantity than in any other part of New England.

"The collecting of 'moss' in New England for commercial purposes is of comparatively recent date, it being obtained almost wholly by Irish emigrants, who, during a period of fifteen or twenty years, have landed upon our shores to pursue an occupation familiar to them in their native land."

Another writer upon the subject (1868) says:

"Up to 1848 all carragheen used in this country was imported from Ireland which gave it the popular name of Irish Moss. It is used as a size for housepainting and is esteemed for medical and culinary purposes. That imported to America was used in making custards and blanc-manges and sometimes sold as high as 75 cents a pound retail.

"In 1849 several parties commenced making a business of gathering and curing chondrus at Scituate.

"The location is near the celebrated Minots Ledge Lighthouse, a locality so dangerous that seven shipwrecks have been reported as visible all at one time."

The uses to which chondrus have been put aside from its employment in making desserts for invalids have been various.

The lower grades have been used as sizing material by painters and paperhangers and also in the manufacture of textiles, paper, felt and straw hats. Another grade has been used extensively by brewers for fining purposes (according to some contributors for giving body to ale and beer). It has been employed for clarifying coffee. Under the name *Alga marina* it has been used for stuffing mattresses, and it has been employed as a cattle food. It has found a place as an ingredient in leather dressings, in veneers, in furniture polishes and in bandolines or hair dressings.

The mucilaginous principle, sometimes referred to as "carrageenin" or "chondrin," is present to the extent of from 60 to 70 per cent. The presence of calcium and sulphates influences and controls the gelatinizing power.

When boiled with water for a period of from three to five hours it will form a jelly with 50 parts of water.

This gelatinizing power is easily destroyed by acids, by alkalies and by certain salts other than calcium.

Flückiger and Hanbury found the mucilage of chondrus to obstinately retain inorganic matter and that even after three times dissolving and reprecipitating it still showed 15 per cent of ash.

The National Formulary Revision Committee should take cognizance of this situation described in this paper and provide standards and tests for the exclusion of the "sulphur-bleached" article for official purposes, and in the meantime the

Federal authorities should require the declaration of the fact that the foreign product is not sun-bleached but is "sulphur-bleached."

It is a curious commentary on the literature of the subject that no American work on Pharmacognosy that we have consulted (seven in all) makes any reference to "sulphur-bleached" chondrus, while no foreign authority that we have seen omits mention of the fact. The secret has been well kept so far as the American Drug Trade is concerned.

REFERENCES.

U. S. P. 1820 to 1920.
Foreign Pharmacopœias as mentioned in article.
1845, "Pharmacopœa Universalis" (Weimar).
1861, "Strumpf's Allgemeine Pharmacopœ."
1866, PROC. A. PH. A., 165.
1868, *A. J. P.*, 417.
1874, "Pharmacographia," Flückiger and Hanbury.
1886, *Pharm. Ztg.*, page 87.
1912, "Pharmacognosie," Tschirsch.
1916, "National Standard Dispensatory," Hare, Caspari, Rusby.
1921, "Origin and History of Pharmacopœial Vegetable Drugs, etc.," John Uri Lloyd.
1921, "Handbuch der Praktischen und Wissenschaftlichen Pharmazie," Hermann Thoms.
1922, *Biochem. J.*, 16, 577.
1923, "Marine Products of Commerce," Donald K. Tressler and collaborators.
1926, N. F. V.
1926, "United States Dispensatory," Wood and LaWall.

CONTRIBUTION FROM THE CONSULTING LABORATORY,
LAWALL AND HARRISSON, PHILADELPHIA.

THE BEHAVIOR OF ETHYL NITRITE IN COPAIBA EMULSIONS.*

BY W. G. CROCKETT, W. M. FRAYSER AND G. V. THOMPSON.

This work was undertaken for the purpose of investigating by means of the nitrometer, the rate of decomposition of ethyl nitrite in Copaiba Mixture, N. F. Preliminary experiments showed, however, that a surprisingly small volume of gas is evolved when 25 cc. of fresh Copaiba Mixture is shaken in a nitrometer with potassium iodide T.S. and diluted sulphuric acid. Other experiments showed that a marked deficiency in gas results when 4 cc. of spirit of ethyl nitrite is mixed with 21 cc. of water before introducing into the nitrometer, but that concordant and approximately theoretical results are obtained when 4 cc. of spirit of ethyl nitrite is pipetted into the nitrometer and followed by 21 cc. of water, and then the reagents.

Further work revealed that emulsions of copaiba, when introduced into a nitrometer containing spirit of ethyl nitrite, behave differently from emulsions of certain fixed oils under the same conditions. Due to the foregoing observations this investigation has been restricted chiefly to the action of copaiba emulsions on spirit of ethyl nitrite after introduction into the nitrometer. Two samples of copaiba were used and will be referred to as copaiba No. 1 and copaiba No. 2.

* Section on Practical Pharmacy and Dispensing, A. PH. A., Toronto meeting, 1932.