welfare to place a high value, commercially, on that which is injurious physiologically. If we estimate a coffee in proportion to the amount of the toxic ingredient, this is not in harmony with the principles regulating the value of dietetics.

Finally, if the pyridine-like body is developed by the roasting process, does not the same principle develop in the roasting of cereals and in chicory? Our experiments seem to indicate that this may be true.

DISCUSSION.

MR. WM. C. KIRCHGESSNER:—I would like to ask, if I may, if you cannot estimate the value of the coffee by the caffein content?

PROF. SAYRE:—You cannot estimate the value of coffee by the caffein content. You can sometimes obtain a very high caffein content from cheap coffee, and from a very expensive coffee you may get a very low yield in caffein content.

MR. KIRCHGESSNER:—Why is it that all the Decaffa Coffees are so dark? I have had men who make it a business of roasting coffee tell me that they could produce Decaffa Coffee at the same price that any other coffee could be sold. They would put the coffee in a roaster, tighten the cap and apply heat and after the coffee was roasted for a certain length of time let the roaster cool, cap up. After taking off cap a dark brown powder would adhere to the top of the roaster which they claimed was the caffein. I got some of this coffee roasted in this way and the people who used it said that they could not see or taste any differences from coffee which they paid twice as much for.

PROF. SAYRE:—I suppose you know that roasters of coffee always expect a loss in caffein in the process of roasting. This constituent is sublimed to a greater or less extent in the operation and it is well known that the crude caffein collects on the walls of the coffee roasters and this sublimate is very valuable because of its caffein content,—because of its richness in this alkaloid.

PROF. KREMERS:—I would like to ask whether the acid gives the aroma to the coffee, or aromatic residuent?

PROF. SAYRE:—It is given by the so-called oil which is associated with pyridine. The pyridine like constituent is associated with the aromatic principles or is a part of them. Dr. Nelson has been working with me on this problem. He is at Harvard during the summer and he hopes in the fall to work out the final result as to this toxic constituent which is present only in very minute quantities. We find it has a very close connection with the aromatic or so-called toxic principle.

PROF. KREMERS:—Do you know of any pyridine content, so-called that is suggestive of the aroma of coffee?

PROF. SAYRE:—No, I do not. It is well known that pyridine *itself* is not suggestive of coffee. But it is well known, however, that you can modify odors by certain combinations, especially when they are present in minute quantities.

THE ASSAY OF OPIUM.

A. R. L. DOHME.

There is no more important nor more frequently used assay process in the pharmacopœia than that of opium. There is no drug used in which the monetary value of variation in assay results is greater, for above all other drugs the price of opium is directly determined by and based upon its assayed strength of morphine. There is hence every reason why this assay process of all processes in the pharmacopœia should tell the truth or as nearly the truth as is possible. Hence the great question before the Revision Committee is the process of assay for opium and again this committee is confronted as it was in 1904 by two opposing factions favoring respectively the U. S. P. method which exhausts the opium by water and the lime method which exhausts the opium by the use of lime. Manufacturers of morphine probably know best the relation of assay of drug to yield of manufacture because

they are getting comparative data along this line all the time. It is admitted that about one *per cent.* of morphine is lost in manufacture whether it be in exhausting the opium for making fluid extract, tincture or morphine salt or by oxidation or other change. I have convinced myself that this loss is not due mainly if at all, to incomplete exhaustion as delicate qualitative tests for the presence of morphine in exhausted drug indicate the absence of morphine in practically all instances. Rather is the loss in my opinion due to oxidation or other chemical change of the morphine in the process incident to the exhaustion of the drug or manufacture of same into its respective preparations.

This view is taken by Debourdeaux, (*Jour. Pharm. Chimi., 1912, II, p. 491*) who found that opium is subject to change when stored for any great length of time. The content of water soluble morphine or morphine salt is increased due to some chemical change in the mass.

It is generally admitted that the present U. S. P. opium assay leaves morphine in the mother-liquors which I have been able not only to detect qualitatively but quantitatively by extracting it with immiscible solvents and determining it volumetrically by a process of assay devised by my laboratory, and to be described below. If you know that by the lime process you obtain from an opium 10 *per cent.* of morphine; by the U. S. P. process, as modified by the present Revision Committee, 11.5 *per cent.* and by our method 12.3 *per cent.* of morphine and if you know by your own test that the lime-process mother-liquors contained about 2.25 *per cent.* of morphine; the U. S. P. method mother-liquors contained about one *per cent.* of morphine and the mother-liquors from our process contained practically no morphine, would you hesitate about voting that the lime method is the best method to be adopted for the pharmacopœia? Yet this is the condition that confronts us all as users of the pharmacopœia, for a majority of the proximate assay committee after once adopting the U. S. P. method by a practically unanimous vote reconsidered their action on the motion of one member and then adopted the lime method of Prof. Stevens, the chairman of the committee, by a vote of 5 to 3, with one member not voting. To be sure, the lime method result above given represents the morphine actually obtained and weighed, to which by the proposed method of assay to be made official in the U. S. P. is to be added 1.12 *per cent.* as a correction factor, making the result 11.12 per cent. Kindly remember that they do not and did not propose to add a correction factor to the result obtained by the U. S. P. process, although any one can prove, as we have done, that there is 0.75 to 1 *per cent.* of morphine left in the U. S. P. mother-liquors. If the committee sees fit to use a correction-factor at all, and personally I think it unwise and unscientific as it admits weakness and error on its face and hence induces doubt, should they not, in their comparative assays in justice to the public and the process have used a correction factor for the U. S. P. method? In that event the comparative figures would read, using the same opium:—

Lime method 11.12 *per cent.* morphine.
U. S. P. method 12.25 *per cent.* morphine.

In 1904 the same thing occurred, and I, as chairman of the committee, appealed the matter to the General Revision Committee of twenty-five. Both myself and Prof. Stevens presented arguments and figures to substantiate our positions and the General Revision Committee supported me by a vote of 16 to 9 and

the U. S. P. method and not the lime method became official in the U. S. P. and has been for ten years. At that time and at this time Prof. Stevens censured me for going outside the committee and giving any results or facts of the committee's work to the General Committee or the public. I do not, however, favor star chamber methods in anything and never have, and feel to-day that every result or piece of work done by the Assay Committee or any other sub-committee of the U. S. P. Revision Committee is the property of the general public and should be made known by publicity to that public. I am sure there is no result, work or vote standing over my name in the files of the Revision Committee during the fourteen years I have been a member of it that I am not willing and glad to have made public and, further than that, I do not propose to remain indifferent and let a process of assaying opium that I consider to be inferior be adopted as the official and legal opium assay process for this country by a small majority of a committee. I propose to fight for what I feel convinced is right until I am convinced by incontrovertible arguments that I am wrong and that privilege I am only too glad to grant to any of my worthy opponents.

In the practical part of this paper I have had the kind assistance of Messrs. H. Engelhardt and O. E. Winters of my laboratory.

For the assay of opium 80 different methods have been recommended, many of which, naturally, have only doubtful value. Most of these methods depend on a crystallizing process, that is, the opium is extracted by a suitable solvent and from the solution either as such or in concentrated form after the addition of an alkali, the morphine is allowed to crystallize. In about 50 (that is about 65%) of these methods water is used as a menstruum and from the aqueous solution after the addition of alcohol or ether or acetic ether or a mixture of these and a slight excess of ammonia water the morphine is allowed to crystallize. In about 14 (that is about 18%) of these methods the opium is extracted with lime water, the calcium salt of morphine thus obtained is decomposed by ammonium chloride and the liberated morphine is allowed to crystallize in the presence of alcohol or ether, or of both. The extraction of opium by lime water has several decided advantages, viz:—calcium hydroxide does not dissolve the greater majority of the opium alkaloids such as narcotine, narceine, etc., as well as meconic acid, and by its use many of the resins present in the opium are eliminated. These, however, do not overcome its several disadvantages, the principal one of which is that it does not extract all or as much morphine from opium as the other methods. I may have the most perfectly devised and scientifically accurate method of assaying gold in copper or nitrogen in the air, but if it yields me less than is actually present or is determinable by other methods it has little practical value to the chemist.

Other methods depend on the property of morphine to be easily oxidized and in them certain reagents which are added to the morphine solution are reduced and the reduction products determined volumetrically.

In other methods, the morphine is estimated either colorimetrically or polarimetrically or with potassium-mercuric iodide solution.

The methods in which the morphine is allowed to crystallize have the one great disadvantage, to wit, that, *with the exception of that of the U. S. P.*, the morphine

is allowed to crystallize from too dilute solutions. It is a very well-known fact that morphine is not entirely insoluble in water or in diluted alcohol or in mixtures of water, alcohol and ether, especially in the presence of an alkali. This solubility is naturally materially increased by the presence of resins and is also dependent on the temperature at which the crystallizing liquid is allowed to stand. In most of these methods the proportion of opium to aqueous liquid is about 1 : 10. while in the U. S. P. method the proportion is only 1 : 5; naturally the results obtained by the latter method are considerably higher than those obtained by the other methods, because less morphine is lost by solubility in the mother-liquors.

Experiments to shake out the morphine with a suitable immiscible solvent such as hot chloroform, amyl alcohol, etc., have repeatedly been reported. By these solvents, however, a great amount of the resins is extracted from the opium also, which render the final titration extremely difficult. About four years ago the chemists of the Bureau of Chemistry at Washington worked out some methods by which the morphine was extracted at first with a mixture of alcohol-chloroform and then with chloroform alone. These methods are rather complicated and have to be adhered to in every detail, but the results are quite satisfactory. The shaking out of the morphine is a very tedious process, inasmuch as at times nearly 20 shakings with the solvent are necessary to remove all the morphine from the solution. Later on, Buchbinder recommended shaking out the alkaloid from the alkaline solution with chloroform containing 5 *per cent.* of alcohol. By this modification the shaking out process is shortened considerably.

In 1912, Anneler (*Archiv. der Pharmacie CCL,* page 187) reported on some experiments made in order to estimate the morphine in pantopon, which is a mixture of the various opium alkaloids in the form of their hydrochlorides. He recommended using a mixture of equal volumes of isobutyl-alcohol and chloroform, having found that 100 parts of such a mixture dissolve 1.7 parts of morphine. This menstruum is without doubt the most convenient solvent for morphine, and since the publication of this article we have applied this mixture exclusively in control assays for estimating morphine both in opium, in galenical preparations of opium, in pills, tablets, elixirs, etc., containing morphine. This shaking out process, like all other processes of this kind, has one great advantage over the crystallization process in that by it the total amount of morphine present in the drug or in the preparation is determined, there is no aliquot part feature; at the same time the assay process is a very rapid one and can be carried out, for instance in the case of opium, easily within about five hours. The details of the process which I apply are the following:—

POWDERED OPIUM.

Four gms. of the powdered opium are macerated with water in the regular way either by allowing to stand over night or by shaking for three hours, then exhausting the opium with water and evaporating the combined filtrate and wash-water to about 50 cc. The solution is then transferred to a separator, made decidedly alkaline with caustic soda solution or caustic potash solution which holds the morphine in solution as alkali morphinate. The solution is then shaken out with several portions of 20 cc. each of ether in order to remove the alkaloids of opium other than morphine, which are present in the free state. The alkaline

solution is then acidulated with sulphuric acid to convert the morphine into morphine sulphate, made again *slightly* alkaline by the addition of ammonia water and is then extracted by adding several portions of a mixture of equal volumes of chloroform and isobutyl-alcohol, until all the alkaloid is removed. The isobutyl alcohol-chloroform solutions are then filtered into a distilling flask, the filter paper washed with fresh isobutyl alcohol-chloroform, the chloroform distilled off under ordinary conditions and the isobutyl-alcohol later under diminished pressure. If the original mixture is distilled under diminished pressure bumping very frequently takes place. This can be avoided by first distilling off the chloroform under ordinary pressure. The residue in the flask is taken up in an excess of standardized acid; the acid solution is well diluted with water, and, after the addition of a few drops of methyl red, the excess of acid is titrated back with standardized alkali. From the amount of acid used, the morphine is calculated volumetrically.

For the assay of *Gum Opium* the following modification should be used: 20 gms. of opium representing an average sample of as many balls is exhausted in the regular way by means of water, the combined aqueous liquids are made up with sufficient water to obtain 500 cc., and 100 cc. of this solution are subjected to the assay process. Thus a fairly representative sample of the opium can be obtained. We append below some comparative results on the same drug by the two methods.

Gum Opium U. S. P. Method	*Gum Opium Shaking Out Method*
Sample I 11.34%	Sample I 12.89%
	12.80%
Sample II 11.37%	Sample II 12.35%
	12.29%
Powdered Opium U. S. P. Method	*Powdered Opium Shaking Out Method*
Opium A 12.24%	Opium A 13.86%
Opium B 11.7 %	Opium B 13.25%

The assays were made in duplicate by two chemists. In order to find out whether or not the method works in the hands of a worker entirely inexperienced with the method, the opium assaying 12.24 *per cent.* of morphine by the U. S. P. method was estimated by this chemist who found 13.58 *per cent.* of morphine, a figure not varying too much from 13.86 *per cent.* as found by the other two chemists.

AS APPLIED TO TINCTURE OF OPIUM.

From 25 cc. of the tincture, the alcohol is expelled by heating; the residual liquid acidulated with sulphuric acid, the solution filtered into a separator and the filter and residue washed with small portions of water until the combined filtrate and wash-water measure 50 cc. This is then treated as the concentrated aqueous extract in the powdered opium assay.

By this process the following comparative results were obtained on the same laudanum sample:—

U. S. P. Method	*Shaking Out Method*
1.23% morphine	1.33% morphine

I have frequently applied the shaking-out method for estimating the morphine in unfinished tinctures of opium, i. e., the strong tincture before being diluted to

the standard. From the amount of morphine obtained I deducted 10 *per cent.* (approximately the amount found in excess over the U. S. P. method by the shaking-out method) and according to the difference thus obtained I diluted the tincture. I invariably found that the tincture thus diluted answered the requirements of the U. S. P. very well. This procedure has saved us a great deal of time in the course of the year we have been using it, since the shaking-out process can easily be carried out in three hours, while the U. S. P. process takes at least 24 hours.

AS APPLIED TO FLUIDEXTRACT OF OPIUM CONCENTRATED.

Five cc. of the fluidextract (four times the strength of the tincture) is deprived of its alcohol by evaporation, the liquid is then acidulated with sulphuric acid and filtered into a separator. The residue on the filter is washed well with several portions of water until the combined filtrate and wash-water measures about 50 cc The assay is then carried out as in the case of the tincture.

AS APPLIED TO FLUIDEXTRACT AND TINCTURE OF OPIUM CAMPHORATED.

The isobutyl alcohol-chloroform method is especially useful in assaying preparations containing only a small amount of opium such as fluidextract of opium camphorated and paregoric. For assaying the fluidextract 25 cc. are used and 50 cc. for estimating the morphine in paregoric. The process is almost identical with that given under fluidextract of opium. Most of the benzoic acid and camphor is eliminated on evaporation and filtering. Any benzoic acid left in suspension does not interfere with the estimation. I append a few results obtained in assaying these camphorated products.

FLUIDEXTRACT OF OPIUM CAMPHORATED.

(Eight times the strength of paregoric without removing the benzoic acid entirely.)

Lot A 0.346% morphine (theoretical strength 0.384%)
Lot B 0.390% morphine (theoretical strength 0.384%)
Lot C 0.372% morphine (theoretical strength 0.384%)

Removing the suspended benzoic acid by first shaking out the acid alkaloidal solution with ether, I found in the same products:—

Lot A 0.374% morphine (theoretical strength 0.384%)
Lot B 0.361% morphine (theoretical strength 0.384%)

Tincture of Opium Camphorated (Paregoric).

Lot D 0.052% morphine (theoretical strength 0.050%)
Lot E 0.056% morphine (theoretical strength 0.050%)

As applied to Extract of Opium.

0.5 gms of the extract was dissolved according to the directions of the U. S. P., the aqueous solution was evaporated to about 50 cc. and the process was then carried out as described under tincture of opium. The results of the assays follows:—

	U. S. P. Method	*Shaking-out Method*
Lot F	18.63% morphine (theo. str. 20%)	20.4% morphine
Lot G	20.04% morphine (theo. str. 20%)	21.7% morphine

As applied to Tablets.

Dr. Engelhardt (*D. A. Apoth. Ztg., 1912,* January and February), has reported in detail on the usefulness of the shaking-out method in assaying morphine sulphate tablets. We have made a number of additional experiments in this direction, and we have also assayed tablets of complex composition, containing morphine salts combined with other drugs. We have found that the shaking-out method gives rather good results with tablets containing other alkaloidal extracts, provided the alkaloids present in these extracts are soluble in ether. It would be beyond the scope of this paper to give in detail all the results which we have obtained. We feel convinced, however, that the shaking-out method is the best yet proposed for quantitatively determining morphine in tablets.

As applied to Pills.

Pills, both simple and complex, can be assayed just as conveniently as tablets. The diluents in the pill mass do not apparently interfere with the accuracy of the assay. For instance, we have applied the shaking-out process to Neuralgic, Gross, N. F. pills which as is well known contain in addition to 1/20 gr. morphine sulphate, 2 gr. quinine sulphate, 1/30 gr. strychnine, 1/20 gr. arsenous acid and 1/2 gr. extract aconite leaves. When applying the shaking-out process, described in this paper, we found the pills to contain 1/23 gr. morphine sulphate, a result which without doubt is very gratifying considering the large proportion of other alkaloids present in the preparation.

We have also applied the shaking-out process to morphine preparations containing a comparatively small amount of the alkaloid, for instance, to Elixir Morphine Aromatic which contains, in addition to several other ingredients one grain of morphine sulphate per fluid ounce. By the isobutyl alcohol-chloroform shaking-out method we found in three different lots of this .936 grain, .938 grain and .94 grain of morphine present in one fluid ounce. The shaking-out method can be applied also with advantage to other substances containing small amounts of morphine, such as poppy heads and preparations thereof.

From an experience of more than a year with the shaking-out method using isobutyl alcohol-chloroform as immiscible solvent, we cannot too strongly recommend this method and we trust that many other chemists will try this method and publish their findings or let us hear from them. It is our conviction that the shaking-out method will eventually be made official in the U. S. P. in order to save time and to estimate the *total amount* and correct amount of morphine present in the drug or in its galenical preparation without using correction factors or aliquot parts. As has been pointed out in the first part of this paper all the methods which have been recommended for the estimation of morphine give results that are too low and that vary too much, due to the varying amount of morphine remaining in solution in the crystallization liquid.

In closing, I would like to mention that we devised and worked out this shaking-out method only after most of the assay work on opium of the Proximate Assays Committee had been completed. In fact, I had practically voted to adopt the U. S. P. process as revised by the committee. When the reconsideration of this action came up, I suggested trying the shaking-out method in comparison with the other two methods before the committee, viz., the U. S. P. and lime methods. It received little or no consideration, however, the Proximate Assay Committee

being unfavorable. The stage was set for lime versus U. S. P. and no one could see the shaking-out process.

In conclusion, I think I have proved that:—

First:—The lime method is inferior in every sense, except shorter time of operation, to the U. S. P. method, and is not worthy of adoption in the pharmacopœia of this country.

Second:—That the shaking-out method devised by us is superior in every sense, will eventually be adopted in the U. S. P. and should be adopted now. If not adopted now, then the modified U. S. P. should be adopted by the Revision Committee.

Third:—That the shaking-out process is adapted to determining morphine in practically all kinds of mixtures as well as practically all forms of medication.

Analytical Laboratory, Sharpe & Dohme, August, 1914.

THE LIME ASSAY OF OPIUM.

A. B. LYONS, M. D.

Of the numerous methods that have been proposed for the morphiometric assay of opium, two only seem to have found favor with the authors or compilers of national Pharmacopœias.

In one, the drug is exhausted with water, the solution concentrated to a small volume, alcohol and ether added together with water of ammonia, the mixture shaken well and allowed to stand for a specified time for separation of the morphine in crystals.

In the other, the powdered opium is mixed with lime and a certain proportion of water, allowed to macerate, with occasional stirring during half an hour, the solution filtered and an aliquot portion of the filtrate treated with ammonium chloride which causes the morphine to separate in crystalline form.

The advantages claimed for the second method are (1) rapidity of execution; (2) superior purity of the morphine obtained, owing partly to the fact that lime combines with morphine forming a very soluble compound—a property not shared with it by narcotine or most of the other alkaloids of opium—partly because the lime throws out of solution certain organic acids and other compounds which otherwise are liable to be thrown down with the morphine; (3) alleged uniformity of results.

Against the lime method it is urged: (1) It involves unavoidably the principle of the aliquot part; (2) crystallization of the morphine is from a more dilute solution than in the first general method, hence more of the morphine is held in solution so that an arbitrary correction is generally prescribed to compensate this loss. (It is generally admitted that there is also loss of morphine in the first assay method, in which no correction factor is generally prescribed;) (3) the assay requires that the opium be in the form of a powder, whereas