FOOD INSPECTION AND ANALYSIS.

CHAPTER I.

FOOD ANALYSIS AND OFFICIAL CONTROL.

INTRODUCTORY.

The general subject of food analysis, in so far as the public health is concerned, is to be considered from two somewhat different standpoints: first, from the outlook of the government, state, or municipal analyst, whose mission it is to ascertain whether or not the food may properly be considered pure or free from adulteration; and second, from the point of view of the food economist, whose aim is to determine its actual composition and nutritive value. The one protects against fraud and injury, the other furnishes data for the arrangement of dietaries and for an intelligent conception of the role which the various nutrients play in the metabolism of matter and energy in the body. The two fields are as a rule distinct each from the other, often involving, in the examination of the food, different methods of procedure.

Official Control of Food.—In view of the importance of the consideration of food with reference to its purity, an ever-increasing number of states have realized the necessity of protecting their citizens from the unscrupulous manufacturers who in various lines are seeking to produce cheaper or inferior articles of food in close imitation of pure goods. Many of the states have laws in accordance with which the sale of such impure or adulterated foods is made a criminal offense, and some, but not all of these, are provided with public analysts and other officers to enforce these laws and punish the offenders. Numerous communities are awake to the importance of municipal control of such commonly used articles of food as milk, butter, and vinegar, and in many cases have machinery of their own for regulating the sale of these foods.
Since January 1, 1907, the federal government has been actively engaged in the enforcement of the national food law of June 30, 1906, through the Bureau of Chemistry of the U. S. Department of Agriculture. In addition to the central laboratories of this Bureau at Washington, a number of branch laboratories have been established in the principal cities of the United States to enforce the provisions of the national law which regulates interstate commerce in foods, as well as their manufacture and sale in the territories and the District of Columbia, and their importation from foreign countries.

**Food Analysis from the Dietetic Standpoint.**—The study of the principles of dietetics has been given increased attention during the last decade in the curricula of many of the technical schools and colleges. Much has been accomplished by certain of the state experiment stations working as a rule in connection with the United States Department of Agriculture along this line. Investigations of this character are especially valuable, and are indeed rendered necessary by the general tendency of the modern physician to regard the hygienic treatment of disease, especially with reference to the matter of diet, as often of far greater importance than the mere administering of drugs.

The food economist studies the varying conditions of age, sex, occupation, environment, and health among his fellow men, with a view to showing what foods are best adapted to supply the special requirements of various classes. The quantity and proportion of protein, fat and carbohydrates, or of fuel value best suited for the daily consumption of a given class or individual having been determined, dietaries are made up from various food materials to supply the need with reference as far as possible to the taste and means of the consumer.

Experiments are made on families, clubs, or individuals, representing various typical conditions of life, and extending over a given period, during which records are kept of the available food materials on hand and received during the term of the experiment, as well as of those remaining at the end. In the case of individuals, additional records may be kept of the amount and composition of the urine and feces. From such data the physiological chemist calculates the amount of nutrients utilized, and studies the metabolism of material in the human body.

Up to this point no very extensive apparatus is required, but if in addition the income and outgo of heat and energy are to be studied, which are important to a complete investigation of the economy of food in the body, the student will require a respiration calorimeter and its appurte-
nances. The calorimeter is so constructed that an individual may be confined therein for a term of days under close observation and with carefully regulated conditions. Such an equipment involves a large expenditure and is to be found in but few laboratories.

It is not the purpose of the present work to go beyond the strictly chemical or physical processes involved in making the analyses by which the proximate components of the foods are determined. For more complete information in the field of dietary studies and the metabolism of matter and energy in the body, the student is referred especially to the investigations of Atwater and his co-workers, as published in the annual reports of the Storrs Experiment Station at Middletown and in the bulletins of the U. S. Department of Agriculture, Office of Experiment Stations, also to studies conducted by Benedict of the Carnegie Institution.

**Commercial Food Analysis.**—The proper preparation of food products has long ceased to be carried on by the hap-hazard rule-of-thumb methods that formerly prevailed. Now in the manufacture of many prepared foods and condiments, especially on a large scale, it has become a necessity to use scientific processes, rendered possible only by the employment of skilled chemists. In fact it is coming to be more and more common for food manufacturers to establish chemical laboratories in connection with their works, in the interests both of economy and of improved production.

Frequently disputed points arise in the enforcement of the food laws that render the services of the private food analyst of great importance both to manufacturer and dealer. Thus a wide field is open to the analyst of foods outside the domain of the government or state laboratory, either in connection with the large food manufacturing plants directly, or in private laboratories for experimental research, or for analytical control work.

**Systematic Food Inspection.**

**Functions of the Official Analyst.**—The public analyst is employed by city, state, or government to pass judgment on various articles of food taken from the open market by purchase or seizure, either by himself or by duly authorized collectors employed for the purpose. The sole object of his examination is to ascertain whether or not such articles of food conform to certain standards of purity fixed in some cases by special law, and in others by common usage or acceptance. Such a public analyst need not concern himself with the dietetic value of the food or whether it is of high or low grade. It is for him to determine simply whether it is genuine or
adulterated within the meaning of the law, and, if adulterated, how and to what extent. Aside from his skill as a chemist, it is often necessary for him to possess other no less important qualifications, chief among which are his ability to testify clearly and concisely in the courts, and to meet at any time the most rigid kind of cross-examination, it being of the utmost importance that he understand thoroughly the nature of evidence.

Standards of Purity for Food Products.—Under an act of Congress approved March 3, 1903, standards of purity for certain articles of food have been established as official standards for the United States by the Secretary of Agriculture. The earlier of these standards were formulated under the Secretary's direction by a committee of the Association of Official Agricultural Chemists. Later, however, a joint committee of that association and of the association composed of state and national food officials has had charge of this work and still later a joint committee of these organizations and the Bureau of Chemistry. Standards have been adopted, covering the entire range of food products.

Nature of the Analytical Methods Employed.—Since usually only a small number of the samples submitted are adulterated, the analyst should, as quickly as possible, separate the pure from the impure, so as to concentrate his attention on the latter. The nature of the processes by which this is done varies with the foods. Experience soon enables one to judge much by even the characteristics of taste, appearance, and odor, though such superficial indications should be used with discretion. One or two simple chemical or physical tests may often suffice to establish beyond a doubt the purity of the sample, after which no further attention need be paid to it.

A sample failing to conform to the tests of a genuine food must be carefully examined in detail for impurities or adulterants. While in most cases usage or experience suggests the forms of adulteration peculiar to various foods, the analyst should be on the alert to meet new conditions constantly arising. His methods are largely qualitative, since technically, he need only show in most cases the mere presence of a forbidden ingredient, though for the analyst's own satisfaction he had best determine the amount, at least approximately.

In reporting approximate quantitative results in court, especially when they are calculated from assumed or variable factors, or when they are the result of judgment based on the appearance of the food under

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* U. S. Dept. of Agric., Off. of Sec., Circ. 19.
the microscope, the analyst should always be conservative in his figures by expressing the lowest or minimum amount of the adulterant, so as to give the defendant the benefit of any doubt. When exact standards are fixed by law, as in the case of total solids or fat in milk, for example, there is of course great necessity for preciseness in quantitative work.

A full analysis of an adulterated food beyond establishing the nature and amount of the adulteration is entirely unnecessary, and in most instances adds nothing to the strength of a contested case, as twenty years' experience in the enforcement of the food laws in Massachusetts has shown.

The responsibility resting upon the analyst is not to be lightly considered, when it is realized that his judgment and findings constitute the basis on which court complaints are made, and the payment of a fine or even the imprisonment of the defendant may be the result of his report. Therefore he should be sure of his ground, knowing that his results are open to question by the defendant. Where court procedure is apt to be involved, a safe rule is for the analyst to consider himself the hardest person to convince that his tests are unquestionable, making every possible confirmatory test to strengthen his position and consulting all available authorities before expressing his opinion; and finally, after being fully convinced that a sample is adulterated, and having so alleged, let him adhere to his statements and not waver in spite of the most rigid cross-examination to which he may be subjected.

While each state or municipality has its own peculiar code of regulations and restrictions concerning the duties of the analyst and other officials, these rules are in the main very similar. For instance, it is usually necessary, except in the case of such a perishable food as milk, for the analyst to reserve a portion of a sample before beginning the analysis, which sample, in the event of proving to be adulterated, shall be sealed, so that in case a complaint is made against the vendor, the sealed sample may, on application, be delivered to the defendant or his attorney.

Adulteration of Food.—Except in special cases a food in general is deemed to be adulterated if anything has been mixed with it to reduce or lower its quality or strength; or if anything inferior or cheaper has been substituted wholly or in part therefor; or if any valuable constituent has been abstracted wholly or in part from it; or if it consists wholly or in part of a diseased, decomposed, or putrid animal or vegetable substance; or if by coloring, coating, or otherwise it is made to appear of greater value than it really is; or if it contains any added poisonous
ingredient. These provisions briefly expressed are typical of the general food laws adopted by most states and by the government, though the verbiage may differ. Laws covering compound foods and special foods vary widely with the locality. As to the character of adulteration, nine out of ten adulterated foods are so classed by reason of the addition of cheaper though harmless ingredients added for commercial profit, rather than by the addition of actually poisonous or injurious substances, though occasional instances of the latter are found.

Authentic instances of actual danger to health from the presence of injurious ingredients are extremely rare, so that the question of food adulteration should logically be met largely on the ground of its fraudulent character. Indeed the commoner forms of adulteration are restricted to a comparatively small number of food products, the most staple articles of our food supply, such as sugar and the cereals, eggs, fresh meat, fresh vegetables and fruit being less often subject to adulteration.

Misbranding.—Under the federal food law and the laws of many of the states misbranding constitutes an offense as well as adulteration. By misbranding is meant any untrue or deceptive statement or design on the label of a food package, either regarding the nature of the contents, or of the place of manufacture or name of manufacturer. One of the commonest forms of misbranding consists in the incorrect statement of weight or measure. Extravagant and untrue claims as to nutritive value have hitherto constituted a frequent form of misbranding.

A Typical System of Food Inspection.—The efficiency of a system of public food inspection is greatly enhanced if the business part of the work, including the bookkeeping and attending to the outside public, be done wholly through some person other than the analyst, as, for example, a health officer, to whom the collectors of samples and the analyst may report independently as to the results of their work, and whose duty it is to determine what shall be done in cases of adulteration. In this way the analyst knows nothing of the data of collection nor the name of the person from whom the sample was purchased, so that he can truthfully state in court that his analysis was unbiased.

Suppose, for example, that three collectors are employed to purchase samples of food for analysis, their duties being to visit at irregular intervals different portions of a state or municipality. Each collector keeps a book in which he enters all data as to the collection of the sample, includ-
Fig. 1.—Inspectors' Lockers. Insuring safe legal delivery of samples collected by tart inspectors. Each locker has a door in the rear accessible, from an anteroom, to the inspector holding key to that locker only.
Fig. 2.—Inspectors' Lockers. Front View. The lockers are accessible to the analyst in the laboratory by a single sliding-sash front, provided with a spring lock. The removable sliding-racks are convenient for returning clean sample bottles.
ing the name of the vendor, assigning a number to each sample, which
number is the only distinguishing mark for the analyst. One collector
may use for this purpose the odd numbers in succession from 1 to 9999,
the second the even numbers from 2 to 10,000, while the third may use
the numbers from 10,000 up. Each of the two former would begin with a
lettered series, as, for instance, A, numbering his samples 1A, 3A, 5A, 7A,
etc., or 2A, 4A, 6A, etc., till he reached 10,000, then beginning on series
B and so on. If the analyst is to be kept in ignorance of the brand or
manufacturer in the case of package goods, the collector must remove
from the original package sufficient of the sample for the needs of the
analyst, and deliver it to the latter in a plain package, bearing simply the
name under which the article was sold and the number. Such precau-
tions are, however, not always practicable and depend largely on local
regulations. The analyst reports the result of the analysis of each sample
with the number thereof on a library card, with appropriate blanks both
for data of analysis and for data of collection, the latter to be filled by
the collector from his book after the analyst has handed in the card with
the data of analysis. This system of recording and reporting analyses
has been successfully used for years by the Department of Food and
Drug Inspection of the Massachusetts State Board of Health.

Legal Precautions.—The laboratory of the public analyst should
preferably be provided with a locker for each collector, to which access
may be had only by that collector and the analyst, so that in the absence
of the latter, or when circumstances are such that the samples cannot be
delivered to him personally, there may be such safeguards with respect
to lock and key as to leave no question in the courts as to safe delivery
and freedom from accidental tampering. With such a system it is un-
necessary for the collector to place under seal the various samples sub-
mitted for analysis. Unless such lockers or their equivalent are employed,
it is best to carefully seal all samples.

Such a system of lockers for use with three collectors is shown in
Figs. 1 and 2. The same careful attention should afterwards be given to
keep the specimens in a secure place both before and during the process
of analysis, and to label with care all precipitates, filtrates, and solutions
having to do with the samples, especially when several processes are
being simultaneously conducted, in order that there may be no doubt
whatever as to their identity. The importance of precautions of this
kind in connection with court work can hardly be too strongly emphasized.
**Practical Enforcement of the Food Law.**—In the case of foods actually found adulterated, there are three practical methods of suppressing their further sale, viz., by publication, by notification, and by prosecution. These may be separately employed or used in connection with each other, according to the powers conferred by law on the commission, board, or official having in charge the enforcement of the law, and according to the discretion of such official.

**Publication.**—Under the laws of some states, the only means of protecting the people lies in publishing lists of adulterated foods with their brands and manufacturers' names and addresses in periodical bulletins or reports. Sometimes it is considered best to publish for the information of the public lists of unadulterated brands as well, and, again, it is held that only the offenders should thus be advertised.

Such publication, by keeping the trade informed of the blacklisted brands and manufacturers, certainly has a decidedly beneficial effect in reducing adulteration, and involves less trouble and expense than any other method. It is obviously an advantage, however, in addition to this to be able in certain extreme cases to use more stringent methods when necessary.

**Notification and Prosecution.**—The adulteration of food is best held in check in localities where under the law cases may be brought in court and are occasionally so brought. The mere power to prosecute is in itself a safeguard, even though that power is not frequently exercised. Under a conservative enforcement of the law, actual prosecution should be made as a last resort. Neither the number of court cases brought by a food commission nor the large ratio of court cases to samples found adulterated are criteria of its good work. Except in extreme cases, it is frequently found far more effective to notify a violator of the law, especially if it is a first offense, giving warning that subsequent infraction will be followed by prosecution. Such a notification frequently serves to stop all further trouble at once and with the minimum of expense. Instances are frequent in Massachusetts where, by such simple notification, widely distributed brands of adulterated foods have been immediately withdrawn from sale.

Massachusetts was the first of all the states to enact pure-food legislation, and since the year 1883 has had a well-established system of inspection, prosecuting cases under its laws through the Food and Drug Department of the State Board of Health. Cases are brought in court with practically no expense for legal services. Complaints are entered by
the collector, or, as he is termed, inspector, who makes complaint not in his official capacity, but as a citizen who under the law has been sold a food found to be adulterated, and who is entitled to conduct his own case, which he does with the aid of the analyst and such other witnesses as he may see fit to employ. Experience is readily acquired by the inspector in conducting such cases in the lower police or municipal courts, where they are first tried, and years ago the services of legal counsel in Massachusetts were dispensed with as superfluous. Where such a practice is in vogue an intelligent inspector must of course be chosen with reference to his ability to do this court work. The food laws are few and simple, as are also the court decisions rendered under them, so that it is no great task for the inspector to become much more familiar with them than the average general lawyer whom he meets in court and who not infrequently consults the inspector for information regarding these laws.

Statistics in the annual reports of the Massachusetts Board show with what uniform success these trials have been conducted. While more often settled in the lower courts, occasional appeal cases are carried to the superior courts, where the services of the regular district attorney are of course availed of in prosecuting the case.

Such a system as the above, while admirable for a state or city after long experience in the enforcement of food laws in the courts, is obviously impracticable with newly established systems of state food inspection.
CHAPTER II.

THE LABORATORY AND ITS EQUIPMENT.

Location.—The selection of a location for a food laboratory cannot always be made solely with reference to its needs and its convenience, but it is more often subject to economic conditions beyond the analyst’s control. Under very best conditions, such a laboratory should be situated in a building designed from the start exclusively for chemical or biological and chemical work. Almost any well-lighted rooms in such a building can be readily adapted for the purpose. When, however, as is frequently the case, rooms for such a laboratory are provided in municipal, government, or office buildings, in which for the most part clerical work is done, the problem of adequately utilizing such rooms so that they may not at the same time prove offensive to or interfere with the comfort of other occupants of the building is sometimes difficult. It is obvious that basement rooms in such a building, as far as ventilation is concerned, are less readily adapted for the requirements in hand than are those of the top floor, though, if the light is good and there are abundant and well-arranged ventilating-shafts, such rooms may be made to serve every purpose. In the basement one may most easily obtain water, gas, and steam, and dispose of wastes without annoyance to one’s neighbors. When, however, it is possible to do so, rooms on the top floor of an office building should be utilized for a food laboratory, for in such rooms the problems of lighting, heating and ventilating are comparatively simple and may usually be solved without regard to other occupants. In such a case ample provision must be made, preferably through shafts which are readily accessible for water-, gas-, steam-, and soil-pipes passing down below.

The actual equipment of the food laboratory depends of course largely on its particular purpose; and while it is manifestly impossible to do otherwise than leave the details to the individual taste and needs of the analyst,
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modified by the means at his disposal, a few general suggestions regarding important essentials may prove helpful. These imply a fairly liberal though not extravagant outlay, with a view to saving both time and energy by convenient surroundings well adapted to the work in hand.

Floor.—The best material for the floor of the working laboratory is asphalt. Such a floor is firm but elastic, is readily washed by direct application of running water, if necessary, and resists well the action of ordinary reagents. An occasional thin coating of shellac with lampblack applied with a brush gives the asphalt floor a smooth, hard surface and may be applied locally to cover spots and blemishes.

Lighting.—The ideal arrangement is with benches for analytical work running north and south, the principal light being from south windows, and with benches for microscopes, balances, colorimeters, and standard solutions along the north wall where the north windows admit a soft light and never direct sunshine.

FIXTURES.

Ventilation by forced draft is a great convenience. For this purpose an exhaust fan driven by an electric motor and controlled in speed by a fractional rheostat is admirable. Such a fan would best be located in a small closed compartment or closet near the centre of the series of rooms designed to be ventilated by it, and this closet should have directly over the fan an outlet-shaft passing through the roof of the building. With such a system, a series of branching air-ducts should radiate from the fan closet, conveniently arranged either above or along the ceiling and communicating with the various hoods, closets, and rooms near the top.

Benches.—The working benches should have wooden or glazed tile tops. White glazed tile, if properly laid, furnish a very clean, sanitary, and resistant surface, besides being often convenient for color tests. If laid on a plank surface, cement should not be applied directly, as it swells the wood before drying out and results in a loose and often uneven surface. Cement may be avoided altogether and the tiles after first soaking in oil may be laid in putty directly on the wood. Tiles may be laid in cement by first covering the plank surface with cheap tin plate, overlapping the edges and securing by tacks. This prevents swelling of the wood. The tin may be covered to advantage with cheap paint. The tiles may then be embedded in a layer of cement spread over the tin surface.

Soft encaustic glazed tiles commonly used for wall finish are not as
effective as hard floor tiles specially glazed, since the former crackle and lose color when subjected to heat. A suitable material for the top of the titration bench is opal plate glass with a polished surface. Jet-black plate glass with a honed surface is admirably suited for the microscope table.

When wooden bench tops are used they may be treated to advantage by staining with the following solutions:

*Solution 1.* 100 grams of anilin hydrochloride, 40 grams of ammonium chloride, 650 grams of water.

*Solution 2.* 100 grams of copper sulphate, 50 grams of potassium chlorate, 615 grams of water.

Apply solution 1 thoroughly to the bare wood and allow it to dry; then apply 2 and dry. Repeat these applications several times. Wash with plenty of hot soap solution, let dry and rub well with vaseline. It is claimed that wood so treated is rendered fire-proof and is not acted on by acids and alkalies. When the finish begins to wear, an application of hot soap solution or vaseline will bring back the deep black color.

Gas and water outlets, sinks and waste pipes should be conveniently arranged, while the space beneath the benches should be utilized for drawers and cupboards. A clear bench width of 24 inches is ample for most work; if wider there is a temptation to allow apparatus to accumulate at the back. At the back of the bench and within easy reach, a raised narrow shelf should be provided to be used exclusively for common desk reagents. This again should not be so wide as to allow the accumulation of useless bottles. A narrow raised guard or beading at the edge of the reagent shelf prevents the bottles from accidently slipping off.

**Hoods.**—Closed hoods with sliding sash fronts are almost indispensable. These hoods should be directly connected with the ventilating shafts or pipes, or with the air-ducts that radiate from the exhaust-fan closet, when such a system is provided. Gas outlets inside the hoods are necessary.

When there is a good draft, either natural or forced, a hooded top over the working bench, such as that shown in Fig. 3, is quite as efficient as a closed hood for most purposes. This is best made of galvanized iron, painted on the outside and treated on the inside with a preparation of graphite ground in oil. Here are best carried out all the processes involving the giving off of fumes and gases, which, if the ventilation is efficient, should pass directly up the flues and not come out in the room.
Sinks and Drains.—The sinks should preferably be of iron or porcelain. If iron, they should at frequent intervals be treated with a coat of asphalt varnish. A great convenience is a hooded sink (Fig. 4) in which foul-smelling bottles, or vessels giving off noxious or offensive fumes
or gases, may be rinsed under the tap while completely closed in. Open-work rubber mats at the bottom of the sinks help to insure against breakage. Open plumbing of simplest design should be used, and a multiplicity of traps should be avoided. Sinks may be variously located for convenience without regard to situation of soil-pipes, if the floor is thick enough to allow an open drain with sufficient pitch to flow readily. Such open drains are much more readily cleaned than closed pipes, and are best constructed by splitting a lead pipe and laying it in an iron box which is sunk into the floor. The edges of the lead pipe are rounded over those of the box as in Fig. 5, filling the joints with hydraulic cement, and the top of the drain is covered by a series of readily removable iron plates.
flush with the top of the floor. Waste-pipes from sinks, still-condensers, refrigerators, and various forms of apparatus involving flowing water may be led into this drain, holes being drilled in the iron cover for their insertion.

Gas, Electricity, and Steam.—While formerly gas, made either in public or private plants, was the sole dependence for laboratory work, to-day gas, electricity, and steam are often on tap in the same laboratory, for some processes one and for others another giving the best results. If only one can be had, gas is usually the cheapest and most satisfactory, but in many office buildings only electricity is available as it may be impracticable to pipe in gas from the city mains and against insurance regulations to make it on the premises from gasoline. Laboratories and

![Diagram of Open Drain-pipe in Floor.](image)

works remote from centers often have an abundance of home-generated electricity and steam, but no gas.

Fortunately electrical heaters for almost every kind of laboratory apparatus, such as furnaces, drying ovens, evaporators, thermostats, Kjeldahl digestors, and stills, are obtainable, although somewhat expensive. An electric current is also of great value in carrying out electrolytic methods and in running motors for driving centrifuges, shaking apparatus, ventilating fans, air pumps, etc. Whenever in an electrically equipped laboratory a free flame is indispensable, which is rarely the case, alcohol or blue flame kerosene oil burners are fairly satisfactory. Steam, when available, may be used to advantage for boiling ether or benzine in connection with continuous fat-extraction apparatus, for furnishing the motive power for driving the Babcock centrifuge, for heating water-baths and hot closets, and, in connection with cold water, to furnish a supply
of hot water when wanted at the sink. The latter application is illustrated in Fig. 4.

**Suction and Blast.**—If the water-pressure is ample, both air-pressure and exhaust for blast-lamps, vacuum filtration, and other purposes are readily available through the agency of the various devices used in connection with the flow of water, as, for instance, the Richards pump. When however, the water pressure is insufficient, other means must be employed for furnishing these much-needed requisites. Fig. 6 illustrates a simple

![Portable Pressure- and Exhaust-pump Run by Electric Motor. Useful for blast-lamps, vacuum filtration, etc.](image)

and almost noiseless pressure and exhaust pump run by a ½-H.P. electric motor, which with the pressure-equalizing tank and the appropriate connections are mounted on a light wheel truck, and readily movable to any part of the laboratory. By simply screwing the plug into an electric-light outlet, either suction or blast may be had at will, depending on the position of a knife-edge switch which determines the direction of the current. By means of a fractional rheostat the speed may be varied and the pressure thus controlled.

**APPARATUS.**

The laboratory is of course to be supplied with the usual assortment of test-tubes, flasks, beakers, evaporating and other dishes of porcelain, platinum and glass, funnels, casseroles, crucibles, mortars, burettes,
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pipettes, graduates, rubber and glass tubing, lamps, ring-stands and various supports, clamps and holders, the nature, number, and sizes of which are determined by individual requirements. Special forms of apparatus peculiar to certain processes of analysis or to the examination of special foods will be described in their appropriate connection. The following apparatus of a general nature may be regarded as indispensable for the proper fitting out of the food laboratory:

Balances.—These should include (1) an open pan balance for coarse weighing, having a capacity up to 1 kilogram and sensitive to 0.1 gram, with a set of weights; and (2) an analytical balance, enclosed in a case, sensitive to 0.001 gram under a load of 100 grams, with an accurate set of non-corrosive weights. The short-beam analytical balance is preferable for quick work, and as constructed by the best modern makers leaves nothing to be desired.

Water-baths.—These are such an important accessory to food analysis that they should, if possible, be specially designed to meet the requirements, though the ordinary copper baths, supported on legs and designed to be heated by gas-burners, as kept in regular stock by the dealers, will sometimes serve the purpose. For nearly all moisture determinations the platinum dishes described on page 110 and the somewhat larger wine-shells of 100 cc. capacity are most used, and for this purpose the top of the bath should have plenty of openings of the right size for these. A very economical construction of bath admirably adapted for the food analyst’s use is shown in Fig. 7, being the form employed by the writer.

The size and number of openings are determined by the number of samples to be simultaneously analyzed. A steam coil within the body of the bath serves to boil the water. Fig. 7 also shows the hood for carrying off the steam and fumes, the sliding front of which is furnished with a hasp and a padlock, so that it may always be kept locked by the analyst whenever he is temporarily absent from the laboratory. This is a useful precaution, when the residues left thereon are from samples which are to form subjects for possible prosecution in court later.

Steam, if available at all seasons of the year, or electric immersion coils furnish a ready means of heating the bath. In the absence of both steam and electricity, the bath must be boiled by gas burners.

Drying-oven.—Water ovens heated by gas and steam ovens are commonly used, although the drying cell seldom reaches a temperature above 98° C. The electric oven shown in Fig. 8 obviates this difficulty, the regulator permitting of adjustment so that full 100°, as well as any de-
sired temperature can be attained. Fig. 9 shows an asbestos-covered, jacketed air-oven, heated by a gas burner, with an efficient form of gas-pressure regulator.

**Water-still.**—An efficient still should be provided, capable of supplying the laboratory with an ample quantity of pure water for analytical purposes. Fig. 10 illustrates a compact form of still, which is particularly economical in view of the fact that a single stream of inflowing cold water first serves to cool the condenser, and, rising, becomes vaporized in the boiler directly connected with the condenser at the top. This apparatus is capable of distilling six gallons of water in twelve hours.

**Universal Centrifuge.**—This convenient apparatus merits a separate brief description, being useful for a wide variety of purposes, such as
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breaking up ether and other emulsions, quickly settling out precipitates, and roughly estimating chlorides, sulphates, phosphates, etc., by the volume of the precipitate in graduated tubes.

Fig. 8.—Freas Electrically Heated Drying Oven with Accurate Temperature Control.

Fig. 9.—Asbestos-covered Air-oven, with Gas-pressure Regulator.

The centrifuge (Fig. 11) is inclosed within a cast-iron case and is driven by an electric motor concealed at the base. The vertical spindle
is provided with interchangeable heads carrying various forms of swinging holders for tubes, bottles, beakers, and separatory funnels. Holders are obtainable for tubes ranging from 2 cc. to 200 cc. capacity, for Squibb's form of separatory funnel of 150 cc. capacity, and for graduated bottles such as are used in determining fat by the Babcock method and in meas-

Fig. 10.—A Convenient Laboratory Water-still with Earthenware Receptacle, Provided with Faucet and Glass Gauge.

uring precipitates, as for example, in Hortvet's method of estimating the amount of lead precipitate formed in solutions of maple sugar or syrup.

The electrical machinery is entirely enclosed, thus obviating the danger of exploding mixtures of vaporized ether and air by sparking—a danger which always must be carefully guarded against in the food laboratory.

The various types of centrifuges designed for the Babcock test (page 124) may also be used for general work especially if fitted with interchangeable heads carrying different forms of holders.
Other Special Apparatus.—The following list includes pieces which are more or less indispensable:

Continuous Extraction Apparatus (Figs. 20, 21, and 22).
Apparatus for Nitrogen Determination (Figs. 26, 27a, and 27b).
Apparatus for Distilling Various Food Products.

![A Universal Electric Centrifuge.](image)

A Babcock or other Milk fat Centrifuge (Figs. 11 and 45).
A Buléro Refractometer (Fig. 38).
An Immersion Refractometer (Fig. 42).
A Microscope and its Appurtenances (Chapter V).
A Polariscope and its Accessories (Figs. 102, 103, and 104).
Specific Gravity Apparatus (Figs. 14, 15, 16, and 17).
Carbon Dioxide Apparatus (Fig. 71).
Melting-point Apparatus (Fig. 93).

Freezing-point Apparatus.

Electrical Conductivity and Hydrogen Ion Concentration Apparatus (Chapter XXII).

Marsh Arsenic Apparatus (Fig. 28).
Electrolytic Apparatus (Fig. 110).
Separatory Funnels and Stand (Figs. 24 and 25).

A Spectroscope, either of the direct-vision variety for the pocket, or the Kirchoff & Bunsen style on a stand.

Spectroscope Cells, parallel-sided, for observation of absorption spectra.
A Photomicrographic Camera and Appurtenances * (pp. 80. to 85).
A Muffle Furnace, gas (Fig. 3), or, preferably, electric (Fig. 19).
An Ebulloscope (Fig. 113).
An Assay Balance, for weighing arsenic mirrors to 0.01 mg.
An Abbé Refractometer (Fig. 39).
A Schreiner Colorimeter (Fig. 30).
A Lovibond Tintometer (p. 67).

REAGENTS.

Under the appropriate methods are described the reagents for carrying out the processes treated of in this volume, together with their strength, mode of preparation when necessary, and other data. Reagents, especially those constantly employed, should be assigned to regular places on the shelves, and should invariably be kept in place when not in use.

Among the standard solutions for volumetric work, none is more frequently of service in the food laboratory than a tenth-normal solution of sodium hydroxide, and a large supply of this reagent, carefully standardized, should be at all times conveniently at hand. Besides being useful for standardizing tenth-normal solutions, it is constantly needed for determining various acids in food products, such as milk, vinegar, butter, lime juice, cream of tartar, liquors, and many others. Time is well spent in carefully adjusting this solution to its exact tenth-normal value, thus simplifying the calculation of results. A large stock bottle (say of two gallons capacity) containing the standard tenth-normal sodium hydroxide, is conveniently mounted with a side-tube burette in connection, in some such manner as shown in Fig. 12. A small connecting side bottle contains a strong solution of sodium hydroxide through which the air that enters the large bottle is passed, thus depriving it of CO₂. In this manner the standard solution may readily be kept of unvarying strength for a year or more.

* A photographic dark room is also necessary if photomicrographic work is to be done.
EQUIVALENTS OF STANDARD SOLUTIONS.

DECINORMAL SULPHURIC ACID. One cc. is equivalent to

<table>
<thead>
<tr>
<th>Substance</th>
<th>Formula</th>
<th>Equivalent (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia gas</td>
<td>NH₃</td>
<td>0.0017 gram</td>
</tr>
<tr>
<td>Ammonia</td>
<td>NH₃OH</td>
<td>0.0035</td>
</tr>
<tr>
<td>Ammonium carbonate</td>
<td>(NH₄)₂CO₃</td>
<td>0.0046</td>
</tr>
<tr>
<td></td>
<td>(NH₄)₂CO₃·H₂O</td>
<td>0.0057</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>CaCO₃</td>
<td>0.0050</td>
</tr>
<tr>
<td>Calcium hydroxide</td>
<td>Ca(OH)₂</td>
<td>0.0037</td>
</tr>
<tr>
<td></td>
<td>Ca(Oh)</td>
<td>0.0038</td>
</tr>
<tr>
<td>Lead acetate</td>
<td>Pb(C₂H₃O₂)₃·3H₂O</td>
<td>0.0189</td>
</tr>
<tr>
<td>Magnesium</td>
<td>MgO</td>
<td>0.0020</td>
</tr>
<tr>
<td>Magnesium carbonate</td>
<td>Mg₂(PO₄)₃</td>
<td>0.0042</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>N₄</td>
<td>0.0014</td>
</tr>
<tr>
<td>Potassium acetate</td>
<td>KC₄H₄O₇</td>
<td>0.0098</td>
</tr>
<tr>
<td></td>
<td>bicarbonate</td>
<td>0.0100</td>
</tr>
<tr>
<td></td>
<td>KH₂CO₃</td>
<td>0.0188</td>
</tr>
<tr>
<td></td>
<td>carbonate</td>
<td>0.0069</td>
</tr>
<tr>
<td></td>
<td>citrate</td>
<td>0.0166</td>
</tr>
<tr>
<td></td>
<td>hydroxide</td>
<td>0.0056</td>
</tr>
<tr>
<td></td>
<td>and sodium tartrate</td>
<td>0.0141</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>NaC₂H₃O₇·3H₂O</td>
<td>0.0136</td>
</tr>
<tr>
<td></td>
<td>benzoate</td>
<td>0.0144</td>
</tr>
<tr>
<td></td>
<td>bicarbonate</td>
<td>0.0084</td>
</tr>
<tr>
<td></td>
<td>borate</td>
<td>0.0191</td>
</tr>
<tr>
<td></td>
<td>carbonate</td>
<td>0.0053</td>
</tr>
<tr>
<td></td>
<td>hydroxide</td>
<td>0.0040</td>
</tr>
<tr>
<td></td>
<td>nitrate</td>
<td>0.0160</td>
</tr>
</tbody>
</table>

DECINORMAL SODIUM HYDROXIDE SOLUTION. One cc. is equivalent to

<table>
<thead>
<tr>
<th>Substance</th>
<th>Formula</th>
<th>Equivalent (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid, acetic</td>
<td>H₃C₂H₂O₂</td>
<td>0.0060 gram</td>
</tr>
<tr>
<td></td>
<td>boric</td>
<td>0.0062</td>
</tr>
<tr>
<td></td>
<td>citric</td>
<td>0.0070</td>
</tr>
<tr>
<td></td>
<td>hydrobromic</td>
<td>0.0081</td>
</tr>
<tr>
<td></td>
<td>hydrochloric</td>
<td>0.00365</td>
</tr>
<tr>
<td></td>
<td>hydrobolic</td>
<td>0.0128</td>
</tr>
<tr>
<td></td>
<td>lactic</td>
<td>0.0090</td>
</tr>
<tr>
<td></td>
<td>malic</td>
<td>0.0067</td>
</tr>
<tr>
<td></td>
<td>nitric</td>
<td>0.0063</td>
</tr>
<tr>
<td></td>
<td>oxalic</td>
<td>0.0063</td>
</tr>
<tr>
<td></td>
<td>phosphoric</td>
<td>0.0049</td>
</tr>
<tr>
<td></td>
<td>sulphuric</td>
<td>0.0098</td>
</tr>
<tr>
<td></td>
<td>tartaric</td>
<td>0.0075</td>
</tr>
<tr>
<td>Potassium bitartrate</td>
<td>KHC₄H₄O₇</td>
<td>0.0188</td>
</tr>
<tr>
<td>Sodium borate</td>
<td>Na₃C₂H₂O₇·10H₂O</td>
<td>0.00955</td>
</tr>
</tbody>
</table>

* To be ignited.
DECINORMAL IODINE SOLUTION. One cc. is equivalent to

- Arsenious oxide, \( \text{As}_2\text{O}_3 \) 0.00405 gram
- Potassium sulphite, \( \text{K}_2\text{SO}_3 \cdot 2\text{H}_2\text{O} \) 0.0007 "
- Sodium bisulphite, \( \text{Na}_2\text{H}_2\text{SO}_3 \) 0.0051 "
- Sulphite, \( \text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O} \) 0.0126 "
- Thiourea, \( \text{Na}_2\text{S}_2\text{O}_3 \cdot 2\text{H}_2\text{O} \) 0.0248 "
- Sulphur dioxide, \( \text{SO}_2 \) 0.0032 "
- Sulphurous acid, \( \text{H}_2\text{SO}_3 \) 0.0011 "

DECINORMAL SODIUM THIOSULPHATE SOLUTION. One cc. is equivalent to

- Bromine, \( \text{Br} \) 0.0080 gram
- Chlorine, \( \text{Cl} \) 0.00355 "
- Iodine, \( \text{I} \) 0.01260 "
- Iron (in ferric salts), \( \text{Fe} \) 0.0056 "

DECINORMAL SILVER NITRATE SOLUTION.* One cc. is equivalent to

- Ammonium bromide, \( \text{NH}_4\text{Br} \) 0.0098 gram
- Chloride, \( \text{NH}_4\text{Cl} \) 0.00535 "
- Chlorine, \( \text{Cl} \) 0.00355 "
- Cyansgen, \( (\text{CN})_2 \) 0.0052 "
- Hydrocyanic acid, \( \text{HCN} \) with indicator 0.0027 "
- " " " " \( \text{HCN} \) to formation of precipitate 0.0054 "
- Hydrobromic acid, \( \text{HBr} \) 0.0080 "
- Potassium bromide, \( \text{KBr} \) 0.0119 "
- Chloride, \( \text{KCl} \) 0.00745 "
- cyanide, \( \text{KCN} \) with indicator 0.0065 "
- " " " " \( \text{KCN} \) to formation of precipitate 0.0130 "
- Sodium bromide, \( \text{NaBr} \) 0.0103 "
- chloride, \( \text{NaCl} \) 0.0035 "

DECINORMAL POTASSIUM BICHROMATE SOLUTION. One cc. is equivalent to

- Ferric carbonate, \( \text{Fe}_2\text{CO}_3 \) 0.0130 gram
- Ferric oxide, \( \text{Fe}_2\text{O}_3 \) 0.0080 "
- Ferrous oxide, \( \text{Fe}_2\text{O}_3 \) 0.01075 "
- sulphate, \( \text{Fe}_2\text{SO}_4 \) 0.0152 "
- " " \( \text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O} \) 0.0275 "
- Iron (ferric) \( \text{Fe} \) 0.0056 "

DECINORMAL POTASSIUM PERMANGANATE SOLUTION. One cc. is equivalent to

- Oxalic acid, \( \text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O} \) 0.0063 gram

---

* Use potassium chromate solution as an indicator, or add till precipitate appears.

† Use a freshly prepared solution of potassium ferricyanide as an indicator, applying a drop of titrated solution to a drop of indicator on a white surface.
The following table from Talbot* shows the reactions of the common indicators used in acidimetry:

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Reaction with Acids</th>
<th>Reaction with Alkalies</th>
<th>Use with Carbonic Acid in Cold Solution</th>
<th>Use with Carbonic Acid in Hot Solution</th>
<th>Use with Ammonium Salt</th>
<th>Use with Organic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmus</td>
<td>Red</td>
<td>Blue</td>
<td>Reliable</td>
<td>Reliable</td>
<td>Reliable</td>
<td>Reliable</td>
</tr>
<tr>
<td>Methyl orange</td>
<td>Pink</td>
<td>Yellow</td>
<td>Unreliable</td>
<td>Unreliable</td>
<td>Unreliable</td>
<td>Unreliable</td>
</tr>
<tr>
<td>Phenolphthalein</td>
<td>Colorless</td>
<td>Pink</td>
<td>Unreliable</td>
<td>Reliable</td>
<td>Reliable</td>
<td>Unreliable</td>
</tr>
<tr>
<td>Lacmoid</td>
<td>Purple-red</td>
<td>Blue</td>
<td>Reliable</td>
<td>Reliable</td>
<td>Reliable</td>
<td>Reliable</td>
</tr>
<tr>
<td>Cochineal</td>
<td>Purple-red</td>
<td>Blue</td>
<td>Reliable</td>
<td>Reliable</td>
<td>Reliable</td>
<td>Reliable</td>
</tr>
<tr>
<td>Rosolic acid</td>
<td>Yellow</td>
<td>Pink</td>
<td>Unreliable</td>
<td>Reliable</td>
<td>Reliable</td>
<td>Unreliable (?)</td>
</tr>
<tr>
<td>Alizarine</td>
<td>Yellow</td>
<td>Red</td>
<td>Unreliable</td>
<td>Reliable</td>
<td>Reliable†</td>
<td>Reliable</td>
</tr>
</tbody>
</table>

* Talbot, *Quantitative Analysis*, page 75
† Reliable with oxalic acid
CHAPTER III.

FOOD, ITS FUNCTIONS, PROXIMATE COMPONENTS, AND NUTRITIVE VALUE.

Nature and General Composition.—Food is that which, when eaten, serves by digestion and absorption to support the functions and powers of the body, by building up the material necessary for its growth and by supplying its wastes. The raw materials that constitute our food-supply are not all available for nourishment, but often contain a proportion of inedible or refuse matter, which it is customary to remove before eating, such as the bones of fish and meat, the shells of clams and oysters, eggshells, the bran of cereals, and the skins, stones, and seeds of fruits and vegetables. The proximate components which make up the edible portion of food include in general water, fat, various nitrogenous bodies consisting chiefly of proteins, carbohydrates, organic acids, and mineral matter. Of these water is hardly to be considered as a nutrient, though it plays an important part in nearly all foods as a diluent and solvent. The fats, proteins, and carbohydrates all contribute in varying degree to the supply of fuel for the production of heat and energy. Besides this universal function, the fats and the carbohydrates serve especially to furnish fatty tissue in the body, while the proteins are the chief source of muscular tissue.

Liebig's classification of foods into nitrogenous, or flesh forthers, and non-nitrogenous, or heat generators, is now no longer accepted as strictly logical, in view of the well-known fact that the nitrogenous materials, besides building up the body, aid in supplying the wastes and yielding energy, and may even be converted into fats or carbohydrates, while the non-nitrogenous aid in furnishing tissue growth in addition to serving as fuel.

The Fat of Food.—Fats and oils consist essentially of the glycerides of the fatty acids, the characteristics of the various edible fats and
oils being treated under their appropriate headings elsewhere. Fat in
human food is supplied by milk and its products, by the adipose tissue of
meat, and in slight extent by the oil of cereals and by the edible table
oils. The term “ether extract” is sometimes used in stating the results
of the analysis of foods and this includes other substances than fat which
when present are extracted by ether, such as chlorophyl and other color-
ing matters, lecithin, alkaloids, etc.

The glycerides occurring in foods are of acids belonging in four series
as follows, the value for n being in parentheses:

A. ACETIC SERIES \( C_nH_{2n}O_2 \).—Butyric (4), caproic (6), caprylic
(8), capric (10), lauric (12), myristic (14), palmitic (16), stearic (18),
arachidic (20), behenic (22), and lignoceric (24).

B. OLEIC SERIES \( C_nH_{2n-1}O_2 \).—Hyppogei: (16), oleic (18), isoleic
(18), rapic (18), and crucic (22).

C. LINOLIC SERIES \( C_nH_{2n-6}O_2 \).—Linolic (18).

D. LINOLENIC SERIES \( C_nH_{2n-3}O_2 \).—Linolenic (18).

E. CLUPANODONIC SERIES \( C_nH_{2n-3}O_2 \).—Clupanodonic (18).

Fats contain not only simple glycerides, consisting of glycerol com-
bined with three equivalents of the same fatty acid, but mixed glycerides
with two or three acids in the same molecule. Other substances present
are free fatty acids, lecithin, cholesterol, phytosterol, stigosterol, coloring
matter, and other matters in minute amount.

NITROGENOUS COMPOUNDS AND THEIR CLASSIFICATION.—These
substances may for convenience be grouped as follows:

A. Proteins, B. Amino-acids, Amides, Amines, etc., C. Alkaloids, D.

A. PROTEINS.—Occurrence.—Under the term proteins are included
numerous bodies consisting, according to our present knowledge, essen-
tially of combinations of \( \alpha \)-amino-acids and their derivatives. Proteins
in one form or another exist in nearly all natural foods both animal and
vegetable, but are supplied chiefly by the flesh of meat and fish, by milk,
cheese, and eggs, and in the vegetable kingdom by grain, seeds, nuts,
and vegetables, especially the legumes. The proportion of crude protein,
often designated merely as “protein,” is commonly estimated by multi-
plying by 6.25 the percentage of nitrogen found in the material analy-
yzed. This is done on the assumption that all of the nitrogen present
in the substance belongs to protein and that the protein contained 16
per cent of nitrogen, neither of which assumptions is usually true, al-
though for most purposes the results are sufficiently accurate. In certain
cases, as for example, wheat flour and milk, special factors (5.70 and 6.38 in the cases cited) are used. Methods depending on the separation of the proteins as such are used in special investigations, but these, with few exceptions, are not adapted for practical purposes.

There is no marked distinction in chemical constitution between animal and vegetable proteins, although some of the types have as yet been found only in one or the other kingdom. The terms "proteids" or "albuminoids" were formerly used generically as synonymous with "protein" to include all nitrogenous bodies of this group, but in 1908 a joint committee on protein nomenclature of the American Physiological Society and the American Society of Biological Chemists recommended that the word "proteid" be abandoned; that "protein" be used to designate the entire group; and that the word "albuminoid" be restricted to a sub-group of proteins. A committee of the Physiological Society of England also made the same recommendation as to the use of the term protein. The classification and most of the definitions here given are those adopted by the American committee.* The examples in most cases were kindly furnished by Dr. T. B. Osborne. For further details the reader is referred to the works of Mathews,† Osborne,‡ Plimmer,§ and Jones,|| also journal articles by Emil Fischer, Kossel, and their students.

I. THE SIMPLE PROTEINS.—Protein substances which yield only α-amino acids or their derivatives on hydrolysis.

Although no means are at present available whereby the chemical individuality of any protein can be established, a number of simple proteins have been isolated from animal and vegetable tissues which have been so well characterized by constancy of ultimate composition and uniformity of physical properties that they may be treated as chemical individuals until further knowledge makes it possible to characterize them more definitely.

(a) Albumins.—Simple proteins soluble in pure water and coagulable by heat.

Examples.—Serum albumin of blood and other animal fluids; lactalbumin of milk; leucosin of the seeds of wheat, rye, and barley; legumelin of leguminous seeds.

† Physiological Chemistry, New York, 1916.
‡ The Vegetable Proteins, London, 1912.
FOOD, ITS FUNCTIONS, PROXIMATE COMPONENTS, ETC.

oils being treated under their appropriate headings elsewhere. Fat in human food is supplied by milk and its products, by the adipose tissue of meat, and in slight extent by the oil of cereals and by the edible table oils. The term “ether extract” is sometimes used in stating the results of the analysis of foods and this includes other substances than fat which when present are extracted by ether, such as chlorophyll and other coloring matters, lecithin, alkaloids, etc.

The glycerides occurring in foods are of acids belonging in four series as follows, the value for n being in parentheses:

A. ACETIC SERIES (\(C_4H_{2n}O_2\)).—Butyric (4), caproic (6), caprylic (8), capric (10), lauric (12), myristic (14), palmitic (16), stearic (18), arachidic (20), behenic (22), and lignoceric (24).

B. OLEIC SERIES (\(C_nH_{2n-1}O_2\)).—Hypogei: (16), oleic (18), isoleic (18), rapic (18), and crucic (22).

C. LINOLIC SERIES (\(C_nH_{2n-4}O_2\)).—Linolic (18).

D. LINOLENIC SERIES (\(C_nH_{2n-5}O_2\)).—Linolenic (18).

E. CLUPANODIC SERIES (\(C_nH_{2n-6}O_2\)).—Clupanodonic (18).

Fats contain not only simple glycerides, consisting of glycerol combined with three equivalents of the same fatty acid, but mixed glycerides with two or three acids in the same molecule. Other substances present are free fatty acids, lecithin, cholesterol, phytosterol, sitosterol, coloring matter, and other matters in minute amount.

NITROGENOUS COMPOUNDS AND THEIR CLASSIFICATION.—These substances may for convenience be grouped as follows:


A. PROTEINS.—Occurrence.—Under the term proteins are included numerous bodies consisting, according to our present knowledge, essentially of combinations of \(\alpha\)-amino-acids and their derivatives. Proteins in one form or another exist in nearly all natural foods both animal and vegetable, but are supplied chiefly by the flesh of meat and fish, by milk, cheese, and eggs, and in the vegetable kingdom by grain, seeds, nuts, and vegetables, especially the legumes. The proportion of crude protein, often designated merely as “protein,” is commonly estimated by multiplying by 6.25 the percentage of nitrogen found in the material analyzed. This is done on the assumption that all of the nitrogen present in the substance belongs to protein and that the protein contained 16 per cent of nitrogen, neither of which assumptions is usually true, although for most purposes the results are sufficiently accurate. In certain
Gelatin in common with most proteins is precipitated from its solution by mercuric chloride, picric acid, and tannic acid. It is readily distinguished from soluble proteins, in that it is not precipitated from its solution by lead acetate, nor by most of the metallic salts that throw down proteins.

(f) **Histones.**—Soluble in water and insoluble in very dilute ammonia, and, in the absence of ammonium salts, insoluble even in an excess of ammonia; yield precipitates with solutions of other portiens, and a coagulum on heating, which is easily soluble in very dilute acids. On hydrolysis they yield a large number of amino-acids, among which the basic ones predominate.

**Examples.**—Thymus histone. Not found in plants.

(g) **Protamins.**—Simpler polypeptides than the proteins included in the preceding groups. They are soluble in water, uncoagulable by heat, have the property of precipitating aqueous solutions of other proteins, possess strong basic properties, and form stable salts with strong mineral acids. They yield comparatively few amino-acids, among which the basic amino-acids greatly predominate.

**Examples.**—Salmin, clupein, and other protamins of fish spermatozoa. Not found in plants.

II. **Conjugated Proteins.**—Substances which contain the protein molecule united to some other molecule or molecules otherwise than as a salt.

(a) **Nucleoproteins.**—Compounds of one or more protein molecules with nucleic acid.

**Examples.**—The nucleins salmin nuclease and clupein nuclease.

(b) **Glycoproteins.**—Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid.

**Examples.**—Mucins; ovomucoid; ovalbumin; ichthulin.

(c) **Phosphoproteins.**—Compounds of the protein molecule with some yet undefined phosphorus-containing substance other than a nucleic acid or lecithins.

**Examples.**—Casein of milk; vitellin of egg yolk.

(d) **Haemoglobin.**—Compounds of the protein molecule with haematin or some similar substance.

**Example.**—Oxyhaemoglobin of red blood corpuscles.

(e) **Lecithoproteins.**—Compounds of the protein molecule with lecithins, (lecithans, phosphatides).

**Examples.**—Lecithalbumin; lecithin-nucleovitellin.
III. Derived Proteins.

1. Primary Protein Derivatives.—Derivatives of the protein molecule, apparently formed through hydrolytic changes which involve only slight alterations of the molecule.

(a) Proteins.—Insoluble products which apparently result from the incipient action of water, very dilute acids or enzymes.

Examples.—Edestan; blood fibrin; insoluble myosin.

(b) Metaproteins.—Products of the further action of acids or alkalis, whereby the molecule is so far altered as to form products soluble in very weak acids and alkalis, but insoluble in neutral fluids.

Examples.—Acid albumin; alkali albumin.

This group will thus include the familiar "acid proteins" and "alkali proteins," not the salts of proteins with acids.

(c) Coagulated Proteins.—Insoluble products which result from (1) the action of heat on their solutions, or (2) the action of alcohol on the protein.

Examples.—Albumin coagulated by heat or alcohol.

2. Secondary Protein Derivatives. Products of the further hydrolytic cleavage of the protein molecule.

(a) Proteoses.—Soluble in water, uncoagulated by heat, and precipitated by saturating their solutions with ammonium or zinc sulphate.

As thus defined this term does not strictly cover all the protein derivatives commonly called proteoses, e.g. heteroproteose and dysproteose.

Subdivision of the Proteoses.—According to the proteins from which they are derived the proteoses may be designated albumose, from albumin, globuloç, from globulin, vitelloç, from vitellin, casose, from casein, etc.

Proteoses are subdivided into proto-proteose, soluble in water (both cold and hot) or in dilute salt solutions, but precipitated by saturation with salt; hetero-proteose, insoluble in water, and deuto-proteose, soluble in water, but not precipitated by saturation with salt.

Vegetable proteoses are sometimes called phyt-albumoses.

Qualitative Tests.—Besides responding to the biuret reaction (p. 34) proteoses are precipitated by nitric acid, the precipitate being soluble on heating, but reappearing on cooling.

Proto-proteose is precipitated from its solution by mercuric chloride and copper sulphate; hetero-proteose is precipitated by mercuric chloride, but not by copper sulphate.

(b) Peptones.—Soluble in water, uncoagulated by heat, and not precipitated by saturating their solutions with ammonium sulphate.
Qualitative Tests.—Besides giving the biuret reaction, peptones are precipitated from their solution by tannic acid, picric acid, phosphomolybdic acid, and by sodium phosphotungstate acidified by acetic, phosphoric, or sulphuric acid.

Peptones are the only soluble proteins not precipitated by saturation with ammonium sulphate.

(c) Peptides.—Definitely characterized combinations of two or more amino-acids, the carboxyl group of one being united with the amino group of the other, with the elimination of a molecule of water.

The peptones are undoubtedly peptides or mixtures of peptides, the latter term being at present used to designate those of definite structure.

Qualitative Tests for Proteins.—Xanthoproteic Reaction.—Concentrated nitric acid containing nitrous acid formed during standing added to a solution of a protein may or may not form a precipitate. It, however, produces a yellow coloration on boiling. Addition of ammonia in excess turns the precipitate or liquid yellow or orange; proteins in suspension also react.

Millon’s Reaction.—Millon’s reagent is prepared by dissolving metallic mercury in twice its weight of concentrated nitric acid, diluting with an equal volume of water, and allowing to settle. When added to a protein solution it produces a white precipitate, which becomes brick-red on heating. Solid proteins give the red color by direct treatment. Sodium chloride prevents the reaction. Various organic substances are precipitated by Millon’s reagent, but these precipitates do not turn red on heating.

Biuret Reaction.—If a solution of a protein in dilute sulphuric acid be made alkaline with potassium or sodium hydroxide and very dilute copper sulphate be added, a reddish to violet coloration is produced, similar to that formed if biuret be treated in the same way, hence the name. An excess of copper sulphate should be avoided lest its color obscure that of the reaction.

In solutions which are strongly colored this reaction is of little use unless modified as follows: A considerable quantity of the dilute copper sulphate solution is added to the solution made alkaline with a large excess of potassium hydroxide, and then solid potassium hydroxide is dissolved to almost complete saturation in the solution. The mixture is then shaken with about one half its volume of strong alcohol. On standing the alcohol separates as a clear layer or a violet or crimson color if proteins are present.

B. AMINO-ACIDS, AMIDES, AMINES, AND ALLIED PRODUCTS.—Under this head are included products derived from acids or bases, the radicles
of which replace one or more hydrogen atoms in ammonia. The most common bodies of this class occurring in foods follow:

I. AMINO-ACIDS—The following are obtained by the hydrolysis of the different proteins: 1. glycocoll; 2. alanine; 3. valine; 4. leucine; 5. glyc-leucine, 6. iso-leucine, 7. serine, 8. cysteine, 9. aspartic acid, 10. glutamic acid, 11. arginine, 12. lysine, 13. cystine, 14. tyrosine, 15. phenylalanine, 16. proline, 17. oxy-proline, 18. histidine, 19. tryptophane. Of these, 1 to 13 inclusive belong to the aliphatic series, 14 and 15 to the carboxyclic series, and 16 to 19 inclusive to the heterocyclic series.

II. AMIDES.—Asparagin occurs in the young shoots of asparagus, lettuce and other green vegetables, and marshmallow root. Glutamine occurs in seeds during sprouting.

III. AMINES.—Choline is found in meat, egg yolk, and certain fungi. Betaine is a constituent of beets, hops, and certain mollusks. Carnitine occurs in meat extract.

IV. CREATINE AND CREATININE.—These are constituents of meat extracts.

V. PURINE BASES.—In the vegetable kingdom these are represented by the caffeine of tea, coffee, and cocoa, and the theobromine of cocoa, in the animal kingdom by xanthine, hypoxanthine, guanine, and adenine of meat and meat extracts. They are also classified with the alkaloids.

C. ALKALOIDS.—This group is characteristic of certain drugs; in foods they are of infrequent occurrence. Aside from the purine bases caffeine and theobromine, the piperine and piperidine of pepper are the only common examples.

D. NITRATES.—These occur mostly in growing parts of the plant and only in traces.

E. AMMONIA.—This occurs in ripened cheese of certain varieties and meat that is undergoing decomposition.

F. LECITHIN.—This is a phosphorized fat occurring in egg yolk and other animal and vegetable substances.

G. CYAN COMPOUNDS.—The bitter cassava contains hydrocyanic acid. Cyanides and sulphocyanides (thiocyanates) are found in small amounts, in various foods. Common examples of sulphocyanides are the pungent principles of mustard and horse radish. Amygdalin of bitter almonds is a glucoside containing the cyan group.

CARBOHYDRATES AND THEIR CLASSIFICATION.—Of the total number of carbohydrates which have been described only a limited number occur in food products and of these a considerable number do not exist in the
original vegetable or animal substance, but are formed during manufacture.

A classification of the common food carbohydrates is given below. Descriptions of the more important individuals appear in chapters X and XIV. Other details will be found in the works of Armstrong,* and Browne,† as well as in special papers by Emil Fischer, Tollens, and their students.

I. MONOSACCHARIDES.—These, also known as simple carbohydrates, are either aldehyde alcohols (aldoses) or ketone alcohols (ketoses) with usually one carbonyl and one or more alcohol groups. One of the hydrogens of the end group CH₂OH may be replaced by an alkyl group, usually methyl. The formulae of the l-forms are mirror images of the d-forms.

(a) Dioxes.—No representative of this group occurs in foods, but an example is here given to illustrate the simplest form of monosaccharide.

Example.—Glycolose \((\text{CH}_3\text{OH} \cdot \text{CHO})\), prepared synthetically.

(b) Methyl Dioxes.—Example.—Dimethylglycolose

\[(\text{CH}_3 \cdot \text{CHOH} \cdot \text{CO} \cdot \text{CH}_3)\],

occurs in vinegar and other fermented products.

c) Trioses.—Example.—Dioxacetone \((\text{CH}_3\text{OH} \cdot \text{CO} \cdot \text{CH}_2\text{OH})\), a ketose, is formed in various fermentation processes.

d) Tetroses.—No example in food products.

e) Methyl Tetroses.—Example.—Apiose

\[(\text{CH}_3\text{OH} \cdot \text{HOCH(\text{CH}_3\text{OH})} \cdot \text{CHOH} \cdot \text{CHO})\],

a constituent of the glucoside apiin of parsley.

(f) Pentoses \((\text{C}_5\text{H}_10\text{O}_5)\).—These sugars occur seldom and in only small amounts in foods, but are prepared from the corresponding pentoses by hydrolysis.

Aldoses.—Examples.—d-Arabinose

\[(\text{CH}_3\text{OH} \cdot (\text{HOCH})_2 \cdot \text{HCOCH} \cdot \text{CHO})\];

l-arabinose \((\text{CH}_2\text{OH} \cdot (\text{HOCH})_2 \cdot \text{HOCH} \cdot \text{CHO})\), a constituent of certain glucosides; l-xylose \((\text{CH}_2\text{OH} \cdot \text{HOCH} \cdot \text{HOCH} \cdot \text{HOCH} \cdot \text{CHO})\); d-ribose \((\text{CH}_2\text{OH} \cdot (\text{HOCH})_2 \cdot \text{CHO})\), a constituent of various nucleic acids.

Ketoses.—Little studied.

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* Simple Carbohydrates, London, 1912.
† Handbook of Sugar Analysis, New York, 1912.
(g) **Methyl Pentoses.** — *Examples.* — Rhamnose
\[(CH_2\_CHOH\_HCOH\_\(\text{HOCH}_2\_\text{CHO}\),
occurs in various glucosides; fucose
\[(CH_2\_\text{CHOH}\_\(\text{HOCH}_2\_\text{HCOH}\_\text{CHO}\),
derived by hydrolysis of fucosan.

(h) **Hexoses** \(\text{C}_6\text{H}_{12}\text{O}_6\).

**Aldoses.** — *Examples.* — d-glucose or dextrose
\[(\text{CH}_4\text{OH}\_\(\text{HOCH}_2\_\text{HCOH}\_\text{HIOCH}\_\text{CHO}\),
abundant in nature, forming with d-fructose invert sugar, occurs in numerous glucosides, formed by hydrolysis of starch, and is one of the chief constituents of commercial glucose; d-mannose
\[(\text{CH}_4\text{OH}\_\(\text{HOCH}_2\_\text{HCOH}\_\text{CHO}\),
found in plant juices, germinating seeds, and molasses; d-galactose
\[(\text{CH}_4\text{OH}\_\text{HOCH}\_\(\text{HICOH}_2\_\text{HOCH}\_\text{CHO}\), a constituent of certain glucosides, occurs free in whey and germinating seeds; l-galactose
\[(\text{CH}_4\text{OH}\_\text{HICOH}\_\(\text{HOCH}_2\_\text{HICOH}\_\text{CHO}\), d, l-galactose or racemic galactose, identified in certain oriental food products.

**Ketoses.** — *Examples.* — d-Fructose or levulose
\[(\text{CH}_4\text{OH}\_\(\text{HOCH}_2\_\text{HCOH}\_\text{CO}\_\text{CH}_2\text{OH}\),
occurs with d-glucose in invert sugar; d-sorbose
\[(\text{CH}_6\text{OH}\_\text{HCOH}_2\_\text{HOCH}\_\text{HCOH}\_\text{CO}\_\text{CH}_2\text{OH}\),
formed by fermentation of the juice of the sorb apple; glutose, found in molasses.

**II. DISACCHARIDES.** — These yield on hydrolysis two monosaccharides. Their constitutional formulas have not been fully decided on.

*Examples.* — Sucrose or common sugar \((\text{C}_12\text{H}_{22}\text{O}_{11})\); maltose \((\text{C}_12\text{H}_{22}\text{O}_{11})\) formed by the action of diastase on starch; lactose or milk sugar \((\text{C}_12\text{H}_{22}\text{O}_{11}\_\text{H}_2\text{O})\); trehalose or mushroom sugar \((\text{C}_12\text{H}_{22}\text{O}_{11}\_2\text{H}_2\text{O})\) melibiose \((\text{C}_12\text{H}_{22}\text{O}_{11}\_\text{2H}_2\text{O})\), formed by action of yeast on raffinos. Of these, maltose, lactose, and melibiose are copper reducing.

**III. TRISACCHARIDES.** — These yield on partial hydrolysis a monosaccharide and a disaccharide.

*Example.* — Raffinose \((\text{C}_{18}\text{H}_{22}\text{O}_{16}\_\text{3H}_2\text{O})\), occurs in sugar beets, cotton seed, etc.

**IV. TETRASACCHARIDES.** — These yield on partial hydrolysis a monosaccharide and a trisaccharide.

*Example.* — Stachyose \((\text{C}_{24}\text{H}_{44}\text{O}_{21}\_\text{4H}_2\text{O})\), found in various roots and in ash manna.
V. POLYSACCHARIDES.—This group includes the pentosans \((\text{C}_5\text{H}_9\text{O}_4)_n\cdot\text{H}_2\text{O}\) and the hexosans \((\text{C}_6\text{H}_{10}\text{O}_5)_n\cdot\text{H}_2\text{O}\). The value of \(n\) is so large that the water may for practical purposes be ignored. For descriptions of the individual pentosans and hexosans see Chapter X.

(a) Pentosans.—Examples.—Araban; metaraban; xylan.

(b) Hexosans.—Examples.—Mannan; galactan; inulin; dextrin; starch; cellulose.

Closely allied to the carbohydrates, if not actually belonging to them, are inositol \((\text{C}_6\text{H}_{12}\text{O}_6)_n\), occurring in muscular tissue, and pectose, found in green fruits and vegetables.

THE ORGANIC ACIDS.—These acids are minor though important constituents of foods. From their conversion into carbonates within the body, they are useful in furnishing the proper degree of alkalinity to the blood and to the various other fluids, besides being of particular value as appetizers. They exist in foods both in the free state and as salts. Acetic acid is supplied by vinegar; lactic acid by milk, fresh meat, and beer; citric, malic, and tartaric acids by the fruits.

MINERAL OR INORGANIC MATERIALS.—These substances occur in food in the form of chlorides, phosphates, and sulphates of sodium, potassium, calcium, magnesium, and iron, and are furnished by common salt, as well as by nearly all animal and vegetable foods. The inorganic salts are necessary to supply material for the teeth and bones, besides having an important place in the blood and in the cellular structure of the entire body.

FUEL VALUE OF FOOD.—In order to express the capacity of foods for yielding heat or energy to the body, the term fuel value is commonly used. By the fuel value of a food material is meant the amount of heat expressed in calories equivalent to the energy which we assume the body could obtain from a given weight of that food material, if all of its nutrients were thoroughly digested, a calorie being the amount of heat required to raise a kilogram of water 1° C. This definition applies to what is known as the large calorie, which is one thousand times as large as the small calorie. Large calories are meant wherever the term occurs in this volume. The fuel value, or, as it is sometimes called, "heat of combustion," may be determined experimentally with a calorimeter, or may be calculated by means of factors based on the result of many experiments showing the mean values for protein, fats, and carbohydrates.

The Bomb Calorimeter.*—This apparatus in its most approved form,
Fig. 13, consists of a water-tight, cylindrical, platinum lined, steel bomb, adapted to hold in a capsule the substance whose heat is to be determined, and containing also oxygen under pressure. This bomb is immersed in water contained in a metal cylinder, which is in turn placed inside of concentric cylinders containing alternately air and water. The heat for igniting the substance is supplied by the electric current passing through wires to the interior of the bomb and acting upon a cleverly devised mechanism therein. The heat developed by the ignition is measured by the rise in temperature of the water surrounding the bomb, as indicated by a very delicate thermometer graduated to hundredths of a degree, certain corrections being made, as, for instance, for the heat absorbed by the metal of the apparatus. A mechanical stirrer serves to equalize the temperature of the water surrounding the bomb.

The Respiration Calorimeter is a combustion apparatus on a large scale, of which a living human being or animal confined in a tight chamber, is a part. The food is carefully weighed and analyzed and the oxygen is supplied in known amount from a cylinder to replace that consumed by oxidation in the lungs. The water and carbon dioxide exhaled are
absorbed in calcium chloride tubes and potash bulbs or their equivalents on a large scale while the excreta is collected, weighed, and analyzed. The heat produced is measured by delicate appliances. In the United States human calorimeters are maintained at the Carnegie Nutrition Laboratory, Boston, under the direction of F. G. Benedict and at The Department of Agriculture, Washington, under the direction of Langworthy. A calorimeter for farm animals is in operation at State College, Pennsylvania, by Armsby.

Calculation of Fuel Value.—The bomb calorimeter is beyond the reach of many laboratories while the respiration calorimeter can be maintained only in specially equipped institutions, hence the expression of fuel values by calculation is the most common method employed. For this the factors of Rubner are generally used, in accordance with which the amount of energy in one gram of each of the three principal classes of nutrients are, for carbohydrates 4.1, for protein 4.1, and for fats 9.3. Expressed in pounds, each pound of carbohydrate or protein has a fuel value of 1860 calories, while each pound of fat has a fuel value of 4220 calories.

For further details on the caloric value of foods and the science of nutrition the works of Jordan,* Lusk,† Sherman,‡ and Snyder§ may be consulted.

‡ Chemistry of Food and Nutrition, New York, 1918.
CHAPTER IV.

GENERAL ANALYTICAL METHODS.

Extent of a Proximate Chemical Analysis.—For purposes of studying the proximate composition of food for its dietetic value, it is nearly always necessary to make determinations of moisture, ash, fat, total nitrogen, and carbohydrates (when present), as well as of the fuel value. In some cases it may be desirable to proceed further, to make an analysis of the ash, for instance, to separate, at least into classes, the various nitrogenous bodies, especially in flesh foods, and perhaps to subdivide the starch, sugar, gums, and cellulose or crude fiber that make up the carbohydrates in the case of cereals.

An analysis is considered complete whenever the purpose for which the examination has been made has been accomplished, and on that purpose depends solely the extent to which the various compounds present shall be subdivided and determined. Such a subdivision may be extended almost indefinitely. For example, a milk analysis may consist simply in the determination of the total solids and (by difference) the water. Again, it may be desirable to divide the milk solids into fat and solids not fat, and in some cases to carry the subdivision still farther and separate the solids not fat into casein, albumin, milk sugar, and ash.

Determinations of one or more of the proximate components natural to food are frequently of great service in proving their purity or freedom from adulteration. For the latter purpose, especially in such foods as milk, vinegar, oils, and fats, the determination of specific gravity is also an important factor. Special methods of a peculiar nature are often necessary in the examination of particular foods, and such methods will be treated subsequently under the appropriate headings. In the present chapter only such general methods as are applicable to a large variety of cases will be discussed.

Expression of Results of a Proximate Analysis.—However complete the division of the various proximate compounds or classes of compounds
which the analyst sees fit to make, the results of his various determinations in a proximate analysis are expected to aggregate about 100%. If every determination be directly made, the result will rarely be exactly 100, but the precision of the work is apt to be judged by its approach to 100.

It is often the custom to determine certain compounds or classes of compounds by difference. Thus in cereals moisture, proteins, fat, crude fiber and ash may be determined by the regular analytical methods, and by subtracting their sum from 100 the difference may be expressed as "nitrogen-free extract" or carbohydrates. It has long been customary in food analysis to calculate the protein by multiplying the total nitrogen by the factor 6.25, and on this basis analyses of thousands of animal and vegetable foods have been made. While the figure thus obtained is an arbitrary one, being at best but a rough approximation of the amount of protein present, yet for many reasons there is much to commend this practice of reporting results. In the first place, in most cases it actually does approach the truth. Again, the nitrogenous ingredients of many foods are so numerous and varied, that for the every-day study of dietaries and food values it would be well-nigh impossible with our present knowledge to subdivide these compounds with any degree of accuracy, and especially with uniformity between different chemists, to say nothing of the time involved.

From the fact that so many valuable analyses have already been expressed on the basis of $N \times 6.25$ for protein, the advantage of comparison with the results thus recorded would seem to be in itself a good reason for continuing the practice, especially until a factor that gives better average results can be adopted. By recording the actual nitrogen found as well as the "protein," old results may at any time be recalculated under new conditions, if found desirable.

In flesh foods, when carbohydrates are known to be absent, the total protein may conveniently be determined by difference. Rather more progress has been made in the separation of the nitrogenous compounds of meats than of the vegetables and cereals, though the methods are by no means accurate or uniform.

Most of the recorded analyses of vegetable foods express the carbohydrates as a whole without attempting to subdivide them, at least further than possibly to express the crude fiber or cellulose separately. A much more intelligible idea of the dietetic value of these foods would be gained by a further separation into starch and sugars.
Preparation of the Sample.—It is at the outset of the utmost importance in all cases that a strictly representative portion of the food to be examined should be submitted to analysis. All refuse matter, such as bones, shells, bran, skins, etc., are removed as completely as possible from the edible portion and discarded.

If the composition of the entire mass cannot be made homogeneous throughout, it may be best to select from various portions in making up the sample for analysis, in order to represent as fair an average of the whole as possible.

Finally the sample, if solid or semi-solid, should be divided as finely as possible, by chopping, shredding, pulp, grinding, or pulverizing according to its nature and consistency.

For disintegrating such substances as vegetables and meats for analysis, the common household rotary chopping-machine is admirably adapted. For pulverizing cereals, tea, coffee, whole spices, and the like, the mortar and pestle may be used, or a rotary disk mill or spice-grinder.

Specific Gravity or Density of Liquids.—Where formerly it was customary to compare the density of liquids with that of water at 4° C. (its maximum density) it is now more common to refer to water at 15.5° C. or 20° C., making the determination at that temperature. A common form of expressing the temperature of the determination and the temperature of the standard volume of water with which that of the substance is to be compared, is the employment of a fraction, the numerator of which expresses the temperature of the determination and the denominator that of the standard volume of water, as \( \frac{15.5°}{4°}, \frac{15.5°}{15.5°}, \frac{100°}{15.5°}, \frac{4°}{4°} \text{ C.}^* \)

When extreme accuracy in the determination of density is required, the pycnometer or Sprengel tube should be employed.

The Hydrometer.—This instrument furnishes the most convenient and ready means of determining the density of liquids where extreme nicety is not required. If well made and carefully adjusted, the hydrometer may be depended on to three decimal places, but before relying on its accuracy, it should be tested by comparison with a standard instrument, or with the pycnometer.

The liquid whose density is to be determined is contained in a jar whose inner diameter should be at least \( \frac{3}{8} \) larger than that of the spindle.

* Unless otherwise stated, all specific gravities in this volume are assumed to be expressed on the basis of \( \frac{15.5°}{15.5°} \).
bulb, and the temperature of the liquid should be exactly 15.5° when the reading is taken.

For best results for use with liquids of varying densities, the laboratory should be furnished with a set of finely graduated hydrometers, each limited to a restricted part of the scale, together with a universal hydrometer coarsely graduated, covering the entire range, to show by preliminary test which of the special instruments should be used.

A convenient set of seven such hydrometers are graduated as follows: 0.700-0.850, 0.850-1.000, 1.000-1.200, 1.200-1.400, 1.400-1.600, 1.600-1.800, 1.800-2.000, while the universal hydrometer has a scale extending from 0.700 to 2.000. Another less delicate set of three only has one for liquids lighter than water and two for heavier liquids. Some instruments have thermometers in the stem. Others require a separate thermometer.

The Westphal Balance (Fig. 14).—This instrument consists of a scale-beam fulcrumed upon a bracket, which in turn is upheld by a supporting pillar. The scale-beam is graduated into ten equal divisions. From a hook on the outer end of the beam hangs a glass plummet provided with a delicate thermometer, the beam being so adjusted that when the dry plummet hangs in the air, the beam is in exact equilibrium, i.e., perfectly horizontal as shown by the indicator on its inner end. If the large rider be placed on the same hook as the plummet and the latter immersed in distilled water of the standard temperature at which the determinations are to be made (say 15.5° C.), the scale-beam should again be in equilibrium if the instrument is accurately adjusted. As commonly made, the weight of the plummet including the platinum wire to which it is attached amounts to 15 grams, and the displacement of its volume to 5 grams of distilled water at 15.5° C., the normal temperature on which the determinations are based. Thus the unit (or largest) rider should weigh 5 grams, while the others weigh 0.5, 0.05, and 0.005 gram respectively.

If, instead of distilled water, the plummet be immersed in the liquid whose density is to be determined, the position of the riders on the scale-beam, when so placed as to bring the same into equilibrium, and when read in the order of their relative size (beginning at the largest), indicates directly the specific gravity to the fourth decimal place.

If the liquid is lighter than water, the large rider is first placed in the notch where it comes closest to restoring the equilibrium of the beam, but with the plummet still underbalanced. The rider next in size is then applied in a similar manner, and, unless equilibrium is exactly re-
stored, the third and the fourth riders successively. If it happens that two riders should occupy the same position on the beam, the smaller is suspended from the larger.

If the liquid is heavier than water, the method employed is the same except that one of the largest or unit riders is in this case always hung from the hook which supports the plummet, while the others cross the beam at the proper points. If carefully made and adjusted, the Westphal balance is capable of considerable accuracy.

A delicate analytical balance can be used in place of the less carefully adjusted Westphal instrument, by hanging the Westphal plummet from one of the scale-hooks of the same, and employing a fixed support for the glass jar that holds the liquid in which the plummet is to be immersed. The support is so arranged that the scale-pan below it can move freely without coming in contact with it. This arrangement is shown in Fig. 15.

The Pycnometer, or Specific-gravity Bottle.—Fig. 16 shows the two
forms of pycnometer commonly made. The plain form has a ground-
glass stopper with a capillary passage through it, the other has a fine ther-
mometer connected with the stopper and a capillary side tube provided
with a ground hollow cap. Both are made in different sizes to hold
respectively 10, 25, 50, and 100 grams of distilled water at the standard

![Diagram of Analytical Balance for Determining Specific Gravity with Westphal Pycnometer.]

Fig. 15.—The Analytical Balance Arranged for Determining Specific Gravity with the Westphal Pycnometer.

temperature. It is convenient to have a counterweight for each pycnom-
eter as fitted with its stopper, thus avoiding much trouble in calculation.
The calculation of results is simplified also if the pycnometers are accurately
constructed to contain exactly the weight of distilled water which they
purport to contain at the standard temperature, but it is rather difficult to
procure such instruments, especially of the form furnished with the ther-
mometer. Most instruments hold approximately the amount specified,
the exact net weight of distilled water which they hold at standard tem-
perature being found by careful test and kept on record. In determining
the density of a liquid, the pycnometer is carefully filled with it at a tem-
perature below the standard, the stopper carefully inserted, and the bottle
wiped dry. Care should be taken that the liquid completely fills the bottle
and is free from air-bubbles. The net weight of the liquid is then taken
on the balance, when the temperature has reached the standard (say 15.5° C.), being careful to wipe off the excess of liquid that exudes from the capillary due to expansion. The net weight of the liquid is divided by that of the same volume of distilled water, previously ascertained under the same conditions at the same temperature; the result being the density of the liquid.

The pycnometer with thermometer attachment is obviously susceptible of a greater degree of accuracy than the other form, since the temperature of the liquid, even though 15.5° C. at the start, soon rises.

Fig. 16.—Types of Pycnometer.

The writer prefers to use the pycnometer provided with the thermometer, but without the hollow cap that covers the capillary side tube, unless liquids like strong acids are to be operated on, that might otherwise injure the balance. By keeping the liquid to be tested for some time in a refrigerator, it acquires a temperature of from 10 to 12° C. It is then transferred in the regular manner to the pycnometer and the thermometer-stopper inserted (but not the hollow cap) and the bottle wiped dry. There is ample time to adjust the balance-weights with extreme care while the temperature of the liquid is rising, leisurely wiping off
at intervals with a soft towel the excess that exudes from the capillary tube, the final weight of the dry bottle and contents being made at the exact temperature of $15.5^\circ$ C.

In taking the tare or adjusting the counterweight of a specific-gravity bottle, the latter should be perfectly clean and dry. It had best be rinsed first with water, then with alcohol, and finally with ether, all traces of the latter being removed by a current of dry air, or otherwise, before weighing.

In making successive determinations of density of a number of different liquids with the same pycnometer, it is sufficient to rinse the bottle once with a little of the liquid to be tested before making each determination, when the various liquids are miscible. When the liquids are immiscible, the bottle should be carefully cleaned in the manner described in the previous paragraph before making each test.

*The Sprengel Tube.*—The Sprengel tube is a variety of pycnometer useful when only a small quantity of the liquid to be tested is available.

It is susceptible of great accuracy. It consists of a U-shaped tube (Fig. 17), each branch of which terminates in a horizontal capillary tube bent outward. One of the capillaries, $b$, has a mark $m$ thereon and has an inner diameter of about 0.5 mm. The diameter of the other capillary, $a$, should not exceed 0.25 mm. The liquid at room temperature is aspirated into the tube so as to fill it completely, the end $b$ being dipped in the liquid while suction is applied at the end $a$. The tube is then placed in a beaker of water kept at the standard temperature, the beaker being of such size that the capillary ends rest on the edge. The temperature of the liquid in the tube may be assumed to be constant when there is no further movement due to contraction in the larger capillary end, $b$. The meniscus of the liquid, when cooled, should not be inside the mark $m$, and is brought exactly to the mark by applying a piece of bibulous paper to the other end, $a$. If a drop or two of liquid has to be added, this may be done by applying to the end $a$ a glass rod dipped in the liquid. When exactly adjusted, the whole is wiped dry and quickly weighed, hung from the arm of the analytical balance. To avoid evaporation by contact with the air, the ends of the capillaries are sometimes ground to receive hollow glass caps not shown in the figure.
Determination of Freezing Point.—The Beckmann Apparatus* consists of a cooling jar provided with a stirrer, an ordinary thermometer registering temperatures below zero, and a siphon for emptying, an air jacket, a freezing tube with a stirrer, and a Beckmann thermometer graduated to 0.01° C.

The reservoir in the top of the Beckmann thermometer is for a reserve supply of mercury. If the capillary tube contains so much mercury that the top of the column when cooled to the freezing point is not within the scale, by gently tapping a portion may be made to drop into the reservoir; if it contains too little a portion may be added in the same manner after inverting the thermometer.

Process.—Place an amount of the sample in the freezing tube sufficient to cover the thermometer bulb and cool in the cooling jar, containing a mixture of crushed ice and salt sufficient to produce a temperature several degrees below zero, until the mercury column ceases to fall and begins to rise. Then quickly transfer the freezing tube to the air jacket and continue the cooling, with gentle stirring, until the mercury column remains constant. Read the temperature with the aid of a lens. Determine the reading for distilled water in the same manner. The difference between the two readings is the freezing point of the sample.

Keister † in the examination of milk recommends as a check removing the freezing tube, after taking each reading, warming with the hands or in water at 40° until the contents melt, and repeating the cooling. He also emphasizes the necessity of controlling the supercooling within narrow limits—from 1° to 1.2° for the apparatus used by him.

Determination of Moisture.—This is usually calculated from the loss in weight at the temperature of boiling water. Platinum dishes (Fig. 51) are well adapted for the drying as the residue can be ignited for the determination of ash. If only the moisture is desired, dishes of other metals or glass weighing bottles may be used. Caps for wide-mouthed, bottles made of tinned lead are convenient and can be thrown away after using. Viscous substances are best spread over asbestos or sand to hasten the drying.

Some materials must be heated above 100° C., while certain saccharine products are dried at 70° C. in vacuo to avoid decomposition. If alcohol, acetic acid, essential oils, or other volatile substances are present the loss includes these as well as moisture. As the water or steam oven seldom

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aking, at the "temperature of boiling water." Figs. 8 and 9 show electric and gas ovens for heating at full 100°. Benedict has shown that tain materials can best be dried at room-temperature over sulphuric d in vacuo. Trowbridge* has shortened this process in the case of at, by gently agitating the desiccator during the drying.

**Fig. 18.—Apparatus for Drying in Hydrogen.**

*Drying in Hydrogen.*—Fig. 18 shows the apparatus devised by Winton† drying cereal products, legumes, cattle foods, etc. The material isighed out on a watch glass and transferred to the drying tube (G),ips of cotton, too small to contain an appreciable amount of moisture, ng used at both ends to prevent mechanical loss. The hydrogen isified by passing through sodium hydroxide solution (A) and dried by phric acid in the jar (B). The acid drops over the glass beads into chamber at the bottom of the jar where the gas bubbles through itore passing out over the beads. A siphon automatically removes the

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excess of acid. The drying tubes pass through the copper tubes of the water oven and are fitted at the posterior ends with capillary exit tubes of 0.5 mm. bore, thus creating a slight pressure and insuring even distribution of current. When the drying is begun the exit tubes should be within the copper tubes to avoid stoppage of the current by condensed moisture, but later they should be pushed out, as in the cut, and each tested by lighting.

**Determination of Ash.**—The residue from the determination of moisture or else a new portion, is burned at a very faint red heat until white or gray, cooled in a desiccator and weighed. A flat bottomed platinum dish is most convenient for the purpose. Platinum, however, is attacked by free chlorine, bromine, and iodine, sulphur and phosphorus, sulphates and phosphates with reducing agents, all sulphides, sodium or potassium hydroxide, nitrate and cyanide, metals, and metallic compounds reduced in fusion, such as lead, tin, zinc, bismuth, mercury, arsenic, and antimony. In such cases porcelain must be used.

The degree of heat employed in ashing should be the lowest possible to insure complete oxidation of the carbon, so as to avoid driving off certain volatile salts that are sometimes present and that would be lost if the heat were too high. At a bright red heat potassium and sodium chloride are slowly volatilized, and calcium carbonate is converted into oxide; furthermore alkali phosphates fuse about particles of carbon, protecting them from oxidation. To avoid overheating it is recommended not to allow the flame to impinge directly against the dish, but to carry out the burning on a piece of asbestos paper supported on a triangle. The asbestos also serves to distribute the heat and to protect the dish from the injurious action of the direct flame on long heating. In order to burn off the last traces of carbon, a second piece of asbestos paper may be placed over the top of the dish, or the incineration may be completed in a gas or electric muffle furnace (Figs. 3 and 19). Heating should be continued till the carbon is all oxidized, which is in most cases indicated by a white
ash. It is, however, sometimes impossible to obtain a perfectly white ash, but the appearance of the ash usually indicates when all the carbon has been burnt off. It is sometimes necessary to stir the contents of the dish with a stiff platinum wire from time to time during the ignition.

Methods for the detection and determination of the various ash ingredients are described in detail in Chapter X. Such cases as are peculiar to certain foods, like the metallic impurities that occur in canned, bottled, and preserved foods under certain conditions, will be considered in their appropriate place.

**Extraction with Volatile Solvents.** — Whenever it is necessary to exhaust a substance of its ether soluble or alcohol-soluble ingredients, some form of continuous extraction apparatus is employed with advantage.

**Preliminary Drying.** — In the case of cereal, legume, and oil seed products, meats, etc., the portion of the material dried in hydrogen, *in vacuo*, or in contact with air in an ordinary oven, for the determination of moisture, may be used for extraction. If volatile oil is present, as in spices, the drying must be performed at room temperature in a desiccator.

Milk and other liquids are absorbed in a roll of bibulous paper, in asbestos, or in sand, previous to drying (Chapter VII). The evaporation may be carried on in shells of thin glass (Hoffmeister Schälchen) which are finally broken previous to extraction, or in tinned lead bottle caps which may be crumpled up and inserted in the extractor.

**The Soxhlet Extractor.** — This apparatus is shown in Fig. 20. The substance to be extracted is subjected to successive treatment with freshly distilled portions of the solvent in the tube *S*. Dry powders are contained in extraction thimbles of filter paper or in filters folded over the end of a flat-bottomed cylinder so as to form a cartridge; liquids, such as milk, previously dried in a paper coil or in a wad of asbestos, are extracted without a filter. The vapor from the solvent, boiling in the flask *F*, passes up through the side tube *a* into the condenser *C*, where it is liquefied and falls drop by drop on the substance.

When the level of the solvent in the tube *S* reaches the top of the siphon the liquid drains off into the tared flask *F*, carrying with it whatever it dissolves. The operation is automatically repeated, the substance being successively extracted with freshly distilled portions of the solvent, which leaves behind in the flask *F* the material in solution.

The heater employed should be a hot plate heated by steam, or; as
shown in Fig. 20, an electric stove, which may be provided with a fractional rheostat for varying the amount of heat. If neither of these is available the extraction flask may be rested on a piece of asbestos paper supported by a lamp stand, the heat being supplied by an ordinary Bunsen burner.

The degree of ebullition is so regulated as to allow the solvent to saturate the sample and siphon over into the flask \( F \) from six to twelve times an hour, the extraction being continued from two to six hours, or until all the ether-soluble material has been removed. Care should be taken also that
the rate of boiling and the rate of condensation are so regulated that no appreciable loss of reagent occurs during the extraction. A strong smell of ether perceptible at the top of the condenser indicates a loss. The solvent is recovered at the end of the extraction by disconnecting the weighing flask at a time when nearly all of the solvent is in the part S and before it is ready to siphon over. The weighing-flask is then freed from all traces of the solvent by drying first on the water-bath and then in the oven, after which it is cooled in the desiccator and weighed, the difference between this and the first weighing representing the weight of the fat or ether extract.

The Johnson Extractor.—This form of apparatus (Figs. 21 and 22) has the advantage of the Srethlet extractor in that it is simpler and employs a much smaller amount of ether. The substance is contained in the inner tube of the extractor (Fig. 21), which is closed at the lower end by one thickness each of filter paper and cheese cloth, held tightly in place by means of a linen thread wrapped several times about the tube in the constriction and tied in a fast knot. This inner tube properly prepared can be used over and over for extractions. The outer tube, also shown in Fig. 21, is of such a size that the inner tube fits loosely within it. A slight bulge on one side prevents trapping by means of the condensed solvent. The extraction flask is preferably of only 30 to 35 cc. capacity. It is attached to the extractor, as is also the extractor to the condenser tube, by means of a carefully bored cork stopper. For ordinary determinations of ether extract the outer tube should have an inside diameter of 26 mm. and the inner tube an outside diameter of 22 mm., only 8 to 10 cc. of the solvent being required. If, however, large amounts of material (25 to 50 grams) are to be extracted, the diameters may be made 32 mm. and 28 mm. respectively and a larger amount of solvent employed.

Where only a few extractions are made, the heating can be performed over (but not on) a metal plate heated by a Bunsen burner, and the condensation effected by an ordinary Liebig condenser. If, however, a considerable number of extractions are carried out, the set apparatus shown in Fig. 22 will be found convenient and also economical of space. It may be attached to the wall or placed at the back of a working desk. The heating, as shown in the cut, is effected by means of two steam pipes, but some form of electric heater answers equally well. The case with glazed door prevents the radiation of heat. At the top is shown the multiple condenser consisting of a copper tank with block tin tubes. Water is introduced at the left and carried off at the right.
The solvent is best poured through the material, thus obviating in large degree the crawling of the extract. The door should be opened several times during the extraction and kept open for a few minutes for the purpose of rinsing down the sides of the tubes by means of the condensed vapors.

Preparation of Solvents.—In taking the so-called ether extract, sometimes reckoned as fat, the solvent employed is either ethyl ether or the cheaper petroleum ether. Whichever reagent is employed, certain precautions are necessary for the purity of the reagent. If ethyl ether is used, it should be entirely freed from moisture and alcohol by first shaking with water to remove the larger portion of the alcohol, allowing it to stand for some time over dry calcium chloride, and then distilling over metallic sodium. The ether thus prepared should be kept till used with sodium in the container, the latter being somewhat loosely corked, to allow escape of the hydrogen formed.

Petroleum ether is variously termed benzine, naphtha, or gasoline. It should be low-boiling, preferably between 35° and 50°, and it is always best to redistil it before using, in order to be sure it is free from residue. As to the choice of the two reagents for use in fat extraction, it may be said that ethyl ether is the solvent most used, but if a large number of determinations are to be made, the lower cost of petroleum ether is to
Fig. 23.—Fractionating-still, Arranged for Petroleum Ether.

Fig. 24.—A Convenient Form of Separatory Funnel.
be considered. A convenient still for fractionating such substances as petroleum ether is shown in Fig. 23.

**Extraction with Immisible Solvents.**—It is frequently necessary to dissolve out a substance from a liquid by shaking it with an immiscible solvent, as, for example, in the extraction of certain preservatives from aqueous or acid solutions with ether, petroleum ether, or chloroform. This can be done by shaking in ordinary flasks, but is attended by some difficulty and loss on decantation. A separatory funnel of the type shown in Fig. 24 is almost indispensable for this kind of extraction. The liquid

![Separatory Funnel Support](image)

and solvent are transferred to the funnel, which is then stoppered and shaken. If the solvent is heavier than water, as in the case of chloroform, it is drawn off from beneath through the outlet-tube of the funnel, or, if the solvent is the lighter, as in the case of ether, the aqueous liquid lying beneath is first drawn off and finally the solvent is poured out through the top. If troublesome emulsions form when shaken, they may frequently be broken up by adding an excess of the solvent and again very gently shaking, or by careful manipulation with a stirring rod, or by centrifuging. If the solvent is ether, and an obstinate emulsion forms, it may frequently be broken by the addition of chloroform. Such a mixture of ether and chloroform sinks to the bottom and may be drawn off as in the case of chloroform alone.
A separatory funnel support, devised by Winton, is shown in Fig. 25. It serves for holding the separatory funnels while drawing from one into another, and also as a support for ordinary funnels. The two shelves are adjustable by means of thumbscrews. The holes in these shelves are somewhat wider than the slots, so that the separatory funnels after being introduced through the latter drop into position and are held firmly while manipulating the stop-cock.

Winton attaches all stop- cocks and stoppers to the funnel by means of small brass chains, thus preventing breaking and interchange of these parts during washing.

**Determination of Nitrogen by Moist Combustion.**—In thus determining nitrogen, the organic matter is first decomposed by digestion with sulphuric acid and an oxidizer, the carbon and hydrogen being driven off as carbon dioxide and water respectively, while the nitrogen is converted into an ammonium salt, from which free ammonia (NH₃) is later liberated by making alkaline. The ammonia is then distilled into an acid solution of known value and calculated by titrating the excess of acid.

In the Kjeldahl process the oxidation is effected by means of a mercury compound, in the Gunning method, by potassium sulphate which forms the bisulphate with the acid.

Neither method in its simplest form is applicable in the presence of nitrates; if these are present, a modification must be used. The Gunning-Arnold method (page 446) is employed for the determination of nitrogen in proper, as the piperin is not completely decomposed by the usual processes.

**The Gunning Method.**—Reagents:

- Standard alkali solution, N/10 NaOH or NH₄OH.*
- Pulverized potassium sulphate.
- Sulphuric acid, concentrated, free from nitrogen.
- Sodium hydroxide, saturated solution.
- Standard acid solution, N/10 H₂SO₄ or HCl.*
- An indicator, cochineal solution (page 28).
- Granulated zinc, passing a 1-mm. mesh.

* Winton employs standard acid of such a strength that 1 cc. is equivalent to 1% of nitrogen, working on a gram of material, and titrates back with standard alkali two and one-half times weaker than the acid. In order to secure accurate readings, burettes of narrow bore (1 cc. = 2.6 cm.) are employed. The alkali burette is so graduated that a reading of 1 corresponds to 2.5 cc., thus allowing for the greater dilution. The advantage of this system is that the per cent of nitrogen is obtained by simply subtracting the alkali reading from the number of cc. of acid employed.
The digestion and distillation are preferably carried out in the same flask, which should be pear-shaped with flat or round bottom and made of moderately thick Jena glass. A convenient size has the following dimensions: length 29 cm., maximum diameter 10 cm., tapering gradually to a long neck, which near the end is 28 mm. in diameter with a flaring edge. Its capacity is about 550 cc.

If desired, the digestion may be conducted in a smaller hard-glass flask of about 250 cc. capacity and of the same shape as the above, and the distillation in an ordinary round-bottomed flask of 500 cc. capacity.

Introduce from 0.5 to 3.5 grams of the sample into the digestion-flask with 10 grams of potassium sulphate and from 15 to 25 cc. of concentrated sulphuric acid. The flask is inclined over the flame and heated gently for a few minutes below the boiling-point of the acid till the frothing has ceased, after which the heat is gradually increased till the acid boils, and the boiling is continued till the contents have become practically colorless or at least of a pale straw color. Wire gauze may be interposed between the flask and flame, but a triangle or a similar support is to be preferred.

The contents of the flask are then cooled, and, if the digestion has been conducted in the larger flask suitable also for distilling, 15 above recommended, 300 cc. of water are added and sufficient strong sodium hydroxide to make the contents strongly alkaline, using phenolphthalein as an indicator. If a separate flask is used for the distillation, the contents of the digestion-flask are transferred thereto with the water and the alkali added. A few pieces of granulated zinc should also be introduced, which by the evolution of gas prevents bumping and the sucking back of the distillate. The flask is then without delay connected with the condenser, the bottom of which is provided with an adapter, dipping below the surface of the standard hydrochloric or sulphuric acid, a measured quantity of which is contained in the receiving-flask. The distillation is then continued till all the ammonia has passed over into the acid, this part of the operation requiring from forty-five minutes to an hour and a half. As a rule the first 250 cc. of the distillate will contain all the ammonia.

The excess of acid in the receiving-flask is then titrated with standard alkali, and the amount of nitrogen absorbed as ammonia is calculated. The reagents, unless known to be absolutely pure and free from nitrates and
ammonium salts, should be tested by conducting a blank experiment with sugar, by means of which any nitrates present are reduced. Any nitrogen due to impurities should be corrected for.

In purchasing sulphuric acid for nitrogen determination it is important to specify that it be "nitrogen-free" as the so-called chemically pure acid often contains a considerable amount of nitrogen.

Modification of Gunning Method to include Nitrogen of Nitrates.—In addition to the reagents used in the simpler Gunning method, sodium thiosulphate and salicylic acid are required.

A mixture of salicylic and sulphuric acids is made in the proportion of 30 cc. of concentrated sulphuric to 1 gram of salicylic. From 30 to 35 cc. of

Fig. 26.—Bank of Stills for Nitrogen Determination by Gunning Process.

the mixture are added to the 0.5 to 3.5 grams of the substance in the di-
occasionally shaking. Then 5 grams of sodium thiosulphate are added, and 10 grams of potassium sulphate, after which the heat is applied, at first very gentle and afterwards increasing slowly till the frothing has ceased. The heating is then continued till the contents have been boiled practically colorless. From this point on, proceed as in the Gunning method.

*The Kjeldahl Method.*—One gram of the air dry substance, or a proportionately larger amount of a moist or liquid substance, and 0.7 gram of mercuric oxide (or an equivalent amount of metallic mercury) are placed in a 550-cc. Jena flask and 20 cc. of sulphuric acid added. The flask is placed in an inclined position over a Bunsen burner, and the mixture heated below boiling for 5 to 15 minutes or until the frothing ceases, after which the heat is raised until the mixture boils briskly. The boiling is continued until the liquid has become nearly colorless and for a half hour in addition. The lamp is then turned out, the flask placed in an upright position, and potassium permanganate slowly added with shaking until the solution takes on a permanent green or purple color.

After cooling, 250 cc. of water are added, then 25 cc. of potassium sulphide solution (40 grams of the commercial salt in 1 liter of water), sufficient saturated sodium hydroxide solution to render the solution alkaline, and finally a few grains of granulated zinc, shaking the flask after each addition. Without delay connect with the distillation apparatus, and proceed as in the Gunning method.

![Fig. 272. - Johnson Digestion Stand for Nitrogen Determination with Lead Pipe for Carrying off Fumes.](image-url)
Apparatus for Nitrogen Determination.—A bank of stills used by the author in nitrogen determination and in other processes is shown in Fig. 26.

The digestion apparatus shown in Fig. 27a is that devised by Johnson, Winton, and Boltwood. The stand is of cast iron, with holes provided with three projections that support the flask. The lead pipe with holes for receiving the ends of the flasks serves to carry off the acid fumes. Sy has devised apparatus for sucking the fumes from the flask into water by means of a filter pump, thus dispensing with a hood.

![Fig. 27b.—Johnson Distilling Apparatus for Nitrogen Determination.](image)

The Johnson distilling apparatus, with accessories by Winton, is shown in Fig. 27b. The distillation tubes, except for the glass traps and bulb receiver tubes, are of block tin, and are cooled in a copper tank filled with water. The receivers for the distillate are ordinary pint milk bottles.

At the left are two bottles with suspended tubes for measuring the potassium sulphide and sodium hydroxide solutions.

Determination of Ammonia. A weighed quantity of the finely divided sample, treated with ammonia-free water and made alkaline with magnesium oxide free from carbonate, is distilled into a measured quantity of standard acid (tenth-normal hydrochloric or sulphuric acid) and the amount of ammonia determined by titration.
Determination of Protein Nitrogen.—Stuizer Method.*—Boil 0.5–2.0 grams of the sample, ground to pass a 1-mm. mesh, with 100 cc. of 1% acetic acid in 95% alcohol, cool, filter, and wash by decantation with warm alcohol. Heat the insoluble matter in the beaker with 100 cc. of water for 10 minutes on a boiling water-bath with stirring, cool, and add copper hydroxide suspension (2% copper sulphate solution containing 0.05% of glycerol, precipitated with an excess of sodium hydroxide, washed by decantation with water containing 0.5% of glycerol, and finally suspended in 10% glycerol) sufficient to contain 0.3–0.4 gram of copper hydroxide as determined by evaporation and ignition. Allow to settle, collect on a paper, wash with water, and determine nitrogen in filter and contents. In the absence of alkaloids heat directly with water and precipitate with the copper reagent.

Determination of Nitrogen in Amino Acids.—Van Slyke Method.†—This method has proved valuable in physiological investigations and is useful in food examination in special cases. The manipulation is quite simple, but the apparatus is somewhat expensive. For further details reference should be made to Van Slyke's original articles or Mathews' Physiological Chemistry.

Determination of the Various Carbohydrates.—Under title of "Cereals" in Chapter X are given in detail methods for separation and determination of sugar, starch, dextrin, crude fiber, etc.

Detection of Poisons.—Metallic impurities present in foods incidental to their preparation, or as adulterants, are considered under title of foods liable to such adulteration. The detection of highly toxic substances, such as arsenic, corrosive sublimate, and alkaloids, added with criminal intent, comes within the province of the medico-legal chemist or toxicologist and is beyond the scope of this work. The methods involved are fully described in the treatises of Autenrieth ‡ and Blyth,§ only those for arsenic, which occurs also as an accidental impurity, being here considered.

Detection and Determination of Arsenic.—Methods of Solution.—Syrups, baking powders and other materials soluble in water or acid do not need preliminary treatment. Beer is treated as described in Chapter XV. Other methods of solution are as follows:

1. Johnson-Chittenden-Gautier Method.||—This method is suitable for meat, vegetables, and the like, the proportion of acids used being

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varied to suit conditions. Heat at 150°–160° C., in a porcelain dish, 100 grams of the finely divided material with 23 cc. of pure concentrated nitric acid, stirring occasionally. When the mixture assumes a deep orange color, remove from the heat, add 3 cc. of pure concentrated sulphuric acid, and stir while nitrous fumes are given off. Heat to 180° and add while hot, drop by drop, with stirring, 8 cc. of nitric acid, then heat at 200° till sulphuric fumes come off and a dry charted mass remains. Pulverize the mass, exhaust with hot water, filter, evaporate to small volume, take up in cold 20°, sulphuric acid and treat by the modified Marsh or Gutzeit method.

2. Sanger Method.*—Digest at room-temperature for some hours 5 to 20 grams of the material in a casserole with about an equal bulk of

![Fig. 28.—Marsh Apparatus for Arsenic.](image)

concentrated nitric acid, add 20 cc. of concentrated sulphuric acid and digest further at a gentle heat until the mixture begins to char. Add about 2 cc. of nitric acid and heat until sulphuric fumes appear, repeating the addition of acid and heating until oxidation appears to be practically complete. Remove all nitric acid by dilution and evaporation to the fuming stage, then dilute with 4 volumes of water. At this point about twice the bulk of saturated sulphurous acid solution may be added and the evaporation repeated, thus reducing to the arsenious condition, but this is not usually necessary.

Methods of Determination.—1. Marsh Test.—The apparatus (Fig. 28) consists of a generating flask with funnel tube, a U-tube containing cotton

moistened with 10% lead acetate solution (to remove hydrogen sulphide), a calcium chloride drying tube, and a hard glass tube of 6 mm. bore, drawn down near the end to a uniform constriction about 4 cm. long and 1 mm. inside diameter and also at the very end to a narrow exit tube. The tube is supported over a three-burner furnace the part in contact with the flame being wrapped with wire gauze.

Introduce into the generating flask from 20 to 30 grams of arsenic free stick zinc and a perforated platinum disk to form an electric couple. Stopper and add through the funnel tube 20°' sulphuric acid sufficient to start the reaction and drive out all air. When danger of explosion is over heat the tube to bright redness. After running the current long enough to prove the absence of arsenic in the reagents add slowly from the funnel tube the solution of the material in 20°' sulphuric acid or the solution obtained by one of the foregoing methods containing about 20°' of that acid, keeping a steady evolution of gas. When the flow slackens add 30°' sulphuric acid and later 40°' acid until all arsenic has been expelled, which usually requires 2 to 3 hours. If no arsenic mirror forms in the constriction of the tube in one hour, further test may be abandoned.

If more than 0.1 mg. of arsenic appears to be present cut off the constriction from the tube and weigh it on an assay balance; then dissolve the arsenic in a solution of sodium hypochlorite. (Antimony being insoluble), wash with water and then with alcohol, dry, cool, and weigh. The loss is arsenic.

If the amount of arsenic is very small Sanger compares the mirror with a series of standard mirrors prepared in the same apparatus using quantities of a standard solution containing from 0.005 to 0.05 mg. of As₂O₃. To prepare the standard solution 1 gram of pure As₂O₃ is dissolved in arsenic-free sodium hydroxide, acidified with sulphuric acid, made up to one liter and 10 cc. of this stock solution further diluted to 1 liter; 1 cc. = 0.01 mg. As₂O₃.

2. Sanger-Black-Gutzeit Method.*—The apparatus (Fig. 29), devised by Bishop, consists of a 30 cc. salt-mouth bottle provided with three upright

tubes one above the other. The lower tube is 7 cm. long, 1 cm. in bore, and contains strips of filter-paper previously soaked in 5% lead acetate solution and dried. The middle tube is of the same size as the lower but shorter. It is loosely filled with cotton moistened with 1% lead acetate solution. The upper tube has a uniform bore of 2.5 mm. and is bent twice so that the upper end is vertical. In this tube is placed a strip of cold-pressed drawing paper 2 mm. wide which has been soaked in 5% alcoholic mercuric chloride (or bromide) and dried.

Place in the evolution bottle 10 grams of stick zinc, a few crystals of stannous chloride, a perforated platinum disk and from 2 to 5 grams of the material or else the extract of the charred or digested material prepared as described in the foregoing sections, containing about 20%, of sulphuric acid. Add enough 20%, (1:4) sulphuric acid to nearly fill the bottle, attach the three tubes and allow to react for 45 minutes. Compare the color on the sensitized strip with that of standard strips obtained with from 0.005 to 0.05 mg. of $\text{As}_2\text{O}_3$ in the same apparatus, using measured quantities of the standard solution described under the Marsh test.

**Colorometric Analysis.**—Certain analytical processes depend on the formation of a compound of the substance to be determined having a definite color, and the calculation of the quantity present from the intensity of the color of the solution, compared with that of a solution containing a known amount. The comparisons may be made in a special form of cylinder or in a colorimeter. The latter has the advantage that a single solution of known strength serves within reasonable limits for matching any shade in the unknown solution, and for any number of determinations, the desired depth of the color being secured by varying the length of the column.

**Schreiner's Colorimeter.**—This apparatus, shown in Fig. 30, consists of two graduated tubes ($B$), containing the standard and unknown colorimetric solutions, the height of the column of liquid in both tubes being changed by two immersion tubes ($A$), which remain stationary while the graduated tubes are raised or lowered in the clamps ($C$). The mirror $D$ reflects the light through the tubes, and the mirror $E$ reflects it again to the eye of the operator at $F$.

In making the comparisons, the tube containing the solution of either known or unknown strength is set at a definite point, and the other tube is raised or lowered until the colors match. If $R$ is the reading of the standard solution of the strength $S$, and $r$ the reading of the colorimetric solution of unknown strength $s$, then $s = \frac{R}{r}S$.

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If desired, standard slides of colored glass, such as accompany the Lovibond tintometer, may be used at G for matching the solution of unknown strength, the value of these slides being determined by comparison with a standard solution.

The Lovibond Tintometer may be used for colorometric chemical analysis, but is not so well suited for this purpose as the Schreiner colorimeter. It is especially designed for determining the color value of liquid and solid technical products, such as beer, wine, oil, flour, paper, etc.

The instrument itself is of simple construction, consisting of an elongated box with an eyepiece at one end and two rectangular openings at the other, one for the solution or substance to be examined, the other for the standard glass slides used for matching the color. Light is reflected through the openings by means of a square piece of opal glass mounted on a jointed standard. Liquids are examined in rectangular cells with glass sides by transmitted light, while powders are pressed into a form and examined by reflected light.

The standard slides used in general work are red, yellow, and blue in even graduation from .006 to 20 tint units which can be combined so as to produce any desired tint or shade of any color. The results are expressed in terms of standard dominant colors (red, yellow, and blue), subordinate colors (orange, green, and violet) obtained by combining equal values of two dominant colors, and neutral tint (black) obtained by combining equal values of the three dominant colors. Thus

\[0.6R + 5.6Y = 0.6O + 5.0Y\]
\[0.08R + 1.5Y + 0.2B = 0.08N + 0.12G + 1.3Y\]
\[1.2R + 1.0B = 1.0V + 0.2R\]

in which \(R\) = red, \(Y\) = yellow, \(B\) = blue, \(O\) = orange, \(G\) = green, \(V\) = violet, \(N\) = neutral tint or black.

Special slides may be obtained for the examination of any desired product. For example, slides of brown shades are furnished for beer, of yellow shades for oils, and so on.
CHAPTER V.

THE MICROSCOPE IN FOOD ANALYSIS.

Microscopical vs. Chemical Analysis.—A very important means of identification of adulterants in many classes of food products is furnished by the microscope, which in many cases affords more actual information as to the purity of food than can be obtained by a chemical analysis. This is especially true of coffee, cocoa, and the spices, wherein the microscope serves to reveal not only the nature of the adulterants, but also not infrequently the approximate amount of foreign matter present. In the case of the cereal and leguminous products so commonly employed as adulterants, a microscopical examination is of paramount importance.

The chemical constants of many of the adulterants of coffee and the spices do not always differ sufficiently from those of the pure foods in which they appear to be distinguished therefrom with accuracy and confidence by a chemical analysis alone. On the other hand, one who is familiar with the appearance under the microscope of the pure foods and of the starches and various ground substances used as adulterants, can, with certainty, identify very minute quantities of these materials, when present, with the same ease that one can recognize macroscopically the most familiar objects about him.

A chemical test may, for example, indicate the presence of starch, but it cannot reveal the particular kind of starch. The microscope will at once show whether the starch present is wheat or corn or potato or arrowroot, since these starches differ almost as much in microscopical appearance as do the physical characteristics of the grains or tubers from which they are obtained. Again, by a chemical analysis an abnormal amount of crude fiber may show the presence of a woody adulterant, but only the microscope will enable one to decide whether the impurity consists of sawdust, chaff, or ground nut shells. Not only in such instances as these is the microscopical examination of greater importance than a chemical analysis, but it is a much quicker guide.

The Technique of Food Microscopy.—The recognition of adulterants by the microscope requires some experience but no more than may be acquired by a chemist who will give the subject serious attention. In
the examination of flour, commercial starch, cocoa, coffee, tea, and the spices for adulteration. A careful study of the powdered substance in temporary water mounting will in most cases prove sufficient to familiarize the food analyst with their characteristics under the microscope. In extended studies standard works on the microscopy of foods should be consulted.

It is not necessary for him to familiarize himself with the details of section cutting, dissecting, or permanent mounting unless he so desires. Such details are given by Behrens,* Chamberlain,† Gage,‡ and Zimmerman.§

Microchemical methods of analysis, a subject quite distinct from food histology, is fully treated by Chamot.||

Standards of Comparison.—For standards the analyst should provide himself with as complete a set as possible of the various materials to be examined, taking care that their absolute purity is established. Wherever possible, he should grind the sample himself from carefully selected whole goods. These, together with samples of the starches and other adulterants, all of known purity, should be contained in small vials carefully stoppered and plainly labeled, arranged alphabetically or in some equally convenient manner in the desk or table on which the microscope is commonly used. The adulterants included in this set of standards should be not only those which experience has shown most liable to be employed, but any which, by reason of their character, might in the analyst’s opinion be used under certain conditions.

APPARATUS.

The Microscope-stand. An expensive or complicated stand is unnecessary. The prime requisites for good work in a microscope-stand are firmness or rigidity, and accuracy in centering. An inexpensive stand possessing these features can be used for the best work, providing the optical parts are satisfactory. It is well, if economy must be practiced, to purchase a simple stand provided with the society screw, and let the larger portion of the allowance go for a high grade of lenses, since many of the attachments inherent in a high-priced stand, though often of convenience, may well be dispensed with.

* Guide to the Microscope in Botany.
† Methods in Plant Histology.
‡ The Microscope and Microscopical Methods.
§ Botanical Microtechnique.
|| Elementary Chemical Microscopy, New York, 1915.
A stand of the so-called continental type (having the horseshoe base) is preferable. A square stage is rather more convenient than the circular form, and the jointed pillar possesses advantages over the rigid variety in ease of manipulation that are certainly worth considering.

The smooth working of both the coarse and fine adjustments should not be lost sight of. If the microscope is to be used exclusively for food work, a substage condenser is unnecessary, hence the construction of the substage may be very simple, unless bacteriological work is to be done as well.

A nose-piece, while not indispensable, is a great convenience for the quick transfer of objectives. A double nose-piece carrying two objectives is ample for routine food work.

The Optical Parts are by far the most important, and should be of superior quality, though not necessarily of the most expensive makers. The food analyst should have at least two objectives, one for high- and one for low power work, and preferably two oculars.

For the routine examination of powdered food substances the writer prefers a ½-inch objective, used with a medium ocular, the combination giving a magnification of from 240 to 330 diameters, according to the ocular employed. For a low-power objective the ½-inch is a conven-
ient size. It is useful as a finder preliminary to examination with the higher power, and, in connection with a low-power eyepiece, is well adapted for the examination of butter and lard, and for use with the polariscope.

An eyepiece micrometer mounted in an one inch ocular is indispensable for measuring starch grains and other elements. It is calibrated by means of a stage micrometer.

**The Micro-polariscope.**—This accessory is useful in the identification of starches and other ingredients, and for ascertaining whether or not fats have been crystallized. The polarizer is held below the stage, while the analyzer is applied above the objective, either in the tube or above the ocular.

![Diagram of Polarizer and Analyzer for the Microscope](image)

**Fig. 32.**—Polarizer and Analyzer for the Microscope.

A common form of construction is one in which the substage is adapted to carry interchangeably the diaphragm tube and the polarizer. If the polariscope is much used, it becomes desirable to provide means for quickly changing the polarizer and diaphragm tube below the stage, and for moving the analyzer in and out of place above the objective. Winton* has devised a microscope-stand with this in view, especially adapted to the needs of the food analyst.

If the polariscope is to be used often, it is convenient to have within easy access two stands, one with the polariscope mounted in place in connection with low-power glasses ready for use, and the other stand provided with the ordinary high- and low-power objectives only.

**Microscope Accessories** include of necessity a large number of slides and cover glasses. The latter should be No. 2 thickness, 3/4 inch, either round or square.

One or more dissecting needles in holders and a small hand magnifying-glass should also be provided.

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Other useful accessories are a mechanical stage, a pair of fine tweezers, knives, scissors, and, if sections are to be cut, a plano-concave razor.

**MICROTECHNIQUE.**

**Preparation of Vegetable Food Products for Microscopical Examination.**—The ground spices and cocoa of commerce are usually of the requisite fineness for direct examination without further treatment. Coffee, chocolate, starches, and similar products should be ground in a mortar fine enough to pass through a sieve with from 60 to 80 meshes to the inch.

A small portion of the powdered sample is taken up on the tip of a clean, dry knife-blade, and placed on the microscope-slide. By means of a medicine-dropper a drop of distilled water is applied, and the wetted powder is then rubbed out under the cover-glass between the thumb and finger to the proper fineness.

The water-mounted slide thus prepared, while useful only for temporary purposes, has proved to be best adapted to the analyst’s requirements for routine microscopical examination of powdered food products for adulteration, partly because water is the best medium in most cases for showing up the structural characteristics of these substances and their adulterants, and partly because it serves best for the “rubbing out” process between thumb and finger under the cover-glass, whereby the sample is brought to the requisite degree of fineness.

Experience will soon show how far this rubbing out should be carried for the best effects. Gentle pressure should be applied, care being taken not to break the cover-glass, especially if the sample contain anything of a gritty nature. The rubbing should be continued till the coarser par,
ient size. It is useful as a finder preliminary to examination with the higher power, and, in connection with a low-power eyepiece, is well adapted for the examination of butter and lard, and for use with the polariscope.

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![Polarizer and Analyzer for the Microscope.](image)

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Microscope Accessories include of necessity a large number of slides and cover glasses. The latter should be No. 2 thickness, $\frac{1}{2}$ inch, either round or square.

One or more dissecting needles in holders and a small hand magnifying-glass should also be provided.

is but a mechanical mixture of various tissues, and that no two portions will show exactly the same composition.

**Characteristic Features of Vegetable Foods under the Microscope.**—
The structural features of a powdered spice, examined microscopically, will be found to differ considerably in appearance from those of a thin, carefully mounted section of the same spice. Instead of the beautiful arrangement of cells and cell contents with the perfect order of various parts as seen in the mounted section, one finds in the powdered sample under the microscope what often appears to be a most confusing mass of fragments of various tissues. For this reason the most striking characteristics seem to vary with different observers, and it is a well-known fact that microscopists differ widely as to conceptions of size, shape, and ordinary appearance, even in the case of certain of the well-known starch grains. It is on this account that, irrespective of the description of others, the analyst should familiarize himself with the microscopical appearance of the foods with which he is dealing, as well as of their adulterants, forming his own standards as to what constitute the recognizable features, from specimens prepared by himself.

In the large variety of ground berries, buds, tubers, barks, etc., from which the spices and condiments are prepared, as well as in the grains, legumes, shells, fruit stones, and other materials forming the most familiar adulterants, the kinds of plant tissues and cell contents which, under the microscope, serve as distinguishing marks or guides for identification are comparatively few in number.

The most common of these varieties of cell tissue and of cell contents to be met with by the food microscopist in his work are briefly the following:

**Parenchyma.**—This is most abundant and widely distributed, forming as it does the thin-walled, cellular tissue of nearly all vegetable food substances. The walls of parenchyma cells are, as a rule, colorless and transparent. The forms of the cells are varied and are often sufficiently characteristic in themselves to identify the substance under examination.

**Sclerenchyma,** or stone cells, are the thick-walled woody cells forming the hard part of nut shells, fruit stones, and seed coverings, occurring also in some fruits and barks. These cells are more often colored and of various shapes but almost always irregular, sometimes elongated, as in coconut shells and olive stones, occasionally nearly rectangular, as in pepper shells, and sometimes polygonal or nearly circular.

In appearance the sclerenchyma cell commonly has a more or less
deep, central or axial cavity, from which small fissures extend through the thick walls. See Fig. 35.

Variously shaped sclerenchyma cells are found in allspice, cassia, pepper, clove stems, nut shells, etc. Stone cells are optically active to polarized light, and between crossed nicols are very conspicuous by their bright appearance.

Fig. 34.—Typical Forms of Various Cell Tissues. Longitudinal section through a clove, showing: \( p \), two forms of parenchyma; \( b \), bast fibers; \( s \), vascular and sieve tissue; \( k' k' \), cells with calcium oxalate crystals. (After Vogl.)

Fig. 35.—Sclerenchyma, or Stone-cell Tissue. A cross-section through the stone-cell layer of the fruit shell of black pepper. (After Vogl.)

*Fibro-vascular Bundles* are composed of three parts: the bast fibers, or mechanical elements, the phloem, and the xylem.
**Bast Fibers** are elongated, pointed sclerenchyma cells, of which flax fibers are examples.

**Sieve Tubes**, the characteristic elements of the phloem, are thin-walled tubes with perforated partitions known as sieve plates.

**Vessels or Ducts** occur in the xylem. They are designated as spiral, annular, reticulated, or pitted, according to the nature of the walls.

**Corky Tissue, or Suberin**, constitutes the thin-walled, spongy cells forming the protective, outer dead layers of the bark. This is found in cassia, and in the barks used as adulterants. Suberin is tested for by potassium hydroxide (p. 80).

**Starch** wherever it occurs furnishes the most characteristic feature of the cell contents, and, as a rule, will at once indicate under the microscope, by the shape and grouping of its granules, the particular substance of which it forms a part. It is very abundantly distributed throughout the vegetable kingdom and occurs in a wide variety of forms. It is particularly conspicuous when viewed by polarized light. Between crossed nicols such starches as corn, potato, and arrowroot show out brightly from a dark background with dark crosses, the bars of which intersect at the hilum of each granule. When a selenite plate is introduced above the polarizer, a beautiful play of colors is seen with various starches, a phenomenon which Blyth applies as a means of identification and classification, but which more modern microscopists regard as of minor importance to distinguishing the various starches morphologically. Starch is found naturally in the cereals, legumes, and many vegetables, in cassia, allspice, nutmeg, pepper, ginger, cocoa, and turmeric. The cereal and leguminous starches from their inertness and cheapness constitute the most common adulterants of the spices and of powdered foods in general. Starch grains are found in the cells of the parenchyma and in other cellular tissues. Iodine is the special reagent (p. 78).

**Gums and Resins** occur in characteristic forms among the cell contents of some of the spices. As an example, the portwine-colored lumps of gum in allspice furnish one of the most ready means of recognizing that spice microscopically. Resin is tested for microchemically with alkanna tincture (p. 79).
THE MICROSCOPE IN FOOD ANALYSIS.

Aleurone or Protein Grains are found in many seeds, but are not especially characteristic. They somewhat resemble small starch grains. Most varieties of protein grains are soluble in water, but some are insoluble. The soluble varieties, which are not apparent in water-mounted specimens, must be examined in absolute alcohol, glycerin, or oil. In leguminous seeds aleurone occurs closely intermingled with starch in the same cells, while in the cereals it occupies the whole cell.

Protein grains are tested for under the microscope by iodine in potassium iodide, which turns them brown or yellowish brown, and by Millon’s reagent, which colors them brick red.

Plant Crystals are not uncommon in the class of substances which the food analyst examines. Among the common forms are the piperin crystals found in pepper. Calcium oxalate occurs in many vegetable products as prismatic crystals, crystal aggregates, or needle-shaped crystals (raphides).

Crystals of calcium carbonate are sometimes met with also, as, for example, in hops. Calcium oxalate crystals are insoluble in acetic acid, while being readily soluble in dilute hydrochloric. Calcium carbonate crystals are soluble with effervescence in both acids. The acid reagents are directly applied to the sample in water-mount under the cover-glass, and the reaction observed through the microscope.

Fat Globules are of common occurrence in many foods and appear of various sizes, sometimes large and conspicuous, and again almost lost sight of because of their minuteness. They are sometimes colorless, as in mace, and sometimes deeply tinted, as in cayenne. Alkanna tincture is used as a reagent for fat (p. 79).

Other Cell Contents of less importance, but which may be identified by the microscope with reagents, are tannic acid (tested for by chloriodide of zinc and ferric acetate (pp. 78 and 79), and various essential oils, for the detection of which alkanna tincture is employed.

REAGENTS IN FOOD MICROSCOPY.

Unless a more extended microscopical investigation of vegetable food substances is contemplated than is involved in the mere identification of adulterants, the analyst will have little need for reagents other than iodine in potassium iodide, chloral hydrate solution, and potassium hydroxide solution, the last two for clearing, but will depend almost entirely on the form and appearance of the various tissues or tissue fragments, as well as on the abundance, shape, and distribution of such distinctive cell contents as the starches, fat globules, or crystals.
Analytical reagents are applied to the water-mounted sample by means of a glass rod or pipette, with which a drop of the reagent is deposited on the sample upon the slide, having previously removed the cover, which is afterwards replaced. Or, without removing the cover-glass, a drop of the reagent is placed in contact with one side of it on the slide. Along the opposite side of the cover is then placed a piece of filter-paper. The latter withdraws by capillary attraction a portion of the water from under the cover-glass, and this is replaced by the reagent, which thus intermingles with the particles of the substance.

The following reagents include those needed in routine work as well as some others suited for studies of the general nature of tissues and cell contents.

A. Analytical Reagents.—*Iodine in Potassium Iodide.*—Two grams of crystallized potassium iodide are first dissolved in 100 cc. of distilled water and the solution is saturated with iodine.

This reagent is indispensable for the identification of starch, especially when the latter is present in minute quantities. Starch granules when moistened with water are turned blue by iodine, the reaction being exceedingly delicate under the microscope, even when the starch granules are very minute and insignificant without the reagent.

Iodine in connection with sulphuric acid is also useful in distinguishing pure cellulose from its various modifications, such as lignin and suberin. For this purpose the water-mounted sample is first permeated with the iodine reagent, after which concentrated sulphuric acid is applied, with the result that all pure cellulose is turned a deep-blue color, while the modified forms of cellulose are colored yellow or brown. The cellulose is first converted by the sulphuric acid into a carbohydrate isomeric with starch, known as amyloid.

Protein grains are colored brown or yellow brown by the action of iodine.

*Chloriodide of Zinc.*—Pure zinc is dissolved in concentrated hydrochloric acid to saturation, and an excess of zinc added. The solution is then evaporated to about the consistency of concentrated sulphuric acid, after which it is first saturated with potassium iodide, and finally with iodine.

This reagent may be used instead of sulphuric acid and iodine for the detection of cellulose, since the zinc chloride converts the cellulose into amyloid, which the reagent colors blue.

Chloriodide of zinc is useful for detecting tannic acid in cell contents. For this purpose the above reagent is much diluted by the addition of
a 20% solution of potassium iodide. In this diluted form, when applied
to the sample, a reddish or violet coloration is imparted to cell contents
having tannin.

*Phenol-hydrochloric Acid* is prepared by saturating concentrated
hydrochloric acid with the purest crystallized carbolic acid. Wood fiber,
or lignin, when treated with a drop of this reagent under the cover-glass,
and exposed for half a minute to the direct sunlight, will be colored an
intense green, which quickly fades.

*Indol.*—Several crystals of indol are freshly dissolved in warm water.
Lignified cell walls assume a deep-red color, when the specimen to be
examined is treated first with a drop of the indol reagent, and afterwards
washed with dilute sulphuric acid, 1:4.

*Millon's Reagent.*—This is prepared by dissolving metallic mercury
in its weight of concentrated nitric acid, and diluting with an equal volume
of water. This reagent, which should be freshly prepared, is of use in
testing for protein compounds, which turn brick red when treated with it,
especially on gently warming the slide.

*Tincture of Alkanna.*—A 70 or 80% alcoholic extract of alkanna root,
when kept in contact with resins, fixed oils, fats, or essential oils for a
short time, stains these cell contents a lively red. The staining is hastened
by the aid of heat. Essential oils and resins are soluble in strong alcohol,
while fixed oils and fats are insoluble, hence the distinction between these
classes of cell contents may be made by the application of alcohol to the
alkanna-stained specimen.

*Ferric Chloride, Ferric Acetate, or Ferric Sulphate,* used in dilute aqueous
solution, are all applicable as reagents for tannic acid, which, when present
in appreciable amount, will be colored green or blue by either of these
reagents.

**B. Clarifying Reagents.**—Many of the harder cellular tissues are too
opaque for careful examination, and may be rendered transparent by clarifying
or bleaching. The simplest and for many purposes the most satisfactory
method for clearing the tissues is by boiling a water mount, replacing
the water lost by evaporation. Proceeding in this manner, there is ordinarily
no danger of the slide or cover-glass breaking; if the boiling is
carried out without a cover-glass, the slide is almost sure to break. A
portion of the powdered sample is either boiled with a drop of the reagent
under the cover-glass or is allowed to soak for hours or even days in the
reagent, using a drop of the same reagent as a medium for examination
on the object-glass, instead of water. The clarifying reagents most com-
monly used are the following:
Chlora Hydrate.—A 60% solution.

Ammonia.—Concentrated, or 28% ammonia is commonly used.

Potassium Hydroxide, used in various degrees of concentration, often in dilute solution, say 5%. This reagent, added to a water mount, causes swelling of the cell wall, and dissolves intercellular substances and protein. It bleaches most of the coloring matters, destroys the starch, and forms soluble soaps with the fats. Potassium hydroxide is also used in testing for suberin, which is extracted from corky tissue on boiling with the reagent, and appears as yellow drops.

Schultze’s Macerating Reagent (concentrated nitric acid and chlorate of potassium) is best used by placing the powder or bit of tissue to be treated in a test-tube with an equal volume of potassium chlorate crystals, adding about 2 cc. of concentrated nitric acid, and warming the tube till bubbles are evolved freely, or until the necessary separation of cells is effected. The sample is then removed and washed with water.

By this treatment, bast and wood fibers as well as stone cells are readily separated from other tissues.

Cuprammonia (Schweitzer’s Reagent).—This is prepared by adding slowly a solution of copper sulphate to an aqueous solution of sodium hydroxide, forming a precipitate of cupric hydroxide, which is separated by filtration, washed, and dissolved in concentrated ammonia. It should be freshly prepared, and is never fit for use unless it is capable of immediately dissolving cotton. Indeed its chief use is as a test for cellulose, which it readily dissolves. In observing this reaction under the microscope, the powdered specimen under the cover glass should be only slightly damp before a drop of the fresh reagent is applied. The cell walls are seen to swell up and gradually become more and more indistinct, till they finally disappear.

Cuprammonia is also used as a test for pectose, which occurs in many cell walls, often intermixed with cellulose. When treated with this reagent, cellular tissue containing pectose is acted upon in such a manner that a delicate framework of cupric pectate is sometimes left behind, after the dissolution of the cellulose with which it is mingled.*

PHOTOMICROGRAPHY.

The photomicrograph serves as a simple means of keeping permanent records of unusual forms of adulteration encountered in the course of routine examination. Besides this, the photomicrograph has at times proved its usefulness as a means of evidence in court, showing as it does with faithfulness the presence of a contested adulterant. It is true

* Poulson, Botanical Micro-chemistry, p. 15.
that from an artistic and didactic standpoint the photomicrograph of a powdered sample is often disappointing, due to the fact that ordinarily much of the field is out of focus, unless a very simple homogeneous subject is photographed, as, for instance, starch. As compared with the carefully prepared drawing of a section, which shows minute details of structure, the photomicrograph portrays what happens to be in focus.

**SUMMARY OF MICROCHEMICAL REACTIONS FOR IDENTIFYING CELLULAR TISSUE AND CELL CONTENTS. BASED ON BEIJERINCK**

<table>
<thead>
<tr>
<th>Cellular Substance</th>
<th>Indigo in Potassium Hydroxide</th>
<th>Chlorides of Zinc</th>
<th>Indigo and Sulphuric Acid</th>
<th>Cuprammonia</th>
<th>Potassium Hydroxide</th>
<th>Concentrated Sulphuric Acid</th>
<th>Schulte’s Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose, cell substance</td>
<td>Yellow or brownish</td>
<td>Violet</td>
<td>Blue</td>
<td>Dissolves</td>
<td>Swells up</td>
<td>Dissolves</td>
<td>Dissolves</td>
</tr>
<tr>
<td>Lignin, wood substance</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow to brownish</td>
<td>Insoluble</td>
<td>Dissolves</td>
<td>Dissolves</td>
<td>Dissolves easily</td>
</tr>
<tr>
<td>Middle lamella, intercellular substance</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow to brownish</td>
<td>Insoluble</td>
<td>Dissolves</td>
<td>Dissolves</td>
<td>Dissolves easily</td>
</tr>
<tr>
<td>Suberin, cork substance</td>
<td>Yellow or brownish</td>
<td>Yellow</td>
<td>Brown</td>
<td>Insoluble</td>
<td>Insoluble in cold.</td>
<td>Insoluble by boiling in drops</td>
<td>Dissolves easily</td>
</tr>
<tr>
<td>Starch</td>
<td>Blue</td>
<td>Brown</td>
<td>Yellow to brownish</td>
<td>Insoluble</td>
<td>Insoluble in cold.</td>
<td>Insoluble by boiling in drops</td>
<td>Insoluble easily</td>
</tr>
<tr>
<td>Protein</td>
<td>Blue</td>
<td>Brown</td>
<td>Yellow to brownish</td>
<td>Insoluble</td>
<td>Insoluble in cold.</td>
<td>Insoluble by boiling in drops</td>
<td>Insoluble easily</td>
</tr>
<tr>
<td>Gums and resins</td>
<td>Reddish</td>
<td>Reddish</td>
<td>Reddish to violet</td>
<td>Insoluble</td>
<td>Saponifies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Essential oils</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Tannin</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Calcium oxalate crystals</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Cellular Substance</th>
<th>Phenol-hydrochloric Acid</th>
<th>Indig.</th>
<th>Ferric Acetate or Salicylate</th>
<th>Alkanna Tincture</th>
<th>Hydrochloric Acid</th>
<th>Acetic Acid</th>
<th>Millon’s Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose, cell substance</td>
<td>Uncolored</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin, wood substance</td>
<td>Green</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle lamella, intercellular substance</td>
<td>Green</td>
<td>Cherry red</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suberin, cork substance</td>
<td>Uncolored</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>Uncolored</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brick red</td>
</tr>
<tr>
<td>Protein</td>
<td>Uncolored</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gums and resins</td>
<td>Uncolored</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Fat</td>
<td>Uncolored</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Essential oils</td>
<td>Uncolored</td>
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<tr>
<td>Tannin</td>
<td>Uncolored</td>
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<tr>
<td>Calcium oxalate crystals</td>
<td>Blue of green</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Insoluble</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>S-lable without effervescence</td>
<td>S-lable with effervescence</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Microscopical Investigation of Vegetable Substances, page 126.
† When treated with the reagent, suberin forms masses of ceric acid, soluble in ether, alcohol, or chloroform.

While the analyst examines microscopically the ordinary powdered spice, for example, he constantly moves with his hand the fine adjustment-screw, bringing into focus different parts of the field successively. This
he does unconsciously, so that he does not realize how far from flat the field actually is till he undertakes to photograph it, when, as a rule, only a small portion is in good focus. It is therefore impossible in one photograph to show successfully many varied forms of tissue or cell contents in the powder, but separate photographs should be made as far as possible with only a little in each. Thus, for example, with a composite subject like powdered cassia bark, it would be very difficult to show starch, stone cells, and bast fibers in one field, all in equally good focus, and, for the best results only, one, or at most two, such varied groups of elements should be shown in one picture.

**Appurtenances and Methods of Procedure.**—The temporary method of water-mounting employed by the analyst in routine examination presents many difficulties from a photographic point of view. The vibrating motion of the particles is very annoying, and some skill is required in using just the right amount of water, in avoiding air-bubbles, in waiting the requisite amount of time before exposing the plate for the vibratory motion to cease, and, on the other hand, avoiding too long delay, which would result in the evaporation of the water, and the consequent breaking up of the field. In the writer's experience, however, in spite of these difficulties, the water-mounting gives decidedly the clearest results, and, with patience on the part of the operator, it is in many ways the most desirable method of mounting for photographic purposes. It is in fact the method employed in making most of the photomicrographs of powdered specimens that appear in the plates at the end of this volume, though a few were mounted in glycerin jelly, and the starches for the polarized-light pictures in Canada balsam. The sections of tissues shown in the plates were mounted some in glycerin and others in glycerin jelly.

Experience has shown that two degrees of magnification well calculated to bring out the chief characteristics of the spices and their adulterants in a photomicrograph are 125 and 250 diameters. The starches, which are the most common of any one class of adulterants, vary very widely in the size of their granules. With these the larger magnification of 250 has been found satisfactory, while the general appearance of the composite ground-spice itself under the microscope, as well as that of such adulterants as ground bark, sawdust, chicory, pea hulls, and the like, is best shown with the lower power of 125.*

* The degrees of magnification adopted in the originals of most of the photomicrographs illustrated in the accompanying plates are accordingly 125 and 250, but in the process of lithographing, the photographs were slightly reduced, so that the actual scales in the reproduction are 110 and 220 respectively.
that from an artistic and didactic standpoint the photomicrograph of a powdered sample is often disappointing, due to the fact that ordinarily much of the field is out of focus, unless a very simple homogeneous subject is photographed, as, for instance, starch. As compared with the carefully prepared drawing of a section, which shows minute details of structure, the photomicrograph portrays what happens to be in focus.

SUMMARY OF MICROCHEMICAL REACTIONS FOR IDENTIFYING CELLULAR TISSUE AND CELL CONTENTS. BASED ON BEIJERINCK.*

<table>
<thead>
<tr>
<th></th>
<th>Indigo in Potassium Iodide</th>
<th>Chloride of Zinc</th>
<th>Indigo and Sulphuric Acid</th>
<th>Currammella</th>
<th>Potassium Hydroxide</th>
<th>Concentrated Sulphuric Acid</th>
<th>Schulte's Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose, cell substance</td>
<td>Yellow to brownish</td>
<td>Violet</td>
<td>Blue</td>
<td>Dissolves</td>
<td>Solves up</td>
<td>Dissolves</td>
<td>Dissolves</td>
</tr>
<tr>
<td>Lignin, wood substance</td>
<td>Yellow</td>
<td>Yellow to brownish</td>
<td>Yellow to brownish</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Dissolves easily</td>
</tr>
<tr>
<td>Middle lamella, intercellular substance</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Insoluble</td>
<td>Dissolves easily</td>
<td>Dissolves easily</td>
<td>Dissolves easily</td>
</tr>
<tr>
<td>Suberin, cork substance</td>
<td>Yellow or brownish</td>
<td>Yellow or brown</td>
<td>Brown</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Starch</td>
<td>Blue</td>
<td>Brown yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>Reddish to violet</td>
<td>Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gums and resins</td>
<td>Reddish to violet</td>
<td>Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>Reddish to violet</td>
<td>Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Essential oils</td>
<td>Reddish to violet</td>
<td>Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>Reddish to violet</td>
<td>Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium oxalate crystals</td>
<td>Yellow to brownish</td>
<td>Yellow to brown</td>
<td>Brown</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>Yellow to brownish</td>
<td>Yellow to brown</td>
<td>Brown</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
</tr>
</tbody>
</table>

|                      | Phenylhydrazine Acid | Indol. | Ferric Acetate or Sulphate | Alkanna Tincture | Hydrochloric Acid | Acetic Acid. | Millon's Reagent.
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose, cell substance</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
</tr>
<tr>
<td>Lignin, wood substance</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
</tr>
<tr>
<td>Middle lamella, intercellular substance</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
</tr>
<tr>
<td>Suberin, cork substance</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
</tr>
<tr>
<td>Starch</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
</tr>
<tr>
<td>Protein</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
</tr>
<tr>
<td>Gums and resins</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
</tr>
<tr>
<td>Fat</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
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<tr>
<td>Essential oils</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
</tr>
<tr>
<td>Tannin</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Uncolored</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
</tr>
<tr>
<td>Calcium oxalate crystals</td>
<td>Blue to green</td>
<td>Blue to green</td>
<td>Blue to green</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
<td>Solves up</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>Blue to green</td>
<td>Blue to green</td>
<td>Blue to green</td>
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<td>Solves up</td>
</tr>
</tbody>
</table>

* Microscopical Investigation of Vegetable Substances, page 156.
† When treated with the reagent, suberin forms masses of crystal acid, soluble in ether, alcohol, or chloroform.

While the analyst examines microscopically the ordinary powdered spice, for example, he constantly moves with his hand the fine adjustment-screw, bringing into focus different parts of the field successively. This
The base is a solid iron plate upon which the microscope rests (any microscope may be used with this camera), and above which the camera bellows is supported on two solid steel rods. The bellows is supported at either end on wooden frames.

The ground glass is provided with a central transparent area, formed by cementing a cover-glass upon the ground glass, and permits the accurate focusing of the most delicate detail by means of a hand magnifying glass. The vertical rods supporting the bellows are attached to metal arms, immovably fixed to a horizontal axis, thus permitting the camera to be tilted to any angle from vertical to horizontal. It is fixed at the desired angle by means of heavy hand-clamps.

In use the camera is placed in a vertical position and the microscope adjusted on the base so that its tube will coincide with the opening in the front of the camera. The connection between microscope and camera is made light-tight by means of a double chamber, which permits considerable vertical motion of the tube of the microscope without readjustment.

A jointed telescoping rod is attached to the upper end of the camera to act as a support, giving perfect steadiness when in a horizontal position, and folding down parallel with the bellows so as to be out of the way when in any other position.

Amplification.—The vertical rods are graduated in inches for determining the amount of amplification, and to show when the ground glass is at right angles to the optical axis. The following simple rule for determining the amount of amplification will give sufficiently accurate results. When photographing without the eyepiece, divide the distance of the ground glass from the stage of the microscope in inches, by the focal length in inches of the objective used. When photographing with the eyepiece, proceed as above and multiply the result by the quotient obtained by dividing 10 by the focus in inches of the eyepiece used.
Adjustment and Manipulation.—The microscope can be placed in any position desired, and the camera adjusted to it. The bellows can then be raised and the microscope used as though no camera were present. When an object is to be photographed, the bellows may be slid into position without in any way disturbing the arrangement of light or object, the final focusing on the ground glass being effected quickly by means of the fine adjustment-screw of the microscope. The exposure having been made, observation through the microscope may be continued without interruption by simply raising the bellows again.

When a water-mounted specimen is to be photographed, the camera and microscope tube should be vertical instead of inclined as shown in the cut.

The camera is best kept in a dark room where the exposures are to be made, the source of light being a 16- or 32-candle-power electric lamp, preferably provided with a ground-glass bulb. The light from this lamp is first carefully centered by moving the reflector of the microscope.

In making pictures, for instance, of the magnification of 250 diameters, the objective, having an equivalent focus of $\frac{1}{4}$ inch, may be used in combination with the one-inch ocular, with the ordinary tube length of microscope. For a lower power, such as 125 diameters, the same objective is employed, but the eyepiece is left out, it being found necessary in this case to remove the upper tube of the microscope, which ordinarily carries the eyepiece, as otherwise the size of the field to be photographed would be restricted. In each case a diaphragm is used in the microscope stage, having an opening of about the same size as that of the front lens of the objective. By means of a stage micrometer scale, the proper position of the camera back is previously determined to give the required magnification.