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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A MEETING of the Society was held at 3.30 p.m. on Wednesday, February 3rd, 1943, at the Chemical Society's Rooms, Burlington House, London, W.1. The chair was taken by Dr. H. E. Cox, Vice-President, and the following papers were presented and discussed:—"A New Colorimetric Method for the Determination of Boron," by Denis Dickinson, M.Sc., F.I.C.; "Methods of Analysis for the Purposes of the Cake and Flour Confectionery (Control and Maximum Prices) Order, S.R. & O., No. 2103 of 1942," by J. R. Nicholls, D.Sc., F.I.C. (representing the Government Laboratory) and R. T. Colgate, D.Sc., F.I.C., L. S. Fraser, B.Sc., A.R.C.S., A.I.C., and E. B. Hughes, D.Sc., F.I.C. (representing the Cake and Biscuit Manufacturers War Time Alliance, Ltd.); "The Photometric Estimation of Potassium by a Modification of the Jacobs–Hoffman Method," by Alfred Eden, M.A., Ph.D., F.I.C.

NEW MEMBERS

THE following have been elected members of the Society:—Peter Alexander, B.Sc. (Lond.); Charles Ernest Gubbins; Stanley Albert Price, B.Sc. (Lond.); Robert Clarence Spalding, B.A. (Cantab.); Gilbert Bruce Wright.*

New Methods for the Separation of Nickel and Cobalt from Iron and of Nickel from Cobalt†

BY B. S. EVANS, M.C., M.B.E., D.Sc., F.I.C.

THE pptn. of cobalt from a mixture with iron and nickel is relatively simple,[1] but the pptn. of nickel (even with glyoxime) in presence of much cobalt is complicated by the stability of the cobalt glyoxime complex tending to make pptn. of the nickel incomplete; also, it partly peptises the nickel ppt., making filtration difficult. In presence of iron the difficulties are enhanced, as iron and cobalt together form a black ppt. with glyoxime. On the other hand, initial pptn. of the iron with ammonia or even with zinc oxide, results in partial co-pptn. of cobalt and nickel, for which single re-pptn. is a very imperfect cure.[2] Potassium ferricyanide, recommended for pptn. of nickel,[3] would doubtless also be applicable to cobalt, but as a reagent it has disadvantages; it is easily reduced, and formation of ferrocyanide would lead to complications; also, it appears to require the addition of ammonium fluoride (to be avoided when glass vessels are used). Potassium cobalticyanide has advantages as a substitute for ferricyanide; it ppts. practically the same cations ($Cu^{\cdot\cdot}$, Ni, Co, Zn, Cd, $Fe^{\cdot\cdot}$, etc.), but is so stable an anion that it can be recovered unchanged from the cations with which it is combined; moreover, it does not introduce any cobalt into the final product. It ppts. $Fe^{\cdot\cdot}$, but not $Fe^{\cdot\cdot\cdot}$ and, as nitric acid does not interfere with the pptn. of nickel or cobalt, this acid can be used to ensure that all iron is in the ferric condition. A single pptn. will then free the cobalt and nickel from all but a trace of (presumably adsorbed) iron. The nickel and cobalt in the ppt. (which contains a little alkali chloride) may be determined by any suitable method; that given below is a modification of the differential titration method previously published.[5]

COBALTICYANIDE METHOD.—For the differential titration finish, or for separate determination of nickel and cobalt, use separate measured portions of the original soln. for the pptn. Oxidise the iron in each portion by boiling with nitric acid, neutralise approx. with sodium hydroxide and add to each 50 ml of 10% potassium nitrate soln. Dilute to *ca.* 100 ml with water and add 10 ml of syrupy phosphoric acid and 5 ml of conc. nitric acid. Next add, with stirring, to each portion (previously cooled) excess of a strong soln. of potassium cobalticyanide in 5% nitric acid, which has been boiled, cooled and filtered. Stir in some filter-paper and leave for 45–60 min. (Addition of 20 ml of 1% gum arabic soln. and a little amyl alcohol before the pulp accelerates and improves the subsequent filtration.) Filter off the ppts. (cobalt, rose-pink; nickel, jade green, mixtures, of intermediate colour) on pulp pressed well down, wash thoroughly (*e.g.*, 8 times) with hot 5% potassium nitrate soln. and re-transfer filter and ppt. to the original beaker, rinsing in with 100 ml of 5%

* Through the Scottish Section. † Communication from the Armaments Research Department.

sodium hydroxide soln. Stir the mixture well, heat to b.p., cool, add 2–3 ml of 20 vol. hydrogen peroxide, stir and leave for 10 min. (not longer) with occasional stirring. Filter off the pulp containing the mixed hydroxides on a small pulp filter and wash with hot 5% sodium chloride soln. Finally, replace the previous receiver by a clean flask and dissolve the ppt. through the filter with a boiling mixture of dil. (1 : 1) hydrochloric acid and saturated sulphur dioxide soln. and wash the filter well with water. The two flasks (one for each of the two lots taken) will now contain all the nickel and cobalt as chlorides with not more than a trace of iron and the cobalticyanide ion will have been completely eliminated.

TITRATION OF TOTAL NICKEL + COBALT.—Evaporate the contents of the first flask almost to dryness, cool and just ppt. the nickel and cobalt with sodium hydroxide. Re-dissolve the ppt. in the min. quantity of nitric acid (sp.gr. 1·2) and add 20 ml of saturated borax soln. If no ppt. forms, too much nitric acid was used; in that event induce pptn. by cautious addition of the min. quantity of dil. sodium hydroxide soln., shaking after each addition. Add 10 ml of 4% potassium iodide soln. and titrate with standard potassium cyanide soln. until the ppt. has just dissolved. The amount of cyanide used is not critical but affords a measure of the excess required. Read the vol. of cyanide (V) and run in excess to bring the total vol. to 1·3 V + 5, leaving the cyanide soln. in the burette for subsequent continuation of the titration. Add 10 ml of 10% sodium carbonate soln., filter off any slight ppt. of iron and wash it with cold water. Draw or force a rapid stream of air through the filtrate for 6 min., add a mixture of 10 ml of dil. (1 : 1) ammonia and 25 ml of 20% ammonium chloride soln., titrate the liquid, which should now be bright, just to opalescence with silver nitrate soln., and just remove the opalescence by adding cyanide soln. dropwise from that left in the burette. Read the total amounts of cyanide and silver nitrate solns. added, and ascertain the value, in terms of silver nitrate, of the cyanide by adding to the titrated liquid a known vol. of cyanide soln. and titrating to the same end-point with the silver nitrate soln. The difference between the silver equiv. of the cyanide used in the determination and the silver actually added is a measure of the nickel and cobalt present.

TITRATION OF NICKEL ALONE.—Boil down the contents of the second flask to *ca.* 10 ml, add excess of ammonia, filter off the trace of ferric hydroxide and wash it with hot water. Then re-dissolve the ppt. in hydrochloric acid, re-ppt. with ammonia, filter into the original filtrate, wash the ppt. with hot water and reject it. Evaporate the mixed filtrates to *ca.* 50 ml and make slightly ammoniacal, add 5 ml of 10% sodium cyanide soln., then a few drops 20 vol. hydrogen peroxide and boil for 5 min. to convert the cobalt into cobalticyanide. Add 20 ml of 20% ammonium chloride soln. and continue boiling for 15 min. Cool slightly, add 10 ml of dil. (1 : 1) ammonia and 10 ml of 20 vol. hydrogen peroxide, and boil very gently for 10 min. with the cover glass on. Cool, add 10 ml of dil. (1 : 1) ammonia and 10 ml of 4% potassium iodide soln., dilute to 200–300 ml with cold water and titrate in the same manner as with the mixed nickel and cobalt; *i.e.*, determine the silver equiv. of the cyanide in the titrated liquid (it varies slightly in different solns.), and take the difference between the silver equiv. of the cyanide used and the silver actually added as a measure of the nickel present (1 ml of a soln. of 5·792 g of pure $AgNO_3$ per litre ≡ 0·0010 g of nickel).

If equal vols. of the original soln. were used for the two titrations the difference corresponds to the cobalt, and, if the above silver nitrate soln. has been used, this figure × 0·803 ≡ mg of cobalt.

RESULTS.—Analysis by this procedure of synthetic mixtures of nickel, cobalt and iron gave the following results:

Ni taken g	Co taken g	Fe taken g	Titration ml			Ni found g	Co found g
0·0099	0·0100	0·1000	Ni+Co	28·30 − 6·25	= 22·05	0·00985	0·0098
			Ni	12·35 − 2·50	= 9·85		
			Co		12·20		
0·0248	0·0050	0·1000	Ni+Co	42·25 − 11·15	= 31·10	0·0248	0·0051
			Ni	31·25 − 6·45	= 24·80		
			Co		6·30		
0·0049	0·0250	0·1000	Ni+Co	44·60 − 8·30	= 36·30	0·0048	0·0253
			Ni	7·65 − 2·85	= 4·80		
			Co		31·50		

Two samples of plating solns. containing iron were analysed by this method and the cobalt was also determined directly by the nitroso-naphthol method.[1] The following results were obtained:

	g per 1000 ml		
Sample	Nickel	Cobalt (by differential titration)	Cobalt by nitroso-naphthol methods
A	9·56	3·00	2·96
B	9·55	3·12	3·09

Recent Work on Differential Titration.—The cyanide used in the original work was the ordinary "double salt"; in a subsequent attempt to standardise the method further a 10% soln. of AnalaR potassium cyanide was used, and somewhat variable and perplexing results were obtained. Apparently pure potassium cyanide is somewhat anomalous, and this has led to the suggestion that it is not the cyanide but the *iso*cyanide. For this reason sodium cyanide was adopted in this work as the reagent for the conversion. The citric acid recommended in the original titration of the nickel[4] has been found to be not only useless but liable to lead to incorrect results and has therefore been abandoned.

SEPARATION OF NICKEL AND COBALT FROM THE SAME SOLUTION.—Occasionally it is necessary (*e.g.*, with a limited supply of sample) to separate nickel and cobalt from the same soln. This is not easy, but the following simple method effects a clean separation:—First remove any iron present by the foregoing or some other method. Add to the soln. containing the nickel and cobalt 20 ml of 20% ammonium chloride soln., excess of ammonia, 5 ml of 10% sodium cyanide soln. and a few drops of hydrogen peroxide. Boil the soln. for 5 min. to convert the cobalt into cobalticyanide, cool slightly, add 10 ml of dil. (1:1) ammonia and 10 ml of 20 vol. hydrogen peroxide and boil gently for 10 min. Then add a little more ammonia and about 0·5 g of solid dimethyl glyoxime, boil again for 1 to 2 min. and cool. When the liquid is quite cold, filter off the nickel glyoxime ppt. and wash with cold water. Deal with the ppt. in any of the ordinary ways, preferably by soln. in acid and subsequent titration with cyanide and silver. Neutralise the filtrate with hydrochloric acid and add 20 ml excess and then excess of 20% cupric chloride soln. Heat to boiling, cool, filter off the copper cobalticyanide on pulp and wash it with 5% hydrochloric acid containing 1% of cupric chloride. Suck off the excess of liquid with a pump, transfer filter and ppt. to a beaker, add 5 ml of conc. sulphuric acid and rinse the filter-funnel with conc. nitric acid into the beaker. Heat the beaker on a hot plate, making small additions of nitric acid when the contents blacken, until the organic matter of the filter is completely destroyed and the sulphuric acid is fuming strongly. The acid should then be dark blue, turning pink on cooling if cobalt is present in significant amount. Cool and take up the contents of the beaker with 100 ml of water, boil until solution is complete, cool and ppt. the copper by passing hydrogen sulphide. Filter off the ppt. and wash it with 5% sodium chloride soln.; boil the filtrate to remove all hydrogen sulphide and cool. Treat the soln. with sodium hydroxide until cobalt hydroxide is just pptd. Then, without delay, just redissolve the ppt. in saturated sulphur dioxide soln. added drop by drop, add 20 ml of saturated borax soln. and titrate as described above for mixed nickel and cobalt. A difference from the titration described is that the somewhat large amount of sodium sulphate present appears to have a peptising effect on the cobalt borate, so that only a slight haze instead of a ppt. appears on addition of borax. This eliminates the end-point of the initial titration, but as the presence of excess of cyanide is equally well indicated by the pronounced lightening of the colour of the soln., the matter is not important.* The following results were thus obtained with synthetic mixtures:

Nickel taken	Cobalt taken	Titration	Nickel found	Cobalt found
g	g	ml	g	g
0·01000	0·00985	Ni 12·35 − 2·35 = 10·00 Co 28·90 − 16·55 = 12·35	0·01000	0·00992
0·01000	0·00985	Ni 11·20 − 1·20 = 10·00 Co 21·75 − 9·45 = 12·30	0·01000	0·00988
0·01500	0·00492	Ni 16·70 − 1·80 = 14·90 Co 12·85 − 6·70 = 6·15	0·0149	0·00494
0·00500	0·01477	Ni 7·05 − 2·00 = 5·05 Co 26·35 − 8·00 = 18·35	0·00505	0·01474

* It may be preferable to filter off the cobalt hydroxide instead of dissolving it; it can then be dissolved through the filter into a clean flask with a small amount of nitric acid containing a little sulphur dioxide. The filtrate, after boiling, can be neutralised and treated with borax, and the determination finished as described. This treatment eliminates the sodium sulphate effect.

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A Colorimetric Method for Estimating the Narcotic Power of Hemp Drugs

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(*Read at the Meeting, November* 4, 1942)

THE dried flowering and fruiting tops of the female Indian hemp plant, *Cannabis sativa* L. (Fam. *Cannabinaceae*), are known as "ganja," and the resinous exudation as "charas." "Bhang" and "hashish" consist of the leaves, small stalks and fruits of the plant. Some or all of the drugs are legitimately used in different parts of India as intoxicants, and are produced and sold under excise supervision and control. In America the use of the drug under the name "marihuana," although officially prohibited, has spread so rapidly in recent years that special powers have been acquired to combat the evil.

Ganja is smoked in a specially constructed "chillum" or earthenware smoking pipe. Charas is taken in many ways, *e.g.*, smoked with tobacco, eaten in sweets, etc. Bhang or hashish is usually taken in sweetened drinks or in confectionery. Marihuana is generally blended with the tobacco of cigarettes. The effects of smoking are described by Dixon.[1] Cannabis, usually as extract or tincture, is used internally as an anodyne, sedative or hypnotic, and externally to alleviate pain, *e.g.*, in "corn cures." The drug shows wide variations in potency and loss of activity when stored in contact with air; it is no longer official in Great Britain or U.S.A.

On extraction with organic solvents, *e.g.*, light petroleum or chloroform, the drugs yield a resinous material, "cannabinone," to which the narcotic properties are due, the residue being physiologically inert. In 1847 Smith[14] found that the active principle of hemp drugs is contained in the high-boiling portion of the resin and that it is not an alkaloid. Later, Wood, Spivey and Easterfield[2] prepared a large physiologically active fraction from the distillate from the charas of the United Provinces (India) and subsequently isolated "cannabinol," $C_{21}H_{26}O_2$, a crypto phenol which forms an acetyl derivative, m.p. 75° C. The bulk of the product from Minnesota (U.S.A.) hemp was shown by Adams *et al.* to consist not of cannabinol, but of a new phenol "cannabidiol," $C_{21}H_{30}O_2$ or $C_{21}H_{32}O_2$. Jacob and Todd[6] showed that Indian hemp does not contain cannabidiol, although an isomeric phenol "cannabol" is present in addition to cannabinol. Neither cannabinol, cannabidiol nor cannabol in the pure state accounts for the characteristic narcotic properties of hemp.[3,4,5,6]

In 1940 Haagen–Smit *et al.*[7] isolated from the most active portion of the Minnesota hemp (b.p. 128–135° C. at 0·005 mm) a colourless crystalline compound, m.p. 128–129° C., which had more than 100 times the physiological activity of the crude drug. Not much information about this principle, "cannin," is yet available. Several tetrahydro-cannabinols have been synthesised[8] and shown to possess hashish activity.[12] Possibly the physiologically active portion of the hemp resin contains a number of such compounds, differing in the position of the ethenoid linkage, and cannin may be such a compound.[6]

Ganja usually contains 15–25% of resin and charas, 35–45%, but the potency of the drug cannot be judged from its resin content, as on continued contact with air the narcotic power of the resin generally diminishes and is finally lost. For this reason a simple method of estimating the narcotic power of hemp drugs is of importance. The methods in use at present depend upon characteristic symptoms developed in certain animals, *e.g.*, dogs or rabbits, to which the drug has been administered, the min. dose per lb. weight of the

animal necessary to produce the symptoms being regarded as an inverse measure of the potency.* In such methods it is assumed that the experimental animals, whether rabbits or dogs of the same weight, respond in the same degree to the same amount of the active principle of the drug—an assumption obviously open to criticism.

Russell, Todd *et al.*[13] consider it probable that the dog and rabbit tests will give comparable results in assessing the hashish activity of synthetic compounds and have no reason to doubt that the Gayer response in the rabbit and the ataxia in the dog are produced by the same principle.

In Bengal, reliance is placed on judgment by veteran smokers after smoking a portion of the sample. This is necessarily only a rough guide, although the uniformity of opinion about the same sample, as expressed by different smokers, is remarkable (*cf.* Table II, p. 72).

Ghose and Bhattacharya have described a polarimetric method, having found that the specific rotatory power of the resin in the drug is a measure of the narcotic power. It is difficult to accept this conclusion because of (*a*) the widely varying resin constituents of these drugs, and (*b*) interference by other optically active substances normally present in the drug.

The specific rotation of cannabinol is [−108°] and that of cannabidiol [−125°], so that it appears doubtful whether the optical rotation observed in a carbon tetrachloride hemp resin extract is a criterion of the physiological activity of the drug unless the ratio between these two compounds and the actual active principle is constant—an unlikely relationship if one considers the proportions in which essential constituents occur in other naturally occurring drugs, *e.g.*, opium.

Although the high colour of the extracts necessitates high dilutions for polarimetric measurements and correspondingly high multiplication factors, the polarisation method may be used to distinguish old and deteriorated drugs from fresh and fully active samples.

Several colorimetric methods have also been outlined in the literature, but these depend on the determination of the quantity of hemp resin present. It has been observed that both Beam's test[10] and the acetaldehyde vanillin test[11] for the identification of Indian hemp depend upon the presence of cannabidiol and not of cannabinol or the actual narcotic principle. Adaptations of these tests would therefore be of no value for estimating the narcotic power of the drug.

The method here described for the estimation of the narcotic power of hemp drugs is based on the observations that (*a*) the active portion of the resin is predominantly phenolic in character and that (*b*) cannabinol, the main inactive phenol present in Indian hemp, is not soluble in dilute alkali solns.

Experiments on a Colorimetric Method.—The phenols were separated from a carbon tetrachloride extract of the drug by dilute alkali, and it was found, by animal tests carried out at the Biological Standardisation Laboratory, Calcutta, that the residue (insol, in alkali) had little or no activity. The alkaline soln. was made up to definite vol., and an aliquot part was treated with diazotised *p*-nitroaniline. The resultant coupled dye, which separated readily, was filtered off and dissolved in a measured vol. of rectified spirit, and the depth of colour was compared with that similarly obtained from a standard sample.

It was found that (*a*) the colour is quite stable, its shades and intensity remaining constant and unimpaired after a week of exposure to air; (*b*) the depth of colour obtained from a sample is proportional to the weight of the drug taken, and (*c*) the depth of colour produced

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* The specific physiological number is the min. weight of ganja or charas resin in mg which will just produce intoxication in a dog (or any experimental animal) weighing 1 lb. and is obtained by dividing the least wt. of the resin in mg required to produce visible intoxication by the wt. of the animal in lbs. The dogs are starved for about 15 hrs., weighed and then dosed with a sample of resin mixed with milk, the dosage usually being approx. 0·5 mg of the resin per lb. of dog weight. A normal resin will generally cause slight intoxication in this proportion. The effect of the drug is to produce inco-ordination in the muscles, generally preceded by some degree of preliminary excitement, drowsiness and a fall in temperature. An intoxicated dog usually takes up a crouching attitude, there are involuntary motions in the hind quarters and the hind leg muscles appear to vibrate. The tail drops, even if it is raised. If these symptoms be very prominent, the dog is over-intoxicated and a fresh expt. has to be made on the same dog with a smaller quantity of the resin after a couple of days. To find out the min. quantity of resin that will just produce symptoms of intoxication, 2 or 3 trials are generally necessary. The dogs are usually examined for intoxication 3–4 hrs. after the resin has been administered.

The defects of the method, apart from the long time required for these expts. are that (1) the dogs become tolerant after some time and have to be changed, (2) some of the dogs show special idiosyncracies, and (3) minor ailments of the dogs may affect the results.

is progressively lowered when the sample is previously exposed to the atmosphere for increasing periods of time, parallel with the observation that the drug progressively loses its potency upon prolonged exposure in air.[13]

PROCEDURE FOR THE TEST.—Exhaustively extract 1 g of charas or 2 g of ganja in a Soxhlet extractor with 50–75 ml of carbon tetrachloride, transfer the extract to a separator and shake with 4 consecutive 20-ml portions of 0·5% aqueous sodium hydroxide soln., and collect the alkali extracts in a 100-ml measuring flask. Adjust the vol. to 100 ml with water and mix well (Solution A). Dissolve *p*-nitroaniline (0·2 g) in 5 ml of 5 *N* hydrochloric acid and cool in ice. To the ice-cold soln. add 5 ml of ice-cold 2% sodium nitrite (A.R.) soln., drop by drop, cooling all the time in ice. The diazotisation is rapid, as is evident from the disappearance of the orange-yellow colour of the soln. (Solution B). To 10 ml of (A) in a beaker cooled in ice, add the diazotised soln. (B). A red dye separates and the liquid becomes pale yellow. Saturate the soln. with common salt, allow the dye to aggregate, filter off in a sintered glass crucible, suck dry, wash with three changes of water and again suck dry. Dissolve the dye on the filter in 5–10 ml of rectified spirit (or colourless methylated spirit) added gradually, and finally rinse down with more spirit until the vol. is 25 ml. Match the colour in a Duboscq colorimeter or in Nessler glasses with that similarly obtained from a sample of standard potency—*i.e.*, a fresh sample from a reliable source.

RESULTS.—The samples of ganja and charas used in the following expts. gave the results shown in Table I.

TABLE I

Sample No.	Description	Resin %	Ash %	Acid-insol. ash %	Sp. rotn. of the resin
	Charas				
1/R	Suspected stock of charas from Neemuch Cantonment	38·1	40·6	28·0	—
2/R	Genuine stock of charas from Neemuch Cantonment	42·9	31·9	20·0	—
3/R	Duplicate genuine charas from Neemuch Cantonment	44·2	33·7	21·0	—
4/R	Charas sample of unknown origin	26·5	62·6	55·9	—
0/R	Genuine charas sample from Hoshiarpur	38·1	41·8	—	—
	Ganja				
5/R	Ganja sample of good stock from Nagpur	20·5	14·7	4·1	−85·4°
6/R	Ganja sample from Nagpur of unknown stock	12·8	15·7	6·7	−86·1°
7/R	Mixture of 50% of 5/R with 50% of inert matter*	—	—	—	—
8/R	Mixture of 75% of 5/R with 25% of inert matter*	—	—	—	—
9/R	Ganja sample of unknown origin	—	—	—	—

* The residue left after complete extraction with carbon tetrachloride.

Table II shows the results obtained by the procedure described, by experienced smokers' assessment (an accepted test in many Excise Laboratories in India) and by administration to dogs. Columns III and VI are based on the resin contents given in Table I.

TABLE II

Sample No. I	Ratio of intensity of colour: on drug as received II	Ratio of intensity of colour: for equal weight of resin III	Smokers' assessment: actual IV	Smokers' assessment: relative to standard V	Smokers' assessment: for equal weight of resin VI	Potency by dog test 1/(sp.ph. No.) VII
			Charas			
10/R (Standard)	1·00	1·00	0·75	1·00	1·00	1·00
1/R	0·81	0·81	0·63	0·83	0·83	1·00
2/R	1·12	0·99	0·75	1·00	0·89	0·95
3/R	1·10	0·95	0·63	0·83	0·73	1·09
4/R	0·69	0·99	0·50	0·67	0·96	0·70
			Ganja			
5/R (Standard)	1·00	1·00	0·75	1·00	1·00	1·00
6/R	0·84	1·35	0·75	1·00	*1·60*	1·06
7/R	0·50	1·00	0·50	0·67	1·33	1·00
8/R	0·75	1·00	0·38	0·50	0·78	1·06
9/R	0·53	—	0·38	0·50	—	—

From the results of 7/R and 8/R it is clear that the colorimetric method gave results proportional to the weight of drug taken. The charas samples 2/R and 3/R were duplicates from the same stock, and as such, the potency ratio as obtained colorimetrically, *viz.*, 112:110, *i.e.*, 100:98, should be nearer the truth than the smokers' assessment, 100:83.

INFLUENCE OF EXPOSURE TO AIR ON THE INTENSITY OF COLOUR PRODUCED.—In order to follow the change in activity of the drug stored in air, 3 g of the charas sample 10/R was exposed in a thin layer in a flat dish to atmospheric action. At successive intervals of a week and fortnight 1 g of the sample was removed, and the colour obtained as described above was compared with that similarly obtained from the standard (No. 10/R) preserved out of contact with air. It was found that the diminution in intensity was 10% after 1 week and 26% after 2 weeks. On the other hand, the alkali extract did not form any dye at all at the end of a fortnight, and the resin similarly exposed to air had lost the major portion of its activity. It is clear, therefore, that the active principle of *Cannabis indica* resin in its natural condition is slowly destroyed on exposure to air, rapidly when the resin had been freed from vegetable matter, etc., and still more rapidly in alkaline solution.

INFLUENCE OF ALKALI EXTRACTION OF THE RESIN ON ITS OPTICAL ACTIVITY.—One g of charas (10/R) was extracted with carbon tetrachloride, and the extract was made up to 100 ml; of this, 50 ml were used directly for determination of optical rotation (−0·20°). The other 50 ml were extracted with 4 successive portions (20 ml each) of 0·5% sodium hydroxide soln., and the carbon tetrachloride soln. was washed with water until free from alkali, and dried over calcium chloride and its rotation (−0·18°) was determined. The slight fall in rotation confirmed the findings of earlier workers, *viz.*, that the optical activity of cannabis resin is mainly due to compounds, such as cannabinol, which are physiologically inactive. The lowering of the optical activity of the resin on storage appears to be due to a change induced in the cannabinol, simultaneously with that which takes place in the active principle.

Further work on the alkali-sol. and insol. portions of the residue and improvements on the procedure is in progress.

CONCLUSIONS.—(1) The active principle of *Cannabis indica* resin can be extracted with 0·5% aqueous sodium hydroxide soln. from a carbon tetrachloride soln. (2) The alkali extract when coupled with diazotised *p*-nitroaniline produces a water-insol. dye. (3) The amount of the dye can be estimated colorimetrically and the results expressed in terms of potency of the drug. (4) The colorimetric results are more consistent than those obtained by tests on animals and the assessment by trained smokers. (5) The optical rotation of hemp resin appears to be largely due to the non-active components present in the drug. (6) It appears that the colorimetric procedure detailed in the report can conveniently be adopted for routine determinations of the narcotic power of hemp drugs, suitable standards being maintained.

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CENTRAL REVENUES CONTROL LABORATORY, NEW DELHI *August*, 1942

DISCUSSION

Dr. J. R. NICHOLLS, who, in the absence of the authors, read the paper on their behalf, said that this was the first occasion on which an attempt had been made to correlate activity of hemp drugs with a chemical test. In this country the drug was not official, but it was occasionally encountered in connection with the

Dangerous Drugs Act. This Act covered not only Indian hemp but also the resins and all preparations of which such resins form the base, and it did not matter whether or not the resins were active. In countries where the drug had a legitimate use it was highly desirable to have some knowledge of the activity of the resins. The production of certain narcotic symptoms in an animal was not necessarily a good guide to the physiological action in man. Although the authors had not shown that the results of their colour test were directly proportional to activity, there appeared to be a definite relation, and particularly important was the fact that samples inactivated by exposure failed to give a reaction. It was unexpected, however, that cannabinol, which was a phenol, was not extracted by dilute alkali under the conditions described. The test would doubtless be of value for rapidly evaluating samples.

Dr. G. V. JAMES has submitted the following comments on the paper:—(*a*) The authors do not appear to have tested pure tetrahydrocannabinol, which has been synthesised and to which Todd ascribes most of the physiological activity of the drug (Ghosh, Todd and Wilkinson (*J. Chem. Soc.*, 1940, 1121, 1393).* (*b*) The effect of cannabidiol on the test does not appear to have been investigated, possibly because the Indian drug contains so very little, but other varieties contain more, the amount diminishing as that of cannabinol increases. In acid conditions such as are obtainable in the laboratory but not in the stomach, cannabidiol undergoes cyclic transformation to give a mixture of optically and physiologically active tetrahydrocannabinols (Adams, *et al.*, *J. Amer. Chem. Soc.*, 1941, **63**, 1971, 1973, 1977). (*c*) The colorimetric test can only be acceptable if consideration is given to the latest chemical and pharmacological developments, such as those of Alles (*J. Pharm. and Exp. Therap.*, 1942, **76**, 21).

Studies in the Analytical Chemistry of Tungsten: II. Separation of Tungstic and Phosphoric Acids

BY D. A. LAMBIE, B.Sc., A.I.C.

THE first Section of this series[1] dealt with the recovery of tungsten from sulphate solns. This paper initiates the study of the problems created by the well-known tendency of tungstic acid towards complex-formation with other acid radicals. Acidification of alkaline tungstate solns. containing, *e.g.*, phosphate, arsenate or silicate produces the corresponding heteropoly acids. These complexes are decomposed by excess of alkali, the salts of the simple acids being regenerated.

Although it has long been known that a complex is formed when solns. containing tungstate and phosphate are boiled with a mineral acid, so that none or only part of the tungstic acid is pptd., it is only a few years since Simpson, Schumb, and Sieminski[2] proved this to be 12-phosphotungstic acid, which they pptd. as cinchonine phosphotungstate, and this on ignition gave $P_2O_5.24WO_3$.

The 2 expts. below confirm that ppts. obtained in acidified solns. of tungstate and phosphate give phosphotungstic anhydride on ignition.

(1) The validity of the claim that double pptn. with *o*-tolidine provides a complete separation of tungstic from phosphoric acid[3] was investigated. A soln. containing 0·1996 g of WO_3 as sodium tungstate and 0·1920 g of P_2O_5 as orthophosphate was diluted to 300 ml, neutralised to methyl orange with 1:1 hydrochloric acid, and treated with 5 ml of excess acid. It was heated to boiling and treated with 25 ml of *o*-tolidine soln. (2 g of base dissolved in 100 ml of 2·5% v/v hydrochloric acid). The liquid was allowed to cool and the crystalline ppt. was collected with the aid of filter-pulp on a close-textured filter-paper, washed with dilute *o*-tolidine soln, ignited and weighed. The green residue weighed 0·2059 g, and obviously was not pure WO_3. It was fused in platinum with sodium carbonate, the melt was leached with water, and the tolidine pptn. was repeated. Again, the ignited residue was green, but weighed only 0·1964 g. Analysed by a method given below, it was found to contain 0·1904 g of WO_3 ($\equiv$ 0·0049 g of P_2O_5 in $P_2O_5.24WO_3$) and 0·0046 g of P_2O_5. Hence this method fails, not only to separate tungstic from phosphoric acid, but also to give a quantitative tungsten recovery.

(2) Ammonium or potassium salts give white crystalline ppts. with acidified solns. of phosphotungstic acid; the same reactions are obtained with acidified solns. of phosphate and tungstate, more readily on warming. A soln. of 0·2624 g of WO_3 and 0·0126 g of P_2O_5, as tungstate and phosphate respectively, was diluted to 100 ml, acidified with 1:1 hydrochloric acid (5 ml excess), and treated while boiling with 50 ml of 20% ammonium chloride soln. The white crystalline ppt. was left to settle overnight, collected on a fine-textured filter, and washed with cold 2% ammonium chloride soln. This ppt., analysed

* A specimen of tetrahydrocannabinol could probably be obtained from Professor A. F. Todd, F.R.S., of Manchester, or Dr. F. Bergel of Roche Products Ltd., Welwyn Garden City, Herts.

by the magnesia method given below, contained 0·1997 g of WO_3 (≡ 0·0051 g of P_2O_5 in $P_2O_5.24WO_3$) and 0·0048 g of P_2O_5.

THE TANNIN-CINCHONINE METHOD.—Since phosphotungstic acid is decomposed by alkali, it was thought probable that a quantitative separation of the two anions would be secured by the tannin-cinchonine method,[4] in which the pptnt. is added to an alkaline tungstate soln. To prove this, an acidified soln. of phosphotungstic acid was treated with tannin soln. This gave no ppt., only a slight brownish colour; when, however, the phosphotungstic acid soln. was rendered alkaline with ammonia prior to the addition of tannin and then acidified, the characteristic brown tungsten complex was pptd.

The P_2O_5 content of a soln. of phosphoric acid (1·6 g of "Analar" acid in 1 litre of water) was determined by Epperson's method[5]; 25 ml contained 0·0265 g and 0·0263 g of P_2O_5; mean, 0·0264 g (≡ 0·00106 g per ml). A sodium tungstate soln., prepared from 1 g of tungstic oxide in a soln. of 1 g of sodium carbonate, was filtered and diluted to 500 ml. The WO_3 content, determined on 25 ml portions by the tannin-cinchonine method, was 0·0501 g of WO_3 per 25 ml (calc., 0·0500 g).

Varying amounts of these solns. equiv. to the quantities of oxides given in Table I were mixed and treated with 50 ml of strong hydrochloric acid (25 ml in Expt. 5). The clear solns. were evaporated to about 5 ml and diluted with 50 ml of 20% ammonium chloride soln., except in Expt. 1, in which, after evaporation to 10 ml, 10 ml of strong nitric acid were added, the evaporation continued to 5 ml, and the soln. diluted to 50 ml with water prior to addition of the ammonium chloride. A heavy white ppt. of ammonium phosphotungstate separated in each expt. except No. 5. Excess of ammonia was then added, and the white ppt. dissolved. The tannin-cinchonine pptn. was carried out on the clear solns. In all the tests the ignited ppt. had the characteristic yellow colour of pure tungstic oxide. The ppts. from Expts. 3–7 were fused with sodium carbonate, the melts were dissolved in water, and the resulting solns. were passed through a small filter to remove insol. matter (trace of Fe_2O_3), and tested for P_2O_5 as follows:—Citric acid (0·1 to 0·2 g), a slight excess of dil. hydrochloric acid, and 10 ml of magnesia mixture were added, and then a 5 ml excess of strong ammonia. In no instance was any phosphoric acid detected. Table I shows that a satisfactory separation and recovery of tungstic oxide was achieved.

TABLE I

Expt.	Tungstic oxide taken g	Phosphorus pentoxide taken g	Tungstic oxide found g	Error g
1	0·0501	0·0212	0·0504	+0·0003
2	0·0501	0·0212	0·0501	nil
3	0·0501	0·0212	0·0500	−0·0001
4	0·0501	0·0212	0·0497	−0·0004
5	0·0020	0·0212	0·0021	+0·0001
6	0·1002	0·0212	0·0995	−0·0007
7	0·1002	0·0265	0·1005	+0·0003

It frequently happens in practice that material containing tungstic and phosphoric acids is obtained in soln. after fusion with alkali. Tests were therefore made to confirm the applicability of the above method under such conditions.

Weighed amounts of dehydrated sodium phosphate (52·8% of P_2O_5 by Epperson's method) and tungstic oxide were fused in platinum with 4 g of sodium carbonate, and the melt was dissolved in water. The tungsten was pptd. by the tannin-cinchonine method, and the ppt. was collected, washed, ignited and weighed. It was then fused with sodium carbonate, and the soln. of the melt was pptd. with magnesia mixture as in the first series of tests. When a ppt. separated it was collected, washed, and dissolved in dil. nitric acid, and any P_2O_5 was pptd. with molybdate mixture and determined by Woy's method.[6] In preliminary tests with about 0·2 g of each oxide, up to 0·5 mg of P_2O_5 was retained in the ppt. and a small quantity of tungstic oxide occasionally failed to be pptd. (in one expt. 1·7 mg). Further work showed that with amounts of WO_3 of the order of 0·1 to 0·2 g, the amount of cinchonine soln. used in the tannin-cinchonine method should be increased from 5 to 10 ml to ensure complete tungsten pptn.

Finally, the following procedure was adopted. Treat the alkaline soln., containing not more than 0·2 g of WO_3, at 60° C. with 50 ml of 20% ammonium chloride soln. and tannin in fresh soln. (10 times the weight of WO_3 presumed to be present, but not less than 0·5 g), and render acid (5 ml in excess) by slow dropwise addition of 1:1 hydrochloric acid during

vigorous agitation. After treatment with filter-pulp dad cinchinone soln. dropwise, continuing the stirring; add 5 ml for quantities up to 0·1 g, and 10 ml for 0·1 to 0·2 g. When the liquid is cold, collect, wash and ignite the ppt. Results:

TABLE II

Expt.	Tungstic oxide taken g	Phosphorus pentoxide taken g	Tungstic oxide found g	Error g
8	0·1021	0·2126	0·1023	+0·0002
9	0·0142	0·2133	0·0143	+0·0001
10	0·0512	0·1070	0·0514	+0·0002
11	0·2010	0·2120	0·2008	−0·0002
12	0·2010	0·1065	0·2008	−0·0002
13	0·0071	0·2143	0·0073	+0·0002

In each expt. the tungstic oxide was found to be free from P_2O_5.

Attempts to recover the phosphoric acid in the filtrate from the tannin ppt. showed that it was necessary to destroy the organic matter by wet combustion (nitric acid and potassium chlorate). In one test (No. 14), 0·1996 g of WO_3 and 0·1920 g of P_2O_5 were taken, and 0·2001 g of WO_3 and 0·1920 g of P_2O_5 were found.

MAGNESIA METHOD.—To avoid the somewhat troublesome wet combustion, I turned my attention to the magnesium ammonium phosphate pptn., but little information on this separation process was found in the literature. Kehrmann[7] proposed pptn. of the phosphoric acid by dropwise addition of magnesia mixture to the ammoniacal soln. of tungstate and phosphate; this method is given by Scott[8]. Hillebrand and Lundell[9] prescribed pptn. from ice-cold ammoniacal tartrate or citrate soln. and dropwise addition of magnesia mixture. In both methods the need for several repetitions of the pptns. to achieve a satisfactory separation is emphasised.

In my own tests the use of citrate or tartrate was not entertained, as it would have rendered the subsequent recovery of the tungsten more difficult. Theoretical considerations led to the view that conditions favouring the slow formation of a coarsely crystalline ppt. should provide the best separation, *viz.*, by dropwise addition of magnesia mixture to the well-agitated hot soln.

A soln. of sodium tungstate prepared by dissolving tungstic oxide in sodium carbonate soln. was standardised as before (1 ml ≡ 0·007983 g of WO_3). A phosphoric acid soln. was also made (1 ml ≡ 0·007680 g of P_2O_5). Varying amounts of these mixed solns. equiv. to the wts. of WO_3 and P_2O_5 in Table III, were acidified with 1:1 hydrochloric acid and treated with 25 ml of 20% ammonium chloride soln. In most expts. a ppt. of ammonium phosphotungstate began to separate within a few min. The soln. was neutralised to methyl orange with 1:1 ammonia, and 5 ml in excess were added. After dilution to 200 ml the liquid was heated just to boiling, removed from the heat, and treated with magnesia mixture added dropwise from a burette (80 to 100 drops per min.) during vigorous agitation until pptn. began; the addition was then interrupted. The soln. was stirred for a few min. until the ppt. crystallised; more magnesia mixture was then added until present in excess, but in no expt. was there less than 10 ml. Finally, 5 ml of strong ammonia (sp.gr. 0·88) was added, and the liquid was left overnight. The ppt. was collected, washed with 5% v/v ammonia, dissolved in dilute hydrochloric acid, and re-pptd. by Epperson's procedure, and the determination was completed by the usual method. The tungsten in the first filtrate was determined by means of tannin and cinchonine; the filtrate from the repptn. was tested for tungsten by the same method, with negative results. As will be seen from Table III, a satisfactory separation was achieved in a single pptn. (the re-treatment was necessary to obtain a phosphate precipitate of correct composition).

TABLE III

Expt.	Tungstic oxide taken g	Phosphorus pentoxide taken g	Tungstic oxide found g	Phosphorus pentoxide found g	Tungstic oxide error g	Phosphorus pentoxide error g
15	0·1996	0·0036	0·1995	0·0030	−0·0001	nil
16	0·1996	0·1920	0·2002	0·1917	+0·0006	−0·0003
17	0·0798	0·0384	0·0798	0·0386	nil	+0·0002
18	0·0798	0·0768	0·0800	0·0767	+0·0002	−0·0001
19	0·0399	0·1920	0·0396	0·1911	−0·0003	−0·0009
20	0·0032	0·1920	0·0033	0·1913	+0·0001	−0·0007

A final test was made to determine the effect of tungsten on Epperson's procedure (*loc. cit.*) for pptng. magnesium ammonium phosphate.

The same vols. of tungstate and phosphate solns. as used in Expt. 16 were diluted to 100 ml, 10 ml of 1:1 hydrochloric acid and 20 ml of magnesia mixture were added, and the phosphoric acid was pptd. by dropwise addition of strong ammonia to the ice-cold liquid. During the addition of ammonia a white turbidity of ammonium phosphotungstate developed while the liquid was still markedly acid, and still persisted when neutrality was reached. When the magnesium ammonium phosphate ppt. was dissolved in dil. acid prior to re-pptn., a white residue remained which on ignition left a green compound weighing 0·0040 g (phosphotungstic anhydride). The determination was completed as usual, giving 0·3139 g. $Mg_2P_2O_7$ ($\equiv$ 0·2002 g of P_2O_5), but obviously contaminated with tungsten (yellowish-green patches). The two filtrates from the magnesium ppts., analysed separately for tungsten, gave 0·1529 and 0·0248 g of WO_3 respectively (total, 0·1777 g). There was thus a large negative error (0·0219 g) in the tungstic acid and a positive error (0·0082 g) in the phosphoric acid determination. Epperson's method is thus inapplicable in the presence of tungstate.

SUMMARY.—The *o*-tolidine method of v. Knorre does not accomplish a quantitative separation of tungstic and phosphoric acids, but accurate results are obtained by the tannin-cinchonine method. A method has been worked out whereby phosphoric acid can be completely separated from tungstic acid by a single pptn. with magnesia mixture.

I wish to thank Dr. W. R. Schoeller for his stimulating criticism.

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THE COOPER TECHNICAL BUREAU, BERKHAMSTED *November*, 1942

Methods of Analysis for the Purposes of the Cake and Flour Confectionery (Control and Maximum Prices) Order, 1942

THE methods of analysis for carrying out the provisions of this Order (S.R. & O., No. 2103, 1942), outlined by Dr. Nicholls, Dr. Colgate, Mr. Fraser, and Dr. Hughes (*cf.* ANALYST, 1943, **68**, 48), were discussed at the meeting on February 3, 1943.

Mr. C. H. F. FULLER commented on the problems of the bakery chemist in controlling the fat and sugar content of bakery products. Tables were given showing the extent of variation that might be expected in some of the major ingredients used:—

	Fat content, %		
	Max.	Min.	Average
Dried whole egg ..	44·6	39·7	41·6
Cake margarine ..	87·2	79·2	84·1
Pastry margarine ..	93·1	87·4	90·4
Wheatmeal flour ..	2·5	1·6	2·0

	Sugar content, %		
	Max.	Min.	Average
Glucose	40	31·3	35·2
Skim milk powder ..	36	31·5	33·7
Treacle	79·2	62·8	71·7
Raisins, Currants, Sultanas	71·0	60·5	65·0

Baking loss could vary from 7% to 16% and moisture loss on storage had also to be considered because the fat and sugar content was determined on the product as sold to the consumer.

Mr. R. D. MASON, as representative of a technical sub-committee of a large trade federation, said that he fully agreed with what Dr. Nicholls had said about the multiplicity of copper reduction methods for the

determination of sugar, and, as each method had its own particular faults and advantages, he thought it very desirable that there should be agreement on one, and possibly an alternative, method. He fully endorsed Mr. Fuller's statement, that apparently no attention had been paid to the vexed question of the amount of loss that occurred in bakery products after baking, and he urged that great consideration should be given to this when assessing the final results. A small practical point, suggested by Mr. Hugh Moir of Messrs. Beatties Bakeries, Ltd., was that, when dealing with heavily fruited cakes, it was desirable to incorporate a small amount of washed purified sand during the preparation of the solution. The abrasive action of the sand broke up the fruit particles and made their contents more accessible to the solvent action of water.

Mr. D. M. FREELAND said that he was pleased that dialysed iron had been mentioned as a clarifying agent in the proposed methods for sugars. The usefulness of this reagent was too little known; it was neutral in reaction and caused no risk of sucrose inversion if a cake extract had to stand for some time before the reducing sugars were determined; it avoided a second filtration, which was required with lead clarifiers. There was the probability that potatoes would soon become an ingredient of cakes, and he had found that, in the analysis of cakes containing potato, the clear sugar extract obtained with the use of dialysed iron showed an enhanced dextrose figure. This might be due to soluble reducing substances other than sugars from the potato. However, due correction for this could be made by the manufacturer who knew the quantity of potato in his cake mixings. The factor 0·97, suggested for correction of vol. due to insol. solids when 10 g of cake were taken for sugars, was likely to assist those manufacturers producing cakes containing sugar and fat percentages towards the top limits of the Statutory Order. The suggestion of Mr. Mason to use sand for disintegration of the cake to aid extraction of sugars would require the establishment of a new correction factor for undissolved solids.

From the production viewpoint, the moisture of the cakes was a useful figure to determine. As a rule, the moisture retained increased with size in the same type of cake. A useful guide to sugar content could be obtained by determining the total dissolved solids from the sugar extract by evaporation of 10 ml in a tared metal dish on a water-bath, drying for 1 hr. at 102° C. and weighing the residue. This applied especially to those cakes bearing the max. quota of sugars, and it was a necessary operation for the determination of a correction factor for undissolved solids in a particular cake.

With regard to the fat determination, the only satisfactory method using ether alone as solvent was to hydrolyse the cake material with dil. hydrochloric acid, neutralise, filter and extract the fat from the dry filter in a continuous extraction apparatus. By this means a perfectly clear fat extract was obtained. The mixed solvent method proposed cleared the way for an easier wet process of extraction, but he doubted if it offered any advantage over dry extraction after hydrolysis.

Dr. R. T. COLGATE said that the meeting would regret the absence of Dr. Hughes, who had collaborated energetically in drawing up the methods presented by Dr. Nicholls and also in the preparation of a long list of fat and sugar percentages in the materials used by cake and flour confectioners at the present time, and of which Mr. Fuller had given some examples. Manufacturers were meeting with very real difficulties in complying with the Order, and the Chemists' Panel of the Cake and Biscuit Manufacturers' War Time Alliance had drawn up the list to arrange that their products complied with the Order, in respect of fat and sugar %. Recommendations were also made advising manufacturers to work to figures 2% lower than the limits stated in the Order. These figures and recommendations had already been published in a number of trade journals. The methods that Dr. Nicholls had given needed no comments, as they were standard and well tried. A small amount of sugar might be produced in the course of manufacture by conversion of starch, but this would be covered if manufacturers worked to the figures recommended.

Mr. G. H. MASON said that one of the difficulties of most bakers was that the fat and sugar percentages of ingredients supplied to them were unknown and might vary. Also, it was impossible in practice to ensure that the distribution of ingredients with a high sugar % was reasonably even throughout small units, *e.g.*, Swiss rolls. Nevertheless, the trade would do its utmost to comply with the requirements of the Order.

Notes

SETTING THE SPEKKER ABSORPTIOMETER

IT is necessary to re-set the Spekker Absorptiometer fairly frequently during routine analysis when it is read by Vaughan's method.[1] This can be avoided by setting the instrument with a neutral filter in the right-hand side of the instrument and by periodically taking readings of the filter. The difference between the actual reading of the filter and the standard reading is added to observed readings when the reading is below, and subtracted when it is above, the standard.

The use of this filter enables the setting to be made in the region of accurate reading of the drum scale and near the same sensitivity at which the "unknowns" are read. It is also possible to obtain a wider range of calibration than can be obtained by Vaughan's method of setting.

The optical density of the filter should be periodically checked. It has been found convenient to do this against a standard solution of approx. the same concentration of material that is normally met with in analysis, as this will tend to minimise any variations in the calibration of the instrument.

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J. H. HIGH

December, 1942

RAPID ESTIMATION OF PARAFFIN ADULTERANTS IN TURPENTINE

TREAT 10 ml of the sample in a 250-ml flask with 30 ml of a mixture of 20 ml of conc. sulphuric acid and 10 ml of water. Connect the flask with a condenser and heat gently over a Bunsen flame until reaction is complete. Then raise the temp. and distil as completely as possible (a second distillation with sulphuric acid is seldom necessary if the residue has been thoroughly carbonised). Transfer the distillate to a separator, run off the oily layer, mix it with 30 ml of conc. sulphuric acid and again distil. Treat the oily layer in a separator twice with sulphuric acid and twice with water and measure the vol.

Allowance for the polymers of turpentine seldom exceeds 0·3 ml. In a typical expt. a mixture of 10 ml of turpentine and 1 ml of paraffin oil (sp.gr. 0·825) yielded 1·3 ml (sp.gr. 0·837).

LABORATORY, 59, VICTORIA STREET, LIVERPOOL, 1 F. ROBERTSON DODD

January, 1943

Ministry of Food

STATUTORY RULES AND ORDERS*

1942—No. 2426. Order, dated November 26, 1942, amending the Threshed Feeding Peas (Control and Maximum Prices) (Great Britain) Order, 1942. Price 1d.

In this Order, "Processed feeding peas" means feeding peas which have been crushed, broken or split or from which the skin has been removed, but does not include any feeding peas which have been polished, hand-picked, cleaned or dressed preparatory to sale as whole peas." *In par.* (2) *of Art. 6 of the Principal Order* (*S.R. & O., 1942, No. 1491*), *the word "feeding"* is inserted in proviso (*c*) after "processed."

No. 2521. Order, dated December 10, 1942, amending the Feeding Stuffs (Regulation of Manufacture) Order, 1942. Price 2d.

The following amendments are made to the Principal Order (*as amended*) (*S.R. & O.*, 1942, *Nos.* 2031 *and* 2258; *cf.* ANALYST, 1942, **67**, 391; 1943, **68**, 15). (*a*) *After the definition of Concentrate* (*Art. 5*) *insert* " 'D" flour' means flour designated as such by or on behalf of the Minister"; (*b*) *after the definition of Molassed feeding stuff insert:* " 'Wheat by-products' means any by-products obtained in the production of flour by milling and includes millers' offals and wheat offals, both as defined in Part II, Schedule IV to the Fertilisers and Feeding Stuffs Act, 1926, as amended, and fine wheatfeed, straight run bran, coarse bran, fine bran, pollards, middlings, and wheat germ." (*c*) *In par.* 3, *Part A, Schedule I, for "wheatfeed" substitute "Wheat by-products other than wheat germ."* (*d*) *For Part A of Schedule II, substitute Part A of the Schedule to this Order.* (*e*) *In Column* 2 *of Part B of Schedule II, for the words "Fine wheatfeed" read "Wheat by-products other than wheat germ."* (*f*) *In par.* 3 *of Part A, of Schedule III, for "Wheatfeed" read "Wheat by-products other than wheat germ."* (*g*) *In par.* 4 *of Part A, Schedule III, for "Flour, damaged and feeding (including sack shakings, flour sweepings and flour scrapings)" read " 'D' Flour; Flour sweepings and sack shakings."*

The Schedule, Part A, gives the conditions as to Compounds for National Cattle Foods, Pig Food, Horse Food, Poultry Foods and Chick Food. "D" flour appears as a constituent or optional constituent of all the Compounds except National Horse Food. Coarse wheat bran is wheat bran containing more than 9·5% of fibre. Where cod liver oil is prescribed as an ingredient of a Compound other substances may be used, provided that (*a*) *their vitamin D content is not less than that of cod liver oil, and* (*b*) *they are warranted in writing by the maker to be as fully effective for poultry in accordance with the Chick Test of the British Standards Institution. The conditions relating to the oil, albuminoid and fibre contents found on analysis are subject to the limits of variation specified in the Fertilisers and Feeding Stuffs Regulations,* 1932, *as amended.*

No. 2663. General Licence, dated December 30, 1942, under the Meat Products and Cooked Meat (Control and Maximum Prices) Order, 1942. Price 1d.

Until March 31, 1943, *the Minister authorises any person to buy or sell free from the restrictions as to meat content and max. prices imposed by Arts.* 3 *and* 8 *of the Principal Order* (*S.R. & O.*, 1942, *No.* 1381) *any meat or fish paste manufactured and packed prior to July* 27, 1942, *ready for retail sale in a non-airtight container made wholly or mainly from glass or earthenware.*

1943—No. 12. Order, dated January 2, 1943, amending the Cake and Flour Confectionery (Control and Maximum Prices) Order, 1942. Price 1d.

The Order defines "unlicensed catering business." In the proviso to Art 2 *of the Principal Order,* (*S.R. & O.*, 1942, *No.* 2103) *nothing prohibits:* (i) *the manufacture of any cake in connection with an unlicensed catering business;* (ii) *the addition by a caterer of any edible substance to any cake for sale as a meal or part of a meal served in the course of his catering business*

— **No. 13. General Licence, dated January 2, 1943, under the Soap (Licensing of Manufacturers and Rationing) (No. 2) Order, 1942.** Price 1d.

Medicated soap may be supplied or obtained free from the restrictions of the Principal Order (ANALYST, 1942, **67**, 291), *provided that it is prescribed by a registered medical practitioner for the needs of a particular person. Medicated soap" means* (*a*) *any of the following complying with the particular formula in the B.P. or B.P. Codex:—Sapo Animalis B.P., Sapo Durus B.P., Sapo Mollis B.P., Sapo Kalinus B.P.C., Sp. Saponatus B.P.C. Sp. Saponis Kalini B.P.C., Liq. Saponis Aethereus B.P.C., Liq. Saponis Antisepticus B.P.C., Liq. Saponis Olei Cocois B.P.C.;* (*b*) *any soap containing as a min. 1% w/w of Mercuric Iodide or 4% w/w of Ichthyol, or 2% w/w of Salicylic Acid, or 2% w/w of Resorcin, or 5% w/w of Sulphur.*

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* A summary of some Orders. Italics signify changed wording. Obtainable from H.M. Stationery Office.

Legal Notes

The Editor would be glad to receive particulars of cases of legal or scientific interest.

FOOD AND DRUGS PROSECUTIONS UNDER THE MERCHANDISE MARKS ACT

EVANS *v.* CLINICAL PRODUCTS, LTD.

ON Dec. 9, 1942, an appeal was heard in the High Court before the Lord Chief Justice, Mr. Justice Humphreys and Mr. Justice Tucker against the decision of the Welshpool (Montgomeryshire) justices, who had dismissed a charge against the respondents for having unlawfully applied a false description on the printed label of a packet of goods (a drug) sold by them, contrary to Sec. 2 [1] (*d*) of the Merchandise Marks Act.

At the hearing of the case before the justices, counsel for the defendants, without discussing the alleged offence, contended that the prosecution must fail because the inspector (the present appellant) who purchased the article for analysis by a Public Analyst had not complied with the requirements of Sec. 70 [1] of the Food and Drugs Act, as to division of the sample into 3 parts, etc.

Mr. Justice Humphreys, giving the decision of the Court, said that the argument for the respondent was largely based upon the hardship that was likely to be caused to an accused being prosecuted in respect of an article which he had sold and which he had had no opportunity of having analysed by his own analyst. An examination of Secs. 60 and 70 of the Food and Drugs Act, however, showed how very limited was the protection afforded to persons selling food and drugs, even under that Act. It was only in cases where the purchaser bought with the intent of submitting the sample to a Public Analyst that he was required to inform the vendor and to divide the sample into 3 parts. There appeared to be nothing in the section to prevent a sampling officer from analysing the sample himself or sending it to a private analyst and subsequently instituting proceedings under the Act against the vendor for selling an article not of the nature, substance and quality demanded, or under Sec. 6 for giving with the article sold a label calculated to deceive. In that event, however, oral proof must be given of all the facts, including the composition, since the certificate of the analyst would not be available in evidence.

The safeguards provided by Sec. 70 were mainly designed to meet the requirements of Sec. 81 "in any proceedings under this Act" to prove the composition of the impugned article by mere production of the certificate of a Public Analyst. Non-compliance with the requirements of Sec. 70 was not a bar to any proceedings except under the Food and Drugs Act. There was nothing in the Merchandise Marks Act, 1887, referring to the procedure laid down in the then existing Food and Drugs Act, 1875, Sec. 14 of which contained directions as to sampling and division of the sample into 3 parts, almost identical with those in the 1938 Act. On the other hand, the Margarine Act, 1887, passed in the same year as the Merchandise Marks Act, provided in Sec. 12 that all proceedings under the Act were to be the same as in Secs. 12–24 of the Food and Drugs Act, 1875, so incorporating Sec. 14. Again, it should be noted that Sec. 9 of the Merchandise Marks Act, 1926, conferring on an official of a local authority compulsory powers for the purchase of food, contained directions for dividing the sample, somewhat similar but differing essentially from those in Sec. 14 of the 1875 Act. Similar provisions were to be found in the Public Health (Preservatives, etc. in Food) Regulations, 1925, as amended in 1926 and 1927, where the provisions of Sec. 14 of the Food and Drugs Act, 1875, are expressly incorporated. The Food and Drugs Act, 1938, formed a code in itself, and in his Lordship's opinion Sec. 70 was a part of the procedure code of that statute and had no relation to the Merchandise Marks Act, 1887.

For these reasons the Court allowed the appeal and remitted the case to the justices to be decided on its merits.

The Lord Chief Justice (Viscount Caldecote) and Mr. Justice Tucker concurred.

The Iron and Steel Institute

SECOND REPORT OF THE MOULDING MATERIALS SUB-COMMITTEE*

THE results of an investigation of possible alternatives to bentonite for the bonding and revivifying of moulding and core sands are described. It has been shown that the bond strength, the ratio of dry strength to green strength, and the range of retention of green strength with increasing moisture content of clays, etc., containing montmorillonite clay substance are almost directly proportional to their montmorillonite content. Bentonite consists essentially of a montmorillonite mineral containing sodium, which is the active constituent, and α-quartz, which is merely a diluent. There are no deposits of bentonite in Britain, but fuller's earth consists substantially of a montmorillonite mineral containing calcium, with calcium carbonate as diluent. Most of the other British clays contain as their active constituent kaolinite, which is a less efficient binder in green sand and clay mixtures than montmorillonite minerals.

FULLER'S EARTH SUBSTITUTES FOR BENTONITE.—With the collaboration of The Fuller's Earth Union, Ltd., selected and treated varieties of fuller's earth occurring in Somerset and Surrey (to which the general designation of Fulbond has been given) have been submitted to laboratory and foundry tests and are now being used extensively to replace bentonite. Fulbond can also be used as a suspensory agent in silica paint in place of bentonite. Apart from fuller's earths, the only British clays so far found to contain much montmorillonite occur in the London clay measures, but these clays also contain a high proportion of siliceous silt, which lowers their bonding power. Details of mechanical tests on mixtures of sand with Colbond (a finely-ground plastic clay from Eire), Benbond (from Essex), Distribond and other English clays, together with the chemical analyses of the clays, are given in a series of tables.

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* Paper No. 3—1942 of the Steel Castings Research Committee (submitted by the Moulding Materials Sub-Committee). Pp. 62, 1942.

X-RAY STUDIES OF CLAYS.—It has been shown (Partridge, *J. Soc. Arts*, 1939, **87**, 227, 255, 283) by X-ray examination that there are two groups of clays. One contains *ca.* 40% of alumina and is of the kaolin type ($Al_2O_3.2SiO_2.2H_2O$); the other contains a higher proportion of silica to alumina, and is typified by pyrophyllite and montmorillonite ($Al_2O_3.4SiO_2.nH_2O$). These clays are built up of layers of hydrated silica and alumina. The structure of the individual layers is the same and the difference between the clays lies in the order and relative proportions in which the layers are arranged. When water is added to montmorillonite it is absorbed in the form of sheets of water mols., thus lengthening the *c* axis of the crystal. It has been suggested, however, that only the first 4 mols. of water enter the cell, the remainder becoming attached to the exterior oxygen surfaces of the particles of clay, the looseness of the attachment permitting easy movement of the particles, which thus become plastic.

X-Ray powder photographs (reproduced in the Report) of various bentonite substitutes show the calcite and quartz lines sharper than those given by montmorillonite, so that the proportions of those constituents appear higher than they really are. The amount of kaolinite in Distribond I, and the amount of montmorillonite in 2 English clays were below the visibility limit of the X-ray.

STEEL MOULDING SANDS.—The investigation has involved grinding and admixture of selected clays with selected sandstones. Some of the sandstones contained 7–12% of partly weathered felspathic matter, and these form a useful base for steel moulding sands; incorporated with 10–20% of clay, they yield products with good general properties and can be diluted with silica sands to give a range of strength and permeability. Weathered sandstones, *e.g.*, those of the Wolsingham (Durham) district, contain a high proportion of "clay" from the decomposition of included felspathic minerals, and, when thoroughly broken down, provide a satisfactory moulding sand. Detailed tables of chemical and mechanical analyses and of the results of tests for green strength and permeability are given. A modified apparatus for the determination of air permeability is described and illustrated.

CORE BINDERS.—Investigations have been made with the aim of reducing the consumption of linseed oil and maize starch products used as binding materials for core sands. It has been found, *e.g.*, that half the linseed oil in a core mixture can be replaced by Truline binder (a synthetic resinous material) without detriment to the strength and collapsibility of the core.

Madras: Report of the Chemical Examiner for 1941

IN his Annual Report Dr. S. Rajagopal Naidu, F.I.C., states that 1685 cases were investigated (1765 in 1940).

HUMAN POISONING.—Of the 432 cases examined (427 in 1940) poison was detected in 230, including opium 43; oleander 26; alcohol 20; datura or mydriatic alkaloids 15; cyanide 12; oduvan 5; madar juice 5; copper or copper sulphate 20; mercury salts 10; nitrites 8; sulphuric acid or sulphates 10.

ANIMAL POISONING.—Poison was detected in 13 of the 33 cases investigated: arsenic 10, aconite 1, oleander 2.

ACTION OF CERTAIN MOULDS ON ARSENIC.—Moulds of most common occurrence in Madras were tested, *viz.*, *Aspergillus glaucus*, *Penicillium glaucum* and *Rhizopus nigricans*. Solns. containing more than 0·10% of arsenic (as As_2O_3) were toxic to these moulds; at lower arsenic concns. growth occurred. *A. glaucus* converted arsenic into volatile compounds with a garlic odour (Gosio gas) even after several sub-cultures, whereas *P. glaucum* and *R. nigricans* lost their original slight activity on sub-culturing. The activity of *A. glaucus* can be demonstrated on arsenical glucose agar or moist sterilised bread. With quantities of 50 to 100μg of arsenic (as As_2O_3) on white bread *ca.* 75% of the arsenic volatilised after 10–15 days, the remainder being fixed in the medium. In one expt. the arsenic lost was accounted for by absorbing the volatile compounds in sulphuric acid and determining the arsenic by the Gutzeit method.

TOXICITY OF PANSUPARI.—The expts. described in the last Report (ANALYST, 1941, **66**, 462) were continued. Intravenous injection of the essential oil of betel leaf into an anaesthetised dog caused an immediate fall in blood pressure and marked depression of respiration resulting in death.

MICROSCOPY OF *Nux Vomica* BARK.—The bark contains a layer of cork cells with a brown pigment. The cortex consists of thin-walled parenchymatous cells, some containing prismatic crystals of calcium oxalate. On the inner side of the cortex there is an unbroken band (*ca.* 0·1 to 0·15 mm broad) of a layer of sclerenchymatous cells with thick striated pitted walls. The bast cells contain innumerable prismatic crystals of calcium oxalate, most of them surrounded by smaller satellite crystals. The medullary rays are *ca.* 5 cells wide. The scanty bast fibres (*ca.* 0·8 mm long, 15μm wide) have a narrow lumen. A few (mostly simple) starch granules (*ca.* 4μm in diam.) occur in both cortex and bast. Sections of the bark mounted in dil. (1:1) nitric acid become orange-red or, if mounted in vanadic sulphuric acid, purple.

SALIVA STAINS.—A piece of cloth, suspected of having been used as a gag, showed under ultra-violet light a faint fluorescence similar to that of saliva dried on cloth. Chemical tests also indicated the presence of amylase, phosphate and chloride.

Diastatic Activity (*Lintner value*) *of Saliva.*—The diastatic activity of different specimens of saliva was studied by the method recommended by the Ministry of Agriculture (Britain) (ANALYST, 1938, **63**, 542), sufficient quantities being taken to give a final value of 20–25 for 5 ml of Fehling's soln. The results showed that the Lintner value varied for different persons and for the same person at different times of the day. The values for fresh saliva were: max. 7·6 (2070 on total solids); min., 0·08 (20 on total solids); aver. *ca.* 2 (*ca.* 350 on total solids). Drying saliva on cotton reduced the value by *ca.* 65% in 1 day, by 85% in 2 days, and by 96% in a week.

IDENTIFICATION OF HAIR.—In 18 cases pieces of hair adhering to weapons were identified as human and in 1 case as sheep's hair.

Differentiation of Sheep's and Goat's Hair.—It is sometimes difficult to distinguish between these when only a small specimen is available. Examination of the medulla may then be helpful. The hair is warmed in 10% caustic soln. on a slide until the first bubble appears (*cf.* Hanausek and Winton, "*Microscopy*

of Technical Products," 1st Ed., p. 134) and examined. The medullary cells in sheep's hair appear like a honeycomb with rounded cells, whilst goat's hair shows a network of flattened cells.

Hairs from the same Person.—Even when two specimens of hair are exactly alike in all respects, it is not possible to be certain that they came from the same person, but when the characteristics differ decidedly the hairs probably came from different persons, although the hair may be of different tints (apart from grey hair) and vary in the relative width of medulla in the same individual. In India, shades of hair are not so varied as in temperate climates. Tints are best observed by mounting the hair in water and examining it under the microscope; ultra-violet light is of little value for establishing identities. Observations are made whether a dark tint of hair is a mixture of blue and black or brown and black, and so forth, and the average diameter and medullary index are noted, any tapering being recorded. The size and shape of cross-sections (prepared as previously described, ANALYST, 1941, **66**, 463), the ratio of long diameter to short diameter, the thickness of the cuticle and the appearance of the medulla are also noted. Consideration of all these factors may show whether two specimens of hair could possibly have belonged to the same person.

Determination of Fat on Hair.—A micro Soxhlet apparatus was devised for the micro-determination of fat or oil on hair. It comprised a glass tube of 3 mm bore bent twice to form a siphon shaped like "**N**," 5 cm high and prolonged 5 cm at its lower end. The upper limb was cut off at the middle of the "**N**" and joined to a tube, *ca.* 7·5 cm long and of 8 mm bore, this wider tube being blown out slightly above the point of junction, so as to held a plug of asbestos or de-fatted cotton. From 20 to 30 mg of hair are weighed out into the apparatus, which is fixed so that its lower end enters the mouth of a micro flask kept on a hot plate. A separating funnel, bent twice at right angles and with its tip drawn out nearly to a capillary, is arranged to deliver ether into the upper opening of the apparatus at such a rate that siphoning occurs each time the ether has evaporated. After sufficient siphoning the ether is completely evaporated and the flask is cooled and weighed. It is then rinsed out several times with light petroleum, the last traces of this are driven off, and the flask is again weighed. The difference between the two weights gives the weight of oil or fat. The petroleum washings are evaporated to dryness, and the residue is examined under ultra-violet light and by chemical tests. Sesame oil (commonly used in Madras) may be detected by Baudouin's test, using cane sugar in hydrochloric acid as the reagent. Applied in this way, the test will detect sesame oil in carbon tetrachloride soln. (1 : 500). The amount of oil thus determined ranged from 2–4% on the hair of different persons. Test expts. with known weights of oil added to hair gave satisfactory results.

FORGED HOROSCOPE.—A horoscope purporting to have been written 45 years ago was on brownish paper which was pliable, not brittle, when folded. Ths presence of turmeric was revealed by the starch granules and the curcumin reaction with sulphuric acid. Lime was also detected. The writing was in blue-black ink which, when treated by Mitchell's test with 5% oxalic acid soln., was partly bleached, while the dye diffused. It was therefore concluded that the writing was not more than 10 years old at the outside and that the paper had been discoloured by application of a mixture of lime and turmeric.

EFFECT OF BURIAL ON COTTON AND GUNNY.—The expts. previously described (ANALYST, 1941, **66**, 463) were continued, the pieces of fabric being buried at depths of 6 in., 1 ft., 2 ft. and 3 ft. and the soil watered daily. Disintegration after 1 month was greatest in the pieces buried at 6 in. and least at the greater depths. Both fabrics had been attacked by moulds (mostly of the *Aspergillus* type), ciliate protozoa and soil nematodes.

CUT TELEPHONE AND TELEGRAPH WIRES.—In cases of theft of telephone wires the question is sometimes raised whether suspected pieces of wire were cut with a particular pair of pliers. The following method of examination is useful. The pliers are secured in a vice with one of the cutting edges horizontal and facing upwards. A long narrow sheet of lead, *ca.* 1 or 2 mm thick and as broad as the cutting edge is long, is pressed firmly by means of a flat piece of wood against the cutting edge. The sheet is then drawn at right angles so as to leave on its surface lands and grooves corresponding with the irregularities in the cutting edge. The lands and grooves thus obtained are compared under a comparator microscope with those on the cut end of the wire. The process is then repeated with the other cutting edge. If torsion has been used during the cutting such markings on the wire may not be present.

The British Pharmacopoeia, 1932: Alterations and Amendments

THE following Amendment of the B.P., 1932, is published at the request of the Secretary to the British Pharmacopoeia Commission.

THE SCHEDULE

EXTRACTUM CASCARAE SAGRADAE SICCUM.—When Extractum Cascarae Sagradae Siccum, or Dry Extract of Cascara Sagrada, is prescribed or demanded an Extract prepared according to the following formula may be dispensed or supplied:—Mix 900 g of cascara sagrada, in coarse powder, with 4000 ml of boiling water, and macerate the mixture during 3 hours. Then transfer it to a percolator, allow it to drain, and exhaust it by percolation, using boiling water as the menstruum and collecting about 5000 ml of percolate. Evaporate the percolate to dryness, reduce the extract to a fine powder, and add sufficient starch, dried at 100° C., to make the product weigh 300 g. Mix the powders thoroughly and pass the Extract through a fine sieve.

FERRI SULPHAS EXSICCATUS.—The requirement for content of $FeSO_4$ is changed from "not less than 80%" to "not less than 77%."

PARAFFINUM MOLLE ALBUM.—When White Soft Paraffin is prescribed, Yellow Soft Paraffin may be dispensed. Yellow Soft Paraffin may be used in place of White Soft Paraffin in making the preparations of the British Pharmacopoeia.

January 11, 1943.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determining the Degree of Cooking in Cereal Products. G. H. Benham. (*Cereal Chem.*, 1942, **19**, 597–605; *J. Inst. Brewing*, 1943, **49**, 39.)—The "alkali number" of a starch or starch cereal (*i.e.*, the ml of 0·1 *N* sodium hydroxide neutralised by 1 g of the sample during 1 hr. digestion at 100° C. under specified conditions; Schoch and Jensen, *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 531) affords an empirical test of the amount of "depolymerisation" undergone during cooking. Expts. described included the examination of various raw and cooked starches, flours and cereal products. The alkali numbers obtained ranged from 7·4 (raw maize starch) to 30 (toasted cereal). Flours gave higher values than the corresponding raw starches, probably owing to the added effect of the proteins. All the true raw materials examined gave alkali numbers below 17, whilst all cooked, pre-heated, flaked or toasted products gave 19 or over. Whilst the value of the Schoch and Jensen method is confirmed, the following modification will give results agreeing within ±0·1 in duplicate determinations. Grind the sample to pass a 60-mesh sieve and digest 0·5 g for exactly 1 hr. at 100° C. (boiling water-bath) with 25 ml of 0·4 *N* sodium hydroxide. Then titrate immediately with 0·2 *N* sulphuric acid, using phenolphthalein as indicator. Make the determination in triplicate, using a blank in which distilled water is substituted for alkali, and calculate the alkali number as twice the titration results for the digested sample and the blank, divided by the dry weight in g of the sample taken.

Comparative Pharmacognosy of *Potentilla anserina* L. and *Potentilla argentea* L. H. W. Youngken, Jr., and E. B. Fischer. (*Amer. J. Pharm.*, 1942, **114**, 372–387.)—*Potentilla anserina* L. has recently been sold contaminated with the closely related sp. *P. argentea*. Authentic specimens of each plant have therefore been examined in the entire, broken and powdered form, and the following characters have been noted:

P. anserina

Roots.—Short or long, adventitious, fibrous or filiform; upper portions solid and fleshy. Numerous root scars and slight longitudinal wrinkles; annular marks and circular scars usually absent. Fractured surface; white or brownish spongy cortical layer and light brown to grey porous woody zone.

Secondary roots show outer layers of cork cells with scattered tannin and resin inclusions, a secondary cortex with intercellular spaces with, in older material, a concentric pattern and alternate compact and loose parenchyma with simple and compound starch grains, resin and tannin deposits appearing in transverse section as concentric circles, and rosettes of calcium oxalate. Stelar tissue narrower than cortical tissue.

Stems.—Creeping, tortuous, stoloniferous and flower-bearing. Stoloniferous stems are bristly-pubescent to sub-glabrous; flower-bearing stems are silky-pubescent. A single flower occurs at the apex. Outer layer of suberised and occasionally lignified epidermal cells with long curled uniseriate, non-glandular hairs, 1 or 2 layers of hypodermal cells with resin and tannin deposits, a narrow zone of loose cortical cells, and a large stelar region. Wood fibres scanty.

Leaves.—Compound; glabrous or pubescent petioles with opposite, alternate oblong-ovate to linear-ovate leaflets up to 6 cm long, with dentate, ciliated margins, obtusely rounded apices and oblique bases. Venation: pinnate, reticulate, impressed on upper surface, upraised with long appressed or raised silky hairs on lower surface. Primary veins reach margin. Large amounts of tannin in epidermis and mesophyll; palisade cells contain large rosettes of calcium oxalate.

Hairs.—Non-glandular, straight with thick cutinised walls on upper surface. Two types on lower surface; long and tortuous with large lumina, and short and straight with narrow lumina.

Flowers.—Petals longer than the outer calyx whorl. Achenes: round, sometimes angular, lenticular or ovate.

P. argentea

Roots.—Primary conical with tortuous and filiform lateral roots. System solid, without spongy, nodose or swollen tuberous portions. Fissures few and not deep. Fractured surface: solid ring of white to yellow wood and darker soft collapsed surface.

Cork tissue wider. Concentric rings not formed by intercellular spaces or the tannin; stelar tissue occupies most of the root cylinder. Secondary xylem forms an inner and outer banp of lignified vessels and wood fibres, giving the cross-section a ringed appearance.

Stems.—Densely covered with stipular remains; on lower stems numerous nodes from which arise pedicels forming multiflowered corymbs or panicles. Older stems are brown to purple-black and glabrous; the brown to greenish-yellow younger stems may be sparsely pubescent. Outermost epidermal cells, not lignified. Numerous long curling to serrate-edged, non-lignified, non-glandular hair on pedicels. Hypodermal cells large and slightly lignified in basal stems. In younger stems each cell of the narrow compact cortex contains resin forming a ring round the inner wall, giving a false fibrous appearance. The stele occupies most of the stem and has a band of pericyclic fibres with thick wall and narrow lumina; wood fibres, numerous.

Leaves.—Digitately compound; long and short silvery pubescent petioles with cuneate to obovate leaflets up to 2 cm long, with pinnately divided margins not ciliated but turned under. The bases are tapering to cuneate rather than oblique. Veins often do not reach margin.

Hairs.—Four types occur: (1) long tortuous, non-glandular, with thin walls, (2) long, glandular with thick serrated walls, (3) short, bristly non-glandular with cutinised walls, (4) short, brown glandular with uniseriate stalks and a 2-celled globular head.

Flowers.—Petals shorter than the outer calyx whorl. Achenes: pear-shaped with a prominent keel.

The chief diagnostic structures are the leaf hairs, the vessel elements (up to 100μ in diam. in *P. anserina*, rarely more than 70μ in *P. argentea*), the tracheids, wood fibres and sclerenchymatous fibres (more abundant in *P. argentea*), and the deposits of resin and tannin (more abundant in *P. anserina*). A. O. J.

Ammonia Content of Meat. J. Gerencsér. (*Közl. Osszehas. élét-es kórtan Köréből.*; *Index to Literature, Food Invest.*, 1942, **14**, 2.)—The ammonia contents of beef and pork obtained direct from the slaughter-house and from shops, and its rate of increase during storage, have been determined. No significant variations in the proportions of ammonia in different muscles of the same animal or in the muscles of animals of different age, sex or fatness were found. It is not possible to estimate the time of slaughtering from the amount of ammonia present. When the ammonia content of the muscle exceeds 30 mg per 100 g Eber's hydrogen sulphide test* will confirm decomposition.

American Oil Chemists' Society's Standards for Oils and Fats. (*Oil and Soap*, 1942, **19**, 140; *B.C.A.*, 1942, B, II, 435.)—The Oil Characteristics Committee of the A.O.A.C. have issued the following "A.O.C.S. Recommended Standards" for 7 oils and fats together with information on trade grades and special tests.

Oils	d_{25}^{25}	n_D^{25}	Iodine val. (Wijs)	Sap. val.	Unsap. %, Max.
Whale (blubber) ..	0·910–0·920	1·470–1·477	110–135	185–202	2·0
Neatsfoot ..	0·907–0·912	1·464–1·465	66–76	190–199	1·0
Teaseed	0·909–0·920	1·466–1·470	80–90	188–196	1·5
Oiticica	0·970–0·978	1·500–1·512	140–180	185–193	1·0
Fats	$d_{15·5}^{99}$	n_D^{40}			
Cacao butter ..	0·856–0·864	1·453–1·458	35–40	190–200	1·0
North American lard	0·858–0·864	45°–52° (Zeiss)	46–70	195–202	1·0
Beef tallow ..	0·860–0·870	46°–49° (Zeiss)	35–48	193–202	0·8

Browne heat test for oiticica oil, max. 17 min.; Bömer value for lard (ether), 71.

Totaquine: Its Source and Uses. Revised U.S.P. XII Standards. Anon. (*Amer. J. Pharm.* 1942, **114**, 350.)—At a conference at Washington on Sept. 1, 1942, it was shown that totaquine, which is essentially a mixture of alkaloids of varying composition obtained from cinchona bark, has definite value as an antimalarial agent, but that the cinchona barks now available, chiefly from Central and S. America, are exceedingly variable in their ratio of quinine to other alkaloids, and that no barks from these sources have the high quinine content of the cultivated barks of the East Indies. In view of these facts, the U.S.P. XII specifications for totaquine were altered to read as follows:—Totaquine is a mixture of alkaloids from the bark of *Cinchona succirubra* Pavon and other suitable species of *Cinchona*. It contains not less than 7% and not more than 12% of anhydrous quinine, and a total of not less than 70% and not more than 80% of the anhydrous crystallisable cinchona alkaloids, the designation "crystallisable alkaloids" referring to cinchonidine, cinchonine, quinidine and quinine. Totaquine of higher quinine content may be reduced to the official standard by admixture either with totaquine of a lower content or with any of the diluents permitted for powdered extracts under *Extracta*. A. O. J.

Effect of the Method of Manufacture on Milk of Magnesia. M. O. Holland. (*Amer. J. Pharm.*, 1942, **114**, 114–133.)—Milk of Magnesia (U.S.P.XI) increases in alkalinity on standing at room temp. and at high temp. Whether made by (*a*) the double decomposition or (*b*) the hydration method, it did not increase appreciably in *p*H when stored at high temp. in quartz or Pyrex containers, but when stored in flint glass at 95°–100° C. (*a*) samples increased in *p*H from 10·18 to 12·66 in 260 hr. and (*b*) others from 9·89 to 10·59 in 258 hr. Storage for 1 year in quartz flasks at room temp. caused the *p*H to rise from 10·18 to 10·53 for (*a*) but caused no appreciable rise for (*b*); in flint glass the corresponding changes were (*a*) 10·18 to 11·92 and (*b*) 9·89 to 10·1. Addition of 0·1% of citric acid prevented any change in alkalinity in either (*a*) or (*b*) samples during 1 year's storage at room temp.; at high temp., the behaviour of (*a*) and (*b*) was identical. The following expt. showed that no buffering effect existed in the filtrate from (*b*) in so far as stabilisation of (*a*) was concerned:—400 g of (*b*) (*p*H 10·35) were filtered, and the filtrate (*p*H 9·66) was evaporated to 20 ml in a hot air-oven at 100° C. Addition of this filtrate to 150 g of (*a*) (*p*H 10·12) gave a product with *p*H 10·13. The increase in alkalinity of this mixture in 211 hr. was comparable with the increase, under the same conditions, in the original material. When the products (*a*) and (*b*) were dried, extracted with water and filtered, the filtrate from (*a*) had a higher *p*H than that of (*b*) and had more titrable alkali. On addition to (*b*) of sodium hydroxide, approx. equal to the difference in titrable alkali, a product similar to (*a*) in effect on flint glass at high temp. was obtained. The theory is advanced that the additional sol. alkali in (*a*) exists either as a double salt or as an adsorbate and that the protective action of citric acid is due to formation of a buffer. This theory accords with hydroxyl ion adsorption occurring when hydroxide particles are pptd. and explains the slow rise in *p*H during 1 year's storage in quartz, since some desorption during slow growth of particle size might be expected. E. B. D.

* Eber's Hydrogen Sulphide Test.—Mix 10–25 g of the finely-divided material with 50 g of dil. (1:10) sulphuric acid in an Erlenmeyer flask loosely plugged with cotton wool, above which is placed a strip of filter-paper soaked in 10% lead nitrate soln. Leave the flask for 24 hr. in the dark at 12°–18° C. in a well-ventilated but not draughty place. Then gum the strip in a book and, after 30 min., compare the colour with standards ranging from faint brown (rarely yellow) to black. Eber's colour scale was an arbitrary one based on the equiv. amounts of hydrogen sulphide liberated from potassium sulphide solns. (No. 1 ≡ 0·002 mg; No. 8 ≡ 0·1 mg of H_2S). The mean values were somewhat higher for tuberculous than for healthy cattle (4·6 as against 3·5). Kidney tissue invariably gave high values (6 to 7) and showed no difference between healthy and diseased animals (*Z. Fleisch u. Milch. Hyg.*, 1897, **7**, 207; 1898, **8**, 41).

Biochemical

Determination of the Gas Content of Animal Tissue. P. F. Scholander. (*J. Biol. Chem.*, 1942, **142**, 427–430.)—The instrument shown in Figure 1 consists of a tube B with a sharp edge at one end

and a rod A at the other by which it can be gripped in the chuck of a hand-drill. Inserted into a groove in the tube is a steel spring (black) fastened by one end to a moveable sleeve C and sharpened at the other to form a knife; it is covered by the fixed sleeve D. With the aid of this instrument a piece of tissue such as muscle is excised as follows. The tube is drilled into the tissue with the knife pulled back, and the knife is then pushed across the opening, and the tube is rotated and pulled out. The cylinder of tissue so removed is dislodged with forceps, dried on filter paper, and transferred, together with a few ml of salt water, to the cylinder of a small, heavy, ice-cooled brass press (Fig. 2). This consists of a cylinder and a loosely-fitting piston. At the base of the cylinder are three fine steel channels formed by syringe needles soldered into three holes in the press. The three channels unite and open through a nozzle which has a fitting for a syringe tip. After squeezing out the salt water by hand, the press is placed between the jaws of a vice and the rest of the salt water and blood are expressed, and finely crushed tissue is extruded out of the nozzle channels as fine threads. An accurately graduated 4-ml syringe containing 3 ml of gas-free ferricyanide solution is fitted to the nozzle, and exactly 0·5 ml of tissue is forced into the syringe. A little mercury is added to facilitate mixing, any gas bubbles are removed and the tip of the syringe is closed with a rubber cap. The nut on the piston stem is then tightened up, the syringe is put into a centrifuge tube holder and, after standing for 10 to 20 mins., it is centrifuged in a small hand centrifuge for some minutes. Two ml of the clear solution are then transferred anaerobically to a Van Slyke apparatus and analysed for carbon dioxide and oxygen in the usual way. The pressure is read at the 0·5 ml mark on the burette. The method can be used for measuring the oxygen capacity of tissue by pressing out the tissue as fine threads directly over a Petri dish, covering the dish with a wet filter paper on a glass plate and, after the muscle haemoglobin is saturated with oxygen, scraping the threads together into a ball and placing this in the press. The analysis is carried out as described above with 0·5 ml of the tissue extruded from the press. A blank is carried out on 3 ml of the ferricyanide solution, 2 ml of which are transferred to the Van Slyke apparatus. The reading of the burette is subtracted from that obtained in presence of the tissue, and the volume % of gas in the tissue is calculated by multiplying the difference (in mm) by a factor dependent on the temperature. For 15, 20, 25 and 30° C., the appropriate factors are 0·236, 0·230, 0·225 and 0·221 for carbon dioxide, and 0·218, 0·214, 0·210 and 0·207 for nitrogen or oxygen. The method is subject to an error of about ±10%, due largely to the uneven composition of the tissue.

F. A. R.

Improvements in the Gasometric Estimation of Carbon Monoxide in Blood. S. M. Horvath and F. J. W. Roughton. (*J. Biol. Chem.*, 1942, **144**, 747–755.)—An improved method of estimating carbon monoxide in blood was recently described by Roughton (Abst., ANALYST, 1941, **66**, 253), in which blood, laked with saponin, is shaken with sodium hydrosulphite in a glycine buffer soln. to bind the oxygen and carbon dioxide, and the nitrogen and dissolved carbon monoxide are expelled. The carbon monoxide bound to the haemoglobin is then liberated by shaking with potassium ferricyanide soln. The method does not always give satisfactory results, and this is now believed to be due to the use of insufficient ferricyanide and too little alkali. In the new method no attempt is made to retain the carbon dioxide by means of an alkaline glycine buffer, but this is liberated, together with the whole of the bound carbon monoxide, by shaking the blood and hydrosulphite soln. with ferricyanide at *p*H 6; the carbon dioxide is then absorbed with sodium hydroxide. Draw 4 drops of capryl alcohol into the chamber of a Van Slyke-Neill apparatus, and measure 2 ml of 1% saponin soln. into the cup. Draw 1–2 ml of blood into the chamber, and add 2 ml of the saponin soln. After 1 min. place in the cup 2 ml of a 2% soln. of sodium hydrosulphite in sat. sodium borate soln. (add 1 g of solid hydrosulphite to 50 ml of 4% sodium borate soln. in a 50-ml flask and shake with only a minute bubble of air in the flask; transfer to a 50-ml burette and store under oil. The soln. must be freshly prepared each day), draw the lower 1·5 ml into the chamber, and cover this with black paper. Lower the mercury to the 50-ml mark and shake for 2 min. Eject the evolved gases, and place in the cup 1·5 ml of air-free freshly prepared 32% potassium ferricyanide soln.; draw in the lower 1·0 ml and discard the rest. Similarly introduce 1·0 ml of air-free phosphate buffer (dissolve 13·6 g of potassium dihydrogen phosphate and 3·5 g of dipotassium hydrogen phosphate in water and dilute to 100 ml) into the chamber, close the tap, and lower the mercury to 1 cm below the 50-ml mark. Shake for 3 min. in all, stopping the motor 3 times and raising the mercury to the 50-ml mark to mix the soln. in the stem of the chamber with the main bulk. Adjust the pressure nearly to atmospheric and place 2·0 ml of air-free 10% sodium hydroxide soln. in the cup. Run 1 ml into the chamber and allow 15–30 sec. for absorption of carbon dioxide. Lower the soln. to the 2·0-ml mark and, after 1 min., read the pressure, p_1. Eject the gas and again read the pressure, p_2. The carbon monoxide content in vols. % = $(p_1-p_2-c) \times$ constant. The correction c is determined by using water in place of blood, and the constant is obtained from Peters and Van Slyke's tables. The average discrepancy between duplicate determinations was 0·055 vol %, and in only 2 out of 20 determinations was it higher than 0·1%. Other methods of carrying out this estimation are:—(1) In place of the acid phosphate buffer use an alkaline phosphate buffer consisting of 3·5 g of dipotassium hydrogen phosphate and 17·1 g of tripotassium phosphate in 100 ml of water. Continue the second shaking for 10 instead of 3 min.; this makes absorption with sodium hydroxide unnecessary; (2) in the original Van Slyke Neill method all the gases are evolved by shaking with acid ferricyanide soln., and the carbon dioxide and oxygen are then absorbed with alkaline hydrosulphite; the carbon monoxide and nitrogen are measured and the nitrogen content, obtained by calculation or, better, determined on a duplicate sample, is subtracted from the value thus obtained. It is advantageous to use a freshly-prepared air-free 5% soln. of pyrogallol in 10% sodium hydroxide soln. in place of the alkaline hydrosulphite soln.

F. A. R.

Determination of Hydroxy and Analogous Groups in Amino Acids. G. Toennies and J. J. Kolb. (*J. Biol. Chem.*, 1942, **144**, 219–227.)—In acetic acid soln., in presence of perchloric acid,

the hydroxy groups of amino acids react quantitatively with acetic anhydride, whereas acetylation of amino groups is suppressed. The extent of the reaction can be determined by adding an amount of anthranilic acid that is more than equimolar to the sum of the free perchloric acid and the available acetic anhydride. Under these conditions the residual acetic anhydride acetylates a proportion of the free amino groups, and the remainder can be determined by titration with perchloric acid. Since the amount of amino groups added in the form of anthranilic acid is known, and that present as amino acid can be determined by direct titration with perchloric acid, the amount of amino groups which have reacted with the acetic anhydride, and therefore the amount of residual acetic anhydride, can be calculated. Thus, since the amount of acetic anhydride added is known, the amount that has reacted with the hydroxy groups, and so the amount of hydroxy groups present, can be calculated. Dissolve about 0·3 m*M* of amino acid, accurately weighed, in 4 ml of acetylating mixture. [First prepare 0·100 *N* acetous perchloric acid by adding to a weighed amount of conc. aqueous perchloric acid, dissolved in acetic acid, an amount of acetic anhydride equimolar to the water accompanying the perchloric acid. Use this to prepare dehydrated acetic acid, adding 100 ml to a mixture of 10 litres of acetic acid and 200 ml of 10·04 *M* acetic anhydride. Next prepare 0·5 *M* anhydrous perchloric acid soln., by dissolving 12·85 g of conc. aqueous perchloric acid (68·42%) in 1 litre of the dehydrated acetic acid and adding 82·65 g of acetic anhydride (95·1%). Mix equal vols. of this soln. and acetic anhydride.] Leave in a desiccator for 2 hr. to complete the reaction of the acetic anhydride with the hydroxy groups, and then pipette 3 ml of the reaction mixture into 4 ml of 0·4–0·45 *M* acetous anthranilic acid soln. [Dissolve an accurately weighed sample of anthranilic acid, previously standardised by titration with perchloric acid, in anhydrous acetic acid, prepared by diluting 20 ml of 10·1 *M* acetic anhydride to 1 litre with acetic acid and 10 ml of aqueous 1·095 *M* perchloric acid.] The exact quantities of each soln. are determined by weighing. After 3½ hr. in the dark to allow for the reaction of the acetic anhydride in the "basic" medium, titrate the soln. with 0·1 *N* acetous perchloric acid in presence of crystal violet indicator. Make a blank determination in the same way, but omit the amino acid. If exactly the same quantities of reagents had been employed in the two determinations and the partial vol. of the amino acid had been negligible, the difference between the two titres would have been equiv. to the amino groups of the amino acid plus the amino groups of the anthranilic acid which have not been acetylated because a certain proportion of the acetic anhydride had already reacted with the hydroxy groups of the amino acid. The amount of these hydroxy groups would then be calculated by subtracting from the difference in the titres the amount of amino group present in the amino acid as determined by direct titration with perchloric acid. Actually, the amounts of the reagents added in the blank and the determination are not the same, so that appropriate corrections must be applied. The method was used to determine the amino and hydroxy groups in 18 amino acids. Most of these gave results between 95 and 102% of the theoretical, and in only 2 instances were the results really unsatisfactory. The thiol group of cysteine reacted imperfectly.

F. A. R.

Titrimetric Micro-determination of Chloride, Sodium and Potassium in a Single Tissue or Blood Sample. W. G. Clark, N. I. Levitan, D. F. Gleason and Greenberg. (*J. Biol. Chem.*, 1942, **145**, 85–105.)—Chloride is determined by absorbing the hydrogen chloride, volatilised by treatment with sulphuric acid in a micro-diffusion apparatus, in potassium hydroxide soln., and titrating the potassium chloride mercurimetrically, with diphenyl carbazone as indicator. Potassium and sodium are determined in the digest. The former is pptd. as the chloroplatinate, which is then reduced with sodium formate, and the liberated chloride is determined mercurimetrically. Sodium is pptd. as sodium zinc uranyl acetate and the dissolved ppt. is titrated with sodium hydroxide soln. For convenience of manipulation, asbestos filter-sticks are employed. These are prepared from 1-mm glass tubing blown out at the end and filled with a small amount of asbestos. With vols. larger than 0·5 ml ordinary calibrated microburettes are used for titrating, but for smaller vols. a hypodermic syringe attached to a vernier micrometer screw mechanism is used. Ordinary calibrated delivery pipettes are used for vols. larger than 1 ml, and Krogh syringe pipettes for smaller vols. The micro-diffusion apparatus consists of an ordinary ground-glass stoppered weighing-bottle with a small phial resting on the bottom, of such a size that it does not touch the stopper or sides of the weighing bottle. Several weighing bottles are fixed into a wooden stand fitted with springs to hold the stoppers in place. *Chloride determination.*—Weigh the samples of tissue rapidly on a torsion balance and drop into the tared weighing-bottles. Dry at 105 to 110° C. for 6–12 hr., or to constant weight. Similarly, transfer known vols. of serum or plasma into the bottles and dry to constant weight. Next insert a phial containing 1 to 2 drops of 50% potassium hydroxide soln., taking care not to touch the glassware with the hands. Lubricate the stopper with conc. sulphuric acid, put 0·5 to 1·0 ml of conc. sulphuric acid in the weighing-bottle, and quickly insert the stopper. Fix the bottle in the wooden block, clamp on the stopper and incubate in an oven at 65°–70° C. for 6 hr. The hydrochloric acid formed diffuses into the phial, which is removed with forceps, and the outside is washed into the weighing-bottle with water. Neutralise the excess potassium hydroxide with 0·2 *N* nitric acid, using phenolphthalein as indicator, and add a slight excess of acid, to give a vol. of *ca.* 1 ml, and make the soln. approx. 0·01 *N* in nitric acid. Add 1 drop of 30% hydrogen peroxide and 1 drop of 1% methyl alcoholic diphenyl carbazone indicator. Titrate the soln. with mercuric nitrate (reagent I) until a permanent light-blue colour develops. The variation in 20 determinations on the same serum was between 100·3 and 102·1μg equivalents per g, and the recovery of chloride added to rat tissues averaged 100·8%. *Sodium determination.*—Transfer the sulphuric acid residue in the weighing-bottle to a platinum crucible, and evaporate to dryness on a hot plate, adding small crystals of pure sucrose to prevent spirting. Heat the dry crucibles overnight in a muffle-furnace at 500 to 520° C. Remove phosphate from muscle ash by adding excess of uranyl nitrate soln. and filtering off the uranyl phosphate; with serum this is unnecessary. Add 5 ml of dil. nitric acid to the cooled ash (or uranyl phosphate filtrate) and stir to dissolve the salts. Remove an aliquot portion, usually 10% of the vol., by means of a Krogh pipette, evaporate to dryness and add 0·06 to 0·08 ml

of 0·01 *N* nitric acid. Add 1 ml of sodium precipitant (reagent 2), and leave for 1 hr. Filter off the sodium zinc uranyl acetate with a filter-stick, add 0·5 ml of wash-liquor 1, filter off the washing, detach any ppt. adhering to the test-tube, wash again with similar wash-liquor and filter. Then wash 5 times with 0·25 to 0·50 ml portions of wash-liquor 2, and finally with two 0·5 to 1·0 ml portions of ether. Push the asbestos out of the filter-stick with a clean wire, wash the tube carefully with CO_2-free water, add 1 drop of methyl alcoholic phenolphthalein, and titrate with standard CO_2-free sodium hydroxide soln. The average of 20 determinations on serum was 130·7 ± 0·2μg-equivalents per g; duplicates did not differ by more than 10%. *Potassium determination.*—Transfer the remainder of the nitric acid soln. to a test-tube with a Krogh pipette and evaporate to dryness. Rinse the sides of the tube and again evaporate to dryness, and to the residue add 0·20 ml of potassium precipitant (reagent 3), 0·20 ml of potassium chloroplatinate soln. (reagent 4), and 2·0 ml of 90% ethanol. Mix, stopper, and leave at constant temp. for 12 hr. Filter off the ppt. through a filter-stick and wash it 4 times with 0·5-ml portions of wash-liquor 3, twice with 0·5-ml portions of wash-liquor 4, and finally twice with 0·5-ml portions of ether. Push out the asbestos, wash the filter-stick 3 times with a drop of water, and add 0·20 ml of 0·2 *N* sodium formate. Rinse the sides of the tube with 1·0 ml of water and leave in boiling water for 3 min. Filter through a micro-funnel and wash three times with 0·2-ml portions of water. To the filtrate add 0·3 ml of 0·2 *N* nitric acid, 1 drop of 30% hydrogen peroxide, and 1 drop of the diphenyl carbazone indicator. Titrate with reagent 1 to the first permanent blue colour (3 chloride atoms ≡ 1 of potassium). The potassium in 10 samples of human serum ranged from 6·32 to 6·47μg-equivalents per g. *Reagent* 1.—Dissolve 1·8 g of mercuric nitrate in water, add 10 ml of *N* nitric acid and make up to 1 litre. Standardise against sodium chloride. *Reagent* 2.—(A) Uranyl acetate ($2H_2O$), 20 g; acetic acid 30%, 6 g; water 49 g. (B) Zinc acetate ($3H_2O$), 30 g; acetic acid 30%, 3 g; water 32 g. Mix A and B, add 1 drop of 0·01 *N* sodium chloride and leave in the dark. Centrifuge or filter before use, if necessary. *Reagent* 3.—Make up 1 g of 20% chloroplatinic acid to 5 ml with *N* hydrochloric acid. *Reagent* 4.—Add an equiv. amount of conc. potassium chloride soln. to chloroplatinic acid, add ethanol, filter off the potassium chloroplatinate and wash several times with 95% ethanol, then with ether and dry. Dissolve 50 mg of the salt in 100 ml of water. *Wash-liquor* 1.—Mix 10 ml of glacial acetic acid and 90 ml of 95% ethanol, and shake with a small amount of sodium zinc uranyl acetate; filter before use. To prepare sodium zinc uranyl acetate, add 10 ml of reagent 2 to 1 ml of a soln. containing 20 mg of sodium chloride, filter and wash with glacial acetic acid, ethanol and ether. *Wash-liquor* 2.—Saturate ethanol with sodium zinc uranyl acetate. *Wash-liquor* 3.—Mix 5 vols. of 95% ethanol and 1 vol. of water saturated with potassium chloroplatinate. *Wash-liquor* 4.—Saturate 95% ethanol with potassium chloroplatinate. F. A. R.

Determination of Amino Acids in Plasma by the Ninhydrin-Carbon-Dioxide Reaction without Removal of Proteins. D. A. MacFayden. (*J. Biol. Chem.*, 1942, **145**, 387–403.)—The production of carbon dioxide from α-amino acids by the action of ninhydrin is highly specific; it is thus possible to determine the amino acids in plasma directly without removal of the proteins. Carbon dioxide, however, is formed from the plasma proteins and urea, but the amounts are small and consistent, and a correction can be applied. The apparatus used is that described by Van Slyke *et al.* (*J. Biol. Chem.*, 1941, **141**, 627), except that 25-ml Pyrex conical flasks are used for the reaction. When in use the flasks are provided with an adapter consisting of a short glass tube with a rubber tube at one end and a rubber stopper at the other. The stopper has a bore of 16 mm for half its length and 5–6 mm for the remainder. The stopper and rubber tube are scrubbed with vaseline and wiped to remove excess. Since rubber adsorbs carbon dioxide when exposed to air for 2 days or more, an untreated adapter would liberate carbon dioxide during an expt., introducing an appreciable error. The carbon dioxide must, therefore, be removed by evacuating the flask, inserting a glass rod in the rubber tube and immersing the flask in boiling water for 30 min. or longer. The rubber stopper and tube should then be stored in an evacuated container. Mix 1 ml of plasma in a reaction flask with 2 ml of citrate buffer of *p*H 2·5 (20·6 g of dry sodium citrate and 191 g of citric acid in 1 litre of water with a few crystals of thymol as preservative) and add 1 drop of caprylic alcohol. Free the contents of the flask from bicarbonate-CO_2 by reducing the pressure below 50 mm and shaking for 2 min., and then remove heat-labile "100° CO_2" by heating the evacuated flask for a few min. at 100° C. Remove the flask from the water-bath, maintaining the suction for a further 2 min., and then add 100 mg of ninhydrin from a calibrated glass spoon. Attach the adapter, previously treated to free it from rubber CO_2, to the flask and immerse in boiling water for *exactly* 10 min. Absorb the carbon dioxide by means of alkali in the Van Slyke–Neill chamber in the usual way, first saturating the 0·5 *N* sodium hydroxide and 2 *N* lactic acid with sodium chloride to reduce the error. Then measure the carbon dioxide by the standard procedure, *immediately* after reaction with ninhyrdin; otherwise there is loss of carbon dioxide through adsorption by the rubber. The result can be calculated in two ways: (1) A correction can be applied for protein carbon dioxide estimated from the plasma protein content. The pressure of carbon dioxide evolved is given by the expression $P_{CO_2} = p_1 - p_2 - c$, where c is the value of $p_1 - p_2$ obtained in a blank analysis. The carboxyl-N* content of the plasma in mg per 100 ml is calculated from the expression mg. COOH-N per 100 ml = P_{CO_2} × factor—(protein CO_2 + urea CO_2), the protein CO_2 + urea CO_2 being calculated as 0·00077 protein N + 0·0026 urea N. With normal plasma the correction for protein and urea is about 0·9 mg of carboxyl-N per 100 ml. The urea nitrogen content is generally low, and a urea carbon dioxide correction of 0·04 mg of carboxyl-N per 100 ml can be assumed. The protein content can be calculated sufficiently accurately from the sp.gr. of the plasma. (2) A correction for the protein carbon dioxide can be made by heating a second time. The evolution of carbon dioxide from protein proceeds at a constant rate for at least 1 hr., so that, if the flask and contents are heated for a further 10 min. after the ninhydrin reaction is complete, the amount of carbon dioxide formed is equiv. to that liberated from the proteins during the initial reaction. The

* "Carboxyl-N" here means α-amino-acid nitrogen.—EDITOR.

pressure of carbon dioxide in the two expts. is given by the expression: 1st heating, $(P_{CO_2})_I = (p_1 - p_2 - c)_I$; 2nd heating, $(P_{CO_2})_{II} = (p_1 - p_2 - c)_{II}$. The pressure P_{CO_2} of carbon dioxide from amino acid carboxyl groups is calculated by subtracting $(P_{CO_2})_{II}$ from $(P_{CO_2})_I$, so that $P_{CO_2} = (p_1 - p_2)_I - (p_1 - p_2)_{II}$, c cancelling out in the subtraction. The carboxyl nitrogen is then calculated as mg. carboxyl-N = $P_{CO_2} \times$ factor minus 0·0026 urea N. The urea correction can be assumed as before, to be equiv. to 0·04 mg of carboxyl nitrogen per 100 ml of plasma except in pathological urines. The recovery of amino acids added to plasma, serum or erythrocytes was between 98·7 and 101·5% in 8 expts. F. A. R.

Ether Extraction Method for the Determination of Urine Phenols. E. G. Schmidt. (*J. Biol. Chem.*, 1942, **145**, 533–544.)—Less than 1% of the "total diazo" value of blood is made up of phenols, but, as the interfering substances are almost exclusively ether-insol. and probably nitrogenous, satisfactory estimates of the amount of phenols present can be obtained after ethereal extraction. Furthermore, separate values for phenols and aromatic hydroxy acids can be obtained by ethereal extraction at different *p*H values. The apparatus used was a West condenser with a loosely fitting glass stopper, a 50 ml extraction tube of the usual type, and a receiver graduated at 3-ml intervals. The ether should be freshly distilled, to avoid formation of a chromogenic substance which reacts with the diazo reagent. The reagent is diazotised *p*-nitroaniline, but as it forms different colours with different phenols and aromatic hydroxy acids, mixtures of phenols must be used as standard. The most useful consists of 10 ml of 0·1% alcoholic phenol soln. mixed with 40 ml of 0·1% alcoholic *p*-cresol soln. and diluted to 100 ml with alcohol. Previously it has been customary to estimate phenols in aqueous solns. obtained by extracting the ethereal extracts with alkali, but a more straightforward method is to determine the phenols directly in the ethereal extract by adding alcohol, water and diazo reagent. All phenols were extracted completely at *p*H 10, whereas hydroxy aromatic acids were extracted not at this *p*H but at *p*H 3 or lower; this was made the basis of a method of estimating the two groups separately. Transfer 20 ml of urine to a 100-ml graduated flask, add water, adjust the *p*H to 10 and make up to vol. Transfer 40 ml to the extraction tube and add freshly distilled ether until it just overflows, and then a few particles of broken porcelain. Put 3 ml of ether in the receiver and, after attaching this to the extraction tube, immerse it in water at 70–75° C. Extract for 2 hr. and then shake gently until ether flows into the receiver up to the 3-ml mark. Dilute the ethereal extract with 5 ml of 95% alcohol, prepare 5 ml of the phenol-*p*-cresol standard and 5 ml of a phenol standard containing 0·003 g of phenol, and add 2 ml of water, 1 ml of diazotised *p*-nitroaniline (add 25 ml of a soln. containing 75 mg of *p*-nitroaniline and 2 ml of conc. hydrochloric acid to 1·5 ml of 5% sodium nitrite soln.) and 3 ml of 5% sodium carbonate soln. to the contents of each tube, and match the colour immediately with that of the standard. Adjust the *p*H of the residue in the extractor to 1 by adding 1 ml of 10 *N* sulphuric acid and add a few particles of powdered porcelain. Place 9 ml of ether in the receiver and continue the extraction for 2 hr. Adjust the extract to the 9-ml mark with ether, dilute with 15 ml of alcohol and remove 8 ml of the mixture for analysis. Prepare 5-ml alcohol standards containing (*a*) 0·1 mg of the phenol-*p*-cresol mixture, (*b*) 0·133 mg of *p*-hydroxybenzoic-*p*-hydroxyphenylacetic acids (1:3) and (*c*) 0·1 mg of *p*-hydroxybenzoic acid. Add to each soln. 2 ml of water, 1 ml of diazotised *p*-nitroaniline and 3 ml of 5% sodium carbonate soln. Compare the colours within 30 min. The value will be arbitrary and depend upon the standards used for the comparison. Conjugated phenols can also be estimated by the method. Transfer the extracted residue to a 400-ml flask, evaporate the ether, and boil gently for 1 hr. Cool, add 11·2 ml of *N* sodium hydroxide and dilute to 80 ml. Extract with ether to remove the phenols, as described above, then adjust the *p*H to 1 by adding acid, and extract the aromatic hydroxy-acids. If required, estimate the total phenols and aromatic hydroxy acids by acidifying the urine to *p*H 1, heating under reflux and extracting. After standing more than 2 days at room temp. urines are not suitable for assay, as after that time conjugated phenols are rapidly hydrolysed. With added phenol, recoveries were satisfactory, but with added *p*-hydroxybenzoic acid they tended to be somewhat high. In normal urine, conjugated phenols averaged about 30 mg of phenol or 64 mg of phenol-*p*-cresol, and very little free phenol was found. About 2/3 of the total aromatic hydroxy acid occurred free and 1/3 conjugated, a total of about 110 mg, estimated as *p*-hydroxybenzoic-*p*-hydroxyphenylacetic acids, being obtained. F. A. R.

Quantitative Determination of Lanthionine. W. C. Hess and M. X. Sullivan. (*J. Biol. Chem.*, 1942, **146**, 15–18.)—Lanthionine can be converted quantitatively into cysteine by hydrolysis with hydriodic acid and the cysteine then estimated by the method of Sullivan and Hess (*Pub. Health Rep. U.S.P.H.S.*, 1931, **46**, 390). Dissolve a sample of alkali-treated protein (*e.g.*, wool) containing 25 mg of lanthionine in 6·0 ml of 57% colourless hydriodic acid containing 1% of potassium dihydrogen phosphite and heat the soln. at 135–140° C. for 4 hr. with a stream of nitrogen passing through it. If necessary, decolorise the hydriodic acid used by heating with 1% of potassium dihydrogen phosphite. Determine the cysteine in an aliquot portion of the hydrolysate by the method of Sullivan and Hess. With pure lanthionine, the average recovery of cysteine was 98%. Mixtures of lanthionine with cystine and methionine in various proportions gave satisfactory results, but due allowance should be made for any cystine present, since it also yields cysteine in the reaction. F. A. R.

Quantitative Strength Determination of Amylase (Diastase) Preparations. E. Lenk. (*J. Soc. Dyers and Col.*, 1942, **58**, 138–141.)—In the determination of diastatic activity by the liquefaction of starch, the changes occurring are considered to be divided into 3 phases, *viz.*, liquefaction (which breaks down the amylopectin), and conversion into dextrin and into maltose. Thus, the outer and inner layers of the starch granule (amylopectin and amylose, respectively) both form maltose, but by liquefaction and dextrinisation, respectively. Diastases are of 2 types: zoo- and phyto-amylases which are of animal (*e.g.*, pancreatic) and vegetable origin, respectively, and the latter are subdivided into malt and bacterial amylases. The properties of these 3 groups differ considerably, *viz.*, optimum temp., 50°, 60° and 80° C.; optimum *p*H 6·81, 5·0–5·6 and 6·81, respectively, at the temps. stated; the zoo-amylases are more readily stimulated by neutral salts. It is shown that the progress of the first phase

may be followed viscosimetrically or by the iodine reaction; the first production of the violet colour corresponds with the min. viscosity and destruction of all the amylopectin. There is no necessity always to use the same starch substrate in order to obtain comparable results, so long as a "starch-factor" (F) is introduced; satisfactory vals. were thus obtained with rice, potato, wheat, rye, arrowroot and sol. starches of different origins. F is determined as follows:—Cool 1% pastes of 3 starches (prepared by heating potato or arrowroot starch on the water-bath for 30 min., or by mixing sol. starch with boiling water), and heat 100 ml with 20 ml of a phosphate buffer soln. (pH 6·81) at 50° C. for 10 min. Add various vols. of a preparation of a pancreatic diastase prepared by mixing 50 ml of the buffer soln. with a soln. of 0·25 g of the sample in 50 ml of 10% sodium chloride soln. and diluting to 500 ml. Allow to react at 50° C. until a mixture of 1 ml of the liquid with 40 ml of 0·005 N iodine changes in colour from red to yellow; add 1 ml of 20% sodium hydroxide soln. (to stop the reaction), and determine the maltose (Fehling-Lenk method). The reaction-constant (K, monomol.-reaction) is given by $\log[a/(a-x)]/t$, where a g is the wt. of starch present and x g the maltose formed in the time t (min.). Since only 75% of the starch is converted, the concn. of amylase (C) may be calculated from $750\ K/d$, where d is the wt. of sample in g. If the time to produce the above end-point is then plotted against d, and a horizontal line parallel with the d-axis is drawn at $t = 15$ min., it will cut the curve for each starch at a val. of d which, if multiplied by C, gives F; vals. of F are: sol. starch, 7·6; tapioca (U.S.A.), 8·35; potato, 8·5; arrowroot, 9·3; "powdered" (U.S.A.), 11·34; pearl (U.S.A.), 19·0. It is thus possible to determine C without the necessity of determining the maltose, *i.e.*, by plotting the log of the time (min.) to attain the end-point against d for 3 vals. of d, and ascertaining at what val. of d the 15-min. axis cuts the curve; then $C = F/d$. It is convenient to mix a soln. of 3 g of sample with 30 ml each of 10% sodium chloride and the buffer solns., and to dilute this to 300 ml, and then further to 1 : 10; increasing vols. of this soln. are added to the substrate (*cf. supra*) at 1-min. intervals. The method may be used to compare all diastases of the same type, but difficulties arise in comparing diastases of different types because different amounts of maltose are produced in a given time; between the blue to violet and red to yellow end-points (*i.e.*, during the dextrinisation phase) the max. and min. amounts of sugar are produced by the malt and bacterial diastases, respectively, pancreatic diastase being intermediate in this phase of activity. Contrary to usual belief, however, malt and pancreatic diastases produce approx. equal amounts of maltose up to the first end-point, whilst between the two end-points the former produces approx. twice as much as the latter; this later portion of the reaction, therefore, cannot be used as a basis of comparison, and since the above formulae are based on the second end-point, the method breaks down. In that event the quantity of sample necessary to produce the first end-point in (*e.g.*) 15 min. is determined. Into each of a series of test-tubes in a cold water-bath put 2 ml of 1% starch soln., 0·5 ml of appropriate buffer soln. (*vide supra*), and various vols. of dil. enzyme soln. Mix, place in a bath at the appropriate temp. (*supra*), and after 16 min. (1 min. for warming-up) pour each mixture into 40 ml of 0·0005 N iodine, and note which corresponds with the change from blue to violet. From this the vol. (v) in ml of 1% starch soln. converted to this end-point by 1 g of sample is calculated. Vals. obtained (tapioca starch substrate) were: malt diastases, 7,300 and 9,100; bacterial diastase, 1,200; pancreatic diastase, 10,000. The val. of C for this end-point may also be calculated from the formula $Fv/100$. J. G.

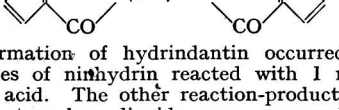
Carotenoids of Yellow Corn Grain. J. W. White, Jr., F. P. Zscheile and A. M. Brunson. (*J. Amer. Chem. Soc.*, 1942, **64**, 2603–2606.)—To obtain an estimate of the pro-vitamin A content of corn it is necessary to effect a separation between the active and inactive carotenoids. Recent investigations have shown that isomerisation products of the naturally occurring carotenoids are present in solutions, and this introduces further complications. In the present paper the identity and absorption spectra of some of these neo type pigments are described, preparatory to devising a system of analysis. Luteol, an unnamed carotene 1, γ-carotene, and a carotenoid possibly identical with monohydroxy-α-carotene were found in yellow corn grain for the first time. The absorption spectra of neocryptoxanthol (which is a provitamin A) and two neozeaxanthols are described. The former has two absorption maxima at 450 and 480$m\mu$, whilst neozeaxanthol I absorbs maximally at 445 and 470$m\mu$ and neozeaxanthol II at 440 and 465$m\mu$.
F. A. R.

Location of Vitamin B_1 in Wheat. A. L. Ward (*Chem. and Ind.*, 1943, **62**, 11–14.)—It was found that the vitamin B_1 content of the wheat berry, estimated from the sum of the contributions of the commonly accepted proportions of bran, endosperm and germ, was considerably less than the actual content determined by expt., and examination of low grade stocks from the milling indicated the presence of some material rich in vitamin B_1 that could not be ascribed to any of the bran layers. Tests were then made upon the complete embryo free from bran and endosperm and upon the grain from which this had been removed, the proportions of the two components of the grain being known. The sum of the contributions of these separate parts was 0·7 I.U. per g, whereas the actual B_1 content of the grain was 1·3 I.U. per g. In subsequent expts. wheat grains were cut transversely into beard ends and germ ends. The sum of the contributions of these two fractions agreed with the actual content of the grain, and similar agreement was found when degermed wheat was subjected to the same procedure. When, however, the germ end of wheat was divided into the embryo and the remainder, the sum of the contributions of the two portions was only *ca.* 50% of the actual content of vitamin B_1. Since the embryo had been removed mechanically by scouring, it was evident that during this process some portion of the grain rich in vitamin B_1 had been lost. It was then found that sixth scourer dust contained as much of the vitamin as the embryo itself, although it consisted mainly of fine outer bran and dirt from the furrow of the grain. Similar expts. with "heavy liftings," as distinct from scourer dust, showed that a rich source of vitamin B_1 is situated either in the outer layers of the embryo or in the layers adhering to the embryo. Finally, wheat grains were dissected by removing first the bran on the outside of the embryo, and then the embryo plant itself with the minimum of adherent scutellum. The scutellum, epithelium and adjacent layer of apparently empty cells were then removed together, the remainder of the grain being degermed wheat. Estimation of the vitamin B_1 in these fractions gave the contribution of each

to the vitamin B_1 content of the whole grain, and this agreed with the actual amount present (1·20 I.U. per g). The fraction comprising the scutellum, epithelium and adjacent layer contained 40·2 I.U. per g, a figure unprecedented in cereal stocks. The embryo plant was then picked out from the grain and the scutellum was obtained free from epithelium, although the separated epithelium was contaminated with some scutellum. These two fractions contained 49·4 and 42·3 I.U. per g, respectively, proving that the scutellum and epithelium are the centres of vitamin B_1 concn. in the grain, but it is pointed out that the result for the epithelium may be too high owing to presence of scutellum, or too low owing to presence of endosperm. In milling, these layers are probably removed with the embryo and knocked off in the subsequent scouring. If milling technique could be altered to prevent the loss of these layers in the offal, it would not be necessary to return the separated germ to the flour to ensure adequate vitamin B_1 content. Thus a flour of 75% extraction containing *ca.* 0·5 I.U. of vitamin B_1 per g could be reinforced by adding 1% of these cell layers and would contain the vitamin B_1 content stipulated for National Wheatmeal. Such flour would be a white flour of 76% extraction, free from bran and germ, equal to wheatmeal in nutritive value and superior thereto in baking and keeping qualities. In these expts. the vitamin was estimated by the method of Jansen (*Rec. Trav. Chim. Pays-Bas*, 1936, **55**, 1046; ANALYST, 1937, **62**, 60), as modified by Booth (Nicholls *et al.*, ANALYST, 1942, **67**, 15). A. O. J.

Improved Photometric Method for Ascorbic Acid. C. Carruthers. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 826.)—In the estimation of ascorbic acid in tissue material containing other reducing substances, *e.g.*, glutathione and cysteine, it is an advantage to add mercuric chloride to inhibit the action of these substances on the 2,6-dichlorophenolindophenol. The ascorbic acid is estimated from the difference in transmission of buffered 2,6-dichlorophenolindophenol before and after reduction. Transmission measurements are made at *p*H 6·5–6·6 and reduction at *p*H 2·5–2·7. A further advantage is increased stability of stock solns. of the dye; although the transmission of the buffered dye soln. changes slowly with time, this change is accompanied by a proportionate change in the ascorbic acid equivalence of the dye as determined by titration with 0·005 *N* sodium thiosulphate; frequent restandardisation is therefore unnecessary. B. S. C.

Reaction of Ninhydrin with Ascorbic Acid and other Endiol Compounds. E. S. West and R. E. Rinehart. (*J. Biol. Chem.*, 1942, **146**, 105–108.)—When solns. of ninhydrin and ascorbic acid are mixed at room temp., an insol. crystalline ppt. is formed. The reaction is very rapid on warming. Reductone and dihydroxymaleic acid also react with ninhydrin to give similar ppts., all of which have the properties of Ruhemann's hydrindantin (*J. Chem. Soc.*, 1911, **99**, 792, 1306):



Max. formation of hydrindantin occurred when two moles of ninhydrin reacted with 1 mole of ascorbic acid. The other reaction-product, which slowly lost carbon dioxide, was presumed to be dehydroascorbic acid. F. A. R.

Influence of Solvent on the Ultra-Violet Absorption Maximum of Vitamin A. K. Morgareidge. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 700.)—The results were obtained with *iso*propanol and cyclohexane, chosen as representative of polar and non-polar solvents respectively. For eleven samples of "oils," in which the vitamin A may be presumed to exist in the esterified form, the average wavelength of the ultra-violet absorption peak was found to be 326·4$m\mu$ in *iso*propanol and 327·4$m\mu$ in cyclohexane. A further ten samples were prepared as the unsaponifiable fractions of the "oils," which should therefore be free from vitamin A esters. The wavelength of max. absorption averaged for this group was 325·3$m\mu$ for *iso*propanol and 327·5$m\mu$ for cyclohexane solvents. A study of the $E^{1\%}_{1\,cm}$ values at various wavelengths in the region of the absorption max. shows that the polar solvent leads to higher values of this "extinction." The article concludes with a discussion of the significance of the factor correlating the spectrophotometric and biological assay for vitamin A. (*Cf.* Adamson and Evers, ANALYST, 1941, **66**, 106.) B. S. C.

Estimation of the Tocopherols and the Tocopherylquinones by the Colorimetric Oxidation-Reduction Method. J. V. Scudi and R. P. Buhs. (*J. Biol. Chem.*, 1942, **146**, 1–6.)—A method of estimating vitamin K was recently described (*J. Biol. Chem.*, 1941, **141**, 451; 1942, **143**, 665; ANALYST, 1942, **67**, 173, 338) in which the tocopherylquinones were estimated at the same time. This method has now been adapted to the estimation of the tocopherols. The quinone, dissolved in butyl alcohol, is reduced with Raney nickel in presence of phenosafranine, and the soln. is pumped into the upper chamber of the apparatus previously described, which contains a standard soln. of 2 : 6-dichlorophenolindophenol in butyl alcohol. The reduction in colour is proportional to the amount of quinone originally present, but, whereas vitamin K hydroquinone reduces the indophenol instantaneously, the tocopheryl-hydroquinones require 40–60 min. for complete reduction. If readings are taken at intervals, the initial reading gives the vitamin K content, whilst the final reading gives the sum of the vitamin K and tocopheryl-quinone. To estimate tocopherols, dissolve a sample, containing 150 to 200μg, in 10 ml of butyl alcohol, and add 0·5 ml of 20% aqueous $AuCl_3.HCl.3H_2O$ solution. Leave in the dark at room temp. for 30 min., add 10 ml of water and 30 ml of hexane, and shake in a separating funnel. Run off the aqueous layer and wash the hexane layer with two 10-ml portions of water, and then with 10 ml of carbonate buffer (*p*H 9·7) and 10 ml of water. Centrifuge the hexane soln. and run it through a layer of anhydrous sodium sulphate. Filter and concentrate an aliquot portion to small vol. *in vacuo* under nitrogen. Add sufficient phenosafranine soln. to give a final concn. of 0·5μg per ml, adjust to a convenient vol. with butyl alcohol—acetate soln. and analyse as described above. The recovery of pure α-tocopherol was 102% ± 3%. To obtain good recoveries it is necessary to use a large excess of gold chloride, since carotenoids and other substances have to be oxidised. The estimation of tocopherol and tocopherylquinone requires two analyses, but when the sample is small the quinone and unoxidised tocopherol can be recovered after the tocopherylquinone estimation by adding two vols. of light petroleum to the butyl alcohol and washing the indophenol from the organic phase with water containing a few drops of dil. sodium

hydroxide soln. After distilling off the light petroleum the butanol soln. is adjusted to 10 ml and oxidised with gold chloride. F. A. R.

Bacteriological

Bacteriological Aspects of the Manufacture of Spray-dried Milk and Whey Powders with some Observations on Moisture Content and Solubility. E. L. Crossley and W. A. Johnson. (*J. Dairy Res.*, 1942, **13**, 5–41.)—The results of different workers by the method described agreed reasonably well. Individual plate counts with 671 powder samples varied widely (200 to 19,500,000 per g). The flora on standard milk agar at 37° C. were of a specialised type and comprised comparatively few species. Thermoduric streptococci of the "*enterococcus*" and "*viridans*" groups predominated, especially *Str. durans* and *Str. thermophilus*; 5 species of micrococci, probably non-thermoduric, also occurred commonly. Aerobic spore-forming bacilli and 3 *Achromobacter* sp. were found regularly, but their numbers were not of practical importance. Spore-forming anaerobic bacilli were present in 14% of the powder samples, probably in very small numbers; they were most frequent during the winter months. Coliform organisms were rarely found in 1 ml, but were isolated from 20 ml of milk reconstituted from 25% of the powder samples; coliforms occurred most frequently during the winter months. False positives due to anaerobes were common, especially in stored powders, and confirmatory tests of presumptive positives were essential. Examination of 198 coliform strains from powders and 164 strains from raw milk showed that *coli* types occurred less frequently in the powders, *aerogenes-cloacae* types occurring more frequently; 48·5% of the powder strains were heat-resistant, as compared with 2·2% of the raw-milk strains. It was shown that the coliform flora of powders were partly due to plant contamination by heat-resistant strains, although some non-heat-resistant strains could survive spray drying. Possibly *coli* types are less resistant to drying than *aerogenes-cloacae* types. Yeasts were rarely found and in 15% of powder samples the numbers of moulds present were negligible. The plate count of powders was not directly related to the raw-milk counts, but was probably affected by the numbers of thermoduric bacteria in the raw milk and the storage conditions before processing. High-temperature—short-time pasteurisation was satisfactory, and the heat treatment required to ensure destruction of pathogens had no adverse effect upon the solubility or nutritional value of the powder produced. Given a heating time of 20 sec., followed by a 3–5 min. holding period, a temp. of 163° F. was satisfactory. Temps. below 159° F. yielded unsatisfactory bacteriological results, but higher temps., up to 167° F., could be employed without seriously decreasing powder solubility. Spray drying destroyed many bacteria, but could not in itself be relied upon to destroy pathogens, since some non-thermoduric species survived. The Kestner system employs a rapid air stream, which enables the thermal advantages of high air-inlet temps. to be utilised. With an outlet-air temp. of 90° C. it was possible to employ an inlet-air temp. of 166° C. with safety, and inlet-air temps. up to 170° C. caused no serious deterioration of powder solubility. Inlet-air temps. below 155° C. (commonest during winter operation) resulted in increased bacterial survival, especially of coliform organisms. Bacteriological cleanliness of the plant is a vital factor. The difficulties of each process are discussed, and it is shown that there were opportunities for contamination of plant by any heat-resistant streptococci (*e.g.*, *Str. durans* and *Str. thermophilus*) and coliform organisms which survived pasteurisation. Furthermore, conditions of operation encouraged multiplication of these organisms. Given good management and close attention to plant hygiene, seasonal variations were small and satisfactory powders were produced from a poor milk supply during hot weather. A variable decline in numbers of bacteria occurred during storage; some species died out more rapidly than others; spore-forming species survived longest. Coliform species disappeared rapidly, particularly *coli I* types. Both the plate count and coliform tests are therefore only of limited value when applied to stored powders, especially when the conditions and duration of storage are unknown. D. R. W.

Streptococci isolated from Raw Retail Milk. E. H. Beahm. (*Amer. J. Hyg.*, 1942, **36**, 147–152.)—Samples of raw milk from 48 Omaha dairies were bacteriologically examined. The counts of haemolytic streptococci ranged from nil to 12,000 per ml. Serological and biochemical tests (described in detail) were applied to representative strains of the isolated haemolytic and non-haemolytic streptococci. The non-haemolytic strains could be placed in the following groups: *S. salivarius*, *S. bovis*, *S. lactis* and *S. fecalis*. Seven of the samples contained haemolytic streptococci belonging to Lancefield's Group A; 24 to Group B; 1 to Group C "human" type; 3 to group D. Milk apparently produced under the most stringent sanitary conditions contained streptococci, and the fact that haemolytic streptococci of human origin can be isolated from milk subjected to rigid inspection is a convincing argument for pasteurisation.

Culture Medium for *Chaetomium globosum*. W. C. Chace and G. S. Urlaub. (*Amer. Dyestuff Rep.*, 1942, **31**, 331–333; *J. Soc. Dyers and Color.*, 1943, **59**, 20.)—The spores of the mould *Chaetomium globosum* are used for testing mildew-proofed fabrics. For the rapid growth of the spores, with little or no development of common air-borne contaminants, the following improved cellulose agar medium has been devised:—Pulp mechanically 10 g of filter-paper to give a fine mash which does not separate on standing, add 3 g of sodium nitrate, 1 g of potassium dihydrogen phosphate, 25 g of magnesium sulphate, 0·25 g of potassium chloride and 15 g of agar-agar. Make up to 1 litre and sterilise. The use of this medium, which is buffered at *p*H 5, reduces the time before sporing by 4 or 5 days.

Agricultural

New Method for the Determination of Total Phosphoric Acid in Soils. J. G. Shrikhande. (*J. Agric. Sci.*, 1942, **32**, 406–412.)—As late as 1931 the only method of proved accuracy for the determination of total phosphoric acid in soil was that of Hall ("*The Soil*," 1921, p. 165), in which the soil is refluxed with conc. hydrochloric acid for 48 hr. Attempted simplifications of the method resulted in arbitrary, tedious or otherwise unsatisfactory procedures. McLean's method (*J. Agric. Sci.*, 1936, **26**, 331) based upon that of Richards and Godden (ANALYST, 1924, **49**, 565) involving digestion with sulphuric and nitric acids proved unsuccessful with some red soils from Ceylon, although

Hall's method gave satisfactory results. The failure of McLean's method was traced to interference by the bases extracted by the more vigorous digesting agent, and, when such bases were removed by a procedure based upon the principles underlying phosphate separation in qualitative analysis, results agreeing closely with those obtained by Hall's method were obtained. Expts. showed that sulphuric acid with selenium as catalyst can replace the sulphuric acid and nitric acid mixture of McLean's method without loss of accuracy and with considerable advantage. During digestion the mixture does not dry up, the ppt. of total phosphates coagulates rapidly, addition of ferric chloride is unnecessary because selenium appears to induce complete pptn. of the total phosphates (presumably as complex seleno-phosphates), excessive dilution is avoided and the same digest serves for phosphate and nitrogen determinations. In presence of selenium, however, it is essential to use sodium acetate instead of the ammonium acetate of the normal procedure to avoid inclusion of complex ammonium selenito-molybdates in the ammonium phosphomolybdate ppt. The addition of copper sulphate or potassium sulphate to the digestion mixture is unnecessary, and it is shown that the extraction of phosphate is complete in 3 hr. *Method.*—Digest 5–10 g of air-dried soil with 20 ml of conc. sulphuric acid and 0·2 g of selenium over a low flame until the liquid begins to clear (*ca.* 20 min.) and then vigorously for 2½–3 hr. Dilute, cool and filter the mixture and wash the residue thoroughly. Neutralise the combined filtrate and washings with 0·88 ammonia (20–30 ml), the end-point being indicated by a curdling of the ppt. Add enough hydrochloric acid just to dissolve the ppt., dilute to 400 ml, and to the boiling soln. add 20 ml of 2 *N* acetic acid and sodium acetate (*ca.* 20 g) until the liquid, after boiling for 10–15 min., remains orange-red. When cold, collect the ppt. on a Whatman No. 42 filter-paper, wash with cold water, dissolve the ppt. in 20–40 ml of hot nitric acid added in small portions, and boil the soln. for ½ hr. to expel volatile selenium compounds. Add 25–30 ml of 50% ammonium nitrate soln. at b.p., then add 30 ml of 6% ammonium molybdate soln. and boil the mixture. If a whitish ppt. forms (indicating insufficient washing of the total phosphate ppt. and insufficient nitric acid in the preceding step) add 10 ml of nitric acid and boil until the ppt. is canary-yellow. Collect the ppt. in a Gooch crucible, wash with 1% nitric acid, dry at 100° C. and ignite gently until the residue is blue-black and until any red film of selenium is volatilised. Each g of residue ≡ 0·038 g of phosphoric anhydride. A. O. J.

Organic

Use of the Ellis-Jones Maleic Anhydride Method in Testing Tung Oil. R. S. McKinney, N. H. Halbrook and W. G. Ross. (*Oil and Soap*, 1942, **19**, 141–143; *B.C.A.*, 1942, B, II, 435.)—Determination of the maleic anhydride value (M.A.V.) by the method of Ellis and Jones (ANALYST, 1936, **61**, 812) has given good results with tung oil, the values for different oils being inversely proportional to the times for the Browne heat test. Unlike Kaufmann's diene method, the M.A.V. method gives results for pure α-elaeostearic acid within 98·1% of theory; hence the amount of this acid in tung oil can be calculated from the M.A.V. (70–75); also, approximately, the proportion of tung oil in admixture with linseed or perilla oil. Thus, *e.g.*, addition of 10% of linseed oil reduces the M.A.V. of tung oil to 63·8.

Application of the Thiocyanogen Value to the Determination of Linolic and Linolenic Acids. N. L. Matthews, W. R. Brodie and J. B. Brown. (*Oil and Soap*, 1941, **18**, 182, 187; *B.C.A.*, 1942, Bii, 20.)—Average thiocyanogen values of (*a*) linolic and (*b*) linolenic acid, determined at 16° ± 0·5° C. (24 hrs.) by means of a 0·2 *N* reagent containing 10% by vol. of carbon tetrachloride, were 96·6 for (*a*) and 163·3 for (*b*). Equations based on these values, in place of Kaufmann's theoretical values, gave good results in the analysis of 5 known mixtures of palmitic, linolic and linolenic acids. The reagent, which is a modification of that of the American Oil Chemists' Society, changes little in reactivity in 3 weeks if stored below 3° C. Certain fatty acids that had been kept for some time gave low thiocyanogen values; as a rule, this was prevented by storing the acids in evacuated sealed ampoules at −20° C.

Separation of Viscose Rayon from Cotton. O. Kirrett. (*Cellulose Chem.*, 1940, **18**, 125–131; *J. Soc. Dyers and Col.*, 1943, **59**, 19.)—The following modification is claimed to be more accurate than the original zincate method of Howlett and Urquhart.—Extract 0·3–0·5 g of the fabric with carbon tetrachloride and then warm it with 0·1 *N* nitric acid to remove any synthetic resins present. Also, if it is dyed with Aniline Black, treat it 3 times alternately with cold 0·06% potassium permanganate soln. for 10 min. and 0·5% oxalic acid soln. at 60° C. for 5 min.; other dyes do not interfere. Next extract the fabric with 30 ml of zincate reagent (*infra*) and 30 ml of water at 13°–14° C. for 1 hr., with vigorous shaking at 5-min. intervals. Filter off the cotton residue and wash it successively with 50 ml of 50% stock zincate soln., 30 ml of 12% sodium hydroxide soln., 100 ml of cold water, 200 ml of 0·2 *N* hydrochloric acid, 1 litre of hot water, 1 litre of cold water, and lastly with alcohol. Dry at 105° C., weigh, and apply a correction of +3·5% to the weight. The method is not applicable if the cotton contains hydrocellulose or oxycellulose; otherwise the results agree well with those obtained by the calcium cyanate method. *Preparation of zincate soln.*—Dissolve 150 g of zinc oxide in a soln. of 300 g of sodium hydroxide in 400 ml of water and dilute to 1 litre.

Determining the Deterioration of Cellulose caused by Fungi. C. A. Greathouse, D. E. Klemme and H. D. Barker. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 614–620.)—The following procedure is suggested as a standardised quantitative method for determining, (*a*) the rate of decomposition of cellulose by micro-organisms; (*b*) the effectiveness of mildew-resisting treatments. Remove from the sample (bleached, 8-oz. Army duck, in the present instance) any sizing, residual waxes, pectins or other nutrient substances present, by treatment in succession with two portions of carbon tetrachloride for 2 hr., and a 0·05% enzyme soln. which renders starch and proteins soluble; rinse and dry. Cut a large number of strips from 10 blocks of cloth (6 in. in warp direction × 1·25 in.), and by unravelling the warp reduce the width of weft to exactly 1·0 in. Select 10 replicate strips by the method of random numbers, so that each represents one of the blocks. The incubation chambers are 16-oz. wide-mouthed, square-type

bottles (6·5 in. long to the neck) with metal screw-tops. Cut a circle (diam., 1·625 in.) out of the centres of the cap and its waxed-paper lining, and insert a circle of glass filter fabric (having the same diam. as the lining) between the latter and the metal. Place a mat of glass fabric (2 × 6 × 0·1 in.) in each bottle, to support the sample strip and so enable it to be thoroughly wetted by the medium without being submerged. Put into each bottle sufficient medium (*vide infra*) just to reach to the top surface of the glass mat when the bottle is horizontal, cap the bottles and heat them at 15 lbs. per sq. in. pressure for 20 min. With slight modifications it is possible to adapt the technique to the continuous renewal of mineral solns. (*e.g.*, for simulating the leaching-out of toxic chemicals). Maintain stock cultures of the organisms used on filter-papers placed on the surface of agar slopes, and 10–14 days before the test transfer some to a filter-paper (which is supported on the surface of a liquid medium on glass beads) in a conical flask; at the correct stage of development shake the flask, so as to produce a suspension of the organisms, dilute with 50 ml of sterile water, and pipette out the suspension (*e.g.*, 1 ml) into the bottles. Incubate for 7 days in the dark at 85–86° F. and 90–92% R.H. The medium contains: dipotassium hydrogen phosphate, 1·3940; magnesium sulphate, 0·7395; ammonium nitrate, 1·0006; calcium carbonate, 0·005; sodium chloride, 0·005; total iron, zinc and manganese sulphates, 0·001 g per litre. It was used with and without agar, and comparisons were made (*vide infra*) against the formula of Rogers, Wheeler and Humfeld (*U.S. Dept. Agr., Tech. Bull.*, 1940, 726). After incubation carefully rinse the fungal growth off the strips, dry them, first in air and then at 80–85° C. for 1 hr., condition at 70° F. and 65% R.H. for 4 hr., and determine the breaking-strength on the Scott tester (*A.S.T.M., Standards on Textile Materials*, 1941). The average loss in breaking-strength, expressed as a departure from the average of the control tests, gives the degree of deterioration. The method has the advantages of accuracy and speed as compared with existing methods, and the method of uniform aeration described reduces sampling errors considerably. The effects of variations in the experimental technique are described in detail. *Metarrhizium* sp. and *Chaetomium globosum* cause rapid decomposition, are easily handled and are satisfactory test organisms in other respects. *Ch. elatum*, *Alternaria* sp., *Hormodendrum* sp. and *Stachybotrys papyrogena* are less satisfactory, and mixtures of organisms are difficult to standardise. The *p*H of the medium has a strong influence on the activities of the fungi, and must be strictly controlled if the determinations are to be comparative; ammonium nitrate assists the buffering capacity, and is preferable to sodium nitrate (*cf.* Rogers, *et al.*) partly because, unlike the latter, it does not develop alkalinity, and partly because it contains nitrogen in 2 inorganic forms. There are indications that the use of agar in the medium (*vide supra*) is undesirable, as agars of different origins have nutrient effects towards the fungi which, although only allowing slow growth, are different in magnitude. J. G.

Inorganic

Scrap[Metal]Identification Tests. Anon. (*Metals and Alloys*, 1942, **16**, 264–265.)—The following spot tests may be used as an aid in the identification of metallic materials. *Nitric acid test.*—Place 1 or 2 drops of conc. nitric acid on the clean metal surface and dilute with 3–4 drops of water after 1–2 min. All classes of steel give brownish drops, except high-chromium (stainless) steels or nichrome, which give no reaction; copper and nickel alloys give a blue or green solution; other non-ferrous alloys mostly react to give colourless drops. *Iron nail test.*—To identify copper, in the nitric acid drop, rub a clean iron nail in it in contact with the specimen; coppering of the nail or the metal surface takes place. *Ammonia test.*—Attack the metal with nitric acid or *aqua regia* and add ammonia until the liquid is strongly alkaline. The following colours are given: nickel steel, red to purple; chromium steel, brown-green; chromium-nickel steel (Staybright type), reddish-blue; other steels and iron, red-brown; nichrome, blue-red; nickel and copper alloys, blue; other non-ferrous alloys, colourless. *Paper test for nickel* (Williams, *Ind. Eng. Chem.*, Jan., 1942).—Place 1 drop of acid mixture (10 ml of sulphuric acid, 10 ml of nitric acid, 10 ml of phosphoric acid, 10 g of citric acid, 25 ml of water) on the metal for 15–30 sec. and then let it be absorbed on a prepared test-paper strip (filter-paper dipped in a soln. of 10 g of citric acid in 25 ml of water with 10 ml of 1% alcoholic dimethyl glyoxime soln. and dried). When potassium hydroxide soln. is dropped on the paper a red colour will form if nickel is present. Iron and other elements do not interfere. *Silver nitrate test for tin.*—To distinguish lead-tin solder from lead or lead-silver solder, treat the clean metal surface with a few drops of 2·5% silver nitrate soln. This blackens the surface, but if tin is present a white ppt. is also produced. *Cupric chloride test.*—To distinguish nickel-chromium alloys with low iron content from nickel chromium steels (*e.g.*, Staybright), place 1 drop of a 10% soln. of cupric chloride in conc. hydrochloric acid on the surface, add 3–4 drops of water slowly after 2 min., and wash off the liquid. The steels show a copper-coloured spot. *Physical tests.*—Indications of a less determinate nature are given by surface appearance, type of lathe chippings, behaviour on melting in a blowpipe flame and test by a magnet. Non-magnetic materials include austenitic, nickel-chromium steel and high chromium-nickel alloy; plain chromium stainless steel and nickel are magnetic and monel metal is slightly magnetic.

S. G. C.

Volumetric Method for Determining Tin. H. H. Willard and T. Y. Toribara. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 716–718.)—The method of Wheeler (ANALYST, 1938, **63**, 883) for the determination of tin in alloys by formation of dioxalatothiometastannic acid and subsequent titration of the sulphur atoms with iodine requires very carefully controlled experimental conditions. The method is modified by forming the stable potassium salt of the complex acid. Add potassium sulphide soln. slowly to a soln. containing the tin, sulphuric acid and excess of potassium oxalate; carry out the reaction at 60° C., with the final *p*H 3·3. Then cool the soln., remove free hydrogen sulphide by passing a stream of carbon dioxide for 30 min., add a slight excess of standard iodine soln. and back-titrate with standard thiosulphate. For fuller details and the application of the method to the analysis of alloys consult the original paper.

L. A. D.

Analysis of Tin-Base Bearing Metal (for Antimony and Copper). J. R. Andrews and A. J. Bender. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 712.)—A rapid volumetric method is given for the determination of antimony and copper, in which both metals are determined on the same portion. The procedure is sufficiently precise under routine conditions where errors of 0·2 to 1·0 mg are tolerable. Tin does not interfere; arsenic, if present will be titrated with the antimony. *Standard solns.*—(1) 0·1 *N* Potassium permanganate; (2) 0·1 *N* sodium thiosulphate. These should be standardised against a standard tin-base bearing metal of about the same composition as the metal to be analysed. The copper actor varies very little, but the antimony factor is appreciably influenced by the amount of antimony present. *Method.*—(1) *Antimony.*—Digest 1 g of the sample in a 250-ml wide-mouthed conical flask with 5 g of potassium sulphate and 15 ml of sulphuric acid. Heat gradually until solution is complete and then cool the flask to 25° C. Add 50 ml of 10% tartaric acid soln. and 10 ml of hydrochloric acid, heat slowly to the b.p. and continue boiling until all sol. matter is dissolved. Dilute with 40 ml of water, cool in ice-water to below 5° C., and titrate with 0·1 *N* permanganate to an end-point which persists for several secs. (2) *Copper.*—Add 3 drops excess of 0·1 *N* permanganate to the above soln. and then ammonia in slight excess (about 50 ml). Exactly neutralise the soln. with 1:1 sulphuric acid and add 2 ml in excess (*p*H 2·5–2·7). Cool to 15° C., add 10 ml of 10% potassium thiocyanate soln. and 15 g of potassium iodide and titrate immediately with 0·1 *N* thiosulphate, using starch indicator at the end-point Back-titrate with 0·1 *N* iodine until the blue colour re-appears. This procedure should be followed closely, as any variations may affect the activity of the antimony and copper tartrate complexes. C. F. P.

Determination of Germanium in Silicate Rocks. A. G. Hybbinette and E. B. Sandell. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 715–716.)—The sample is decomposed with hydrofluoric, nitric and sulphuric acids, and, after removal of all hydrofluoric acid, the germanium is distilled as tetrachloride and estimated colorimetrically after reaction with ferrous ammonium sulphate and ammonium molybdate reagent. *Reagents.*—*Ammonium molybdate.*—Dissolve 6·0 g of tetrahydrate in 35 ml of water, add a cooled mixture of 16·0 ml of conc. sulphuric acid with 35 ml of water and dilute to 100 ml. *Ferrous ammonium sulphate.*—Dissolve 10 g of cryst. salt in water containing 1·5 ml of 6 *N* sulphuric acid and dilute to 500 ml. *Sodium acetate.*—Dissolve 67·5 g of trihydrate in acid. Bubble air slowly through the soln., heat water and dilute to 200 ml. *Ammonium molybdate and ferrous ammonium sulphate reagent.*—Add successively with shaking 10 ml of ammonium molybdate soln., 10 ml of ferrous ammonium sulphate soln. and 25 ml of sodium acetate soln. to 50 ml of water. Dilute to 100 ml, leave for 5 min. and then use immediately as it is not stable. *Sodium hydroxide.*—Dissolve 25 g of pure sodium hydroxide, as free as possible from silica, in 100 ml of water. *Standard germanium solution.*—Dissolve a suitable amount of pure germanium oxide in a few ml of water containing a drop of sodium hydroxide soln., make slightly acid with sulphuric or hydrochloric acid and dilute. A suitable strength is 0·1 g per litre of germanium or germanium oxide. Reagents should be protected from silica contamination by storage in well-waxed vessels. *Procedure.*—Treat 1 g of rock powder in a platinum dish with a few ml of water, 6 ml of sulphuric acid (1:1), 0·5 to 1 ml of conc. nitric acid and 10 ml of hydrofluoric acid. Evaporate slowly until the sulphuric acid fumes, cool, add a few ml of water and again evaporate to "fuming." Repeat the dilution and evaporation at least twice, never allowing more than slight "fuming." Transfer the sample to the 100 ml flask of an all-glass distillation apparatus (*e.g.*, Scherrer, *J. Res. Nat. Bur. Stds.*, 1936, **16**, 255) with chloride-free 1:1 sulphuric acid and water; the final vol. of liquid in the flask should be about 50 ml, 35 ml being 1:1 sulphuric to 140° C. and maintain at that temp. by gradual addition of water. Distil and discard 150 ml of liquid, cool the flask, add 15 ml of water and distil in a stream of air until the temp. rises to 120° C. Collect the distillate in 1 ml of sodium hydroxide soln. and 1 ml of water in a waxed taper-ended tube. Neutralise with 1:1 hydrochloric acid (phenolphthalein), add 1 drop of sodium hydroxide soln. and acidify with 0·1 ml of 1:1 acetic acid. Add 10 ml of ammonium molybdate-ferrous ammonium sulphate reagent, mix and dilute to 25 ml. Compare with a blank similarly prepared from 1 ml of sodium hydroxide soln. If the test soln. has a more intense colour than the blank (due to silicon in the distillate, carried over by residual hydrofluoric acid), the distillation at 140° C. must be continued until a negative test results. Then cool the distilling flask, add 2 ml of 1:1 hydrochloric acid and 2 ml of water, and distil as above until the temp. is 120° C. Collect the distillate in a waxed tube containing 2 ml of sodium hydroxide soln., neutralise with 1:1 hydrochloric acid and dilute to 25 ml. Cool the distilling flask, again add 2 ml of acid and 2 ml of water and repeat the procedure. Neutralise 2 ml of sodium hydroxide soln. and dilute to 25 ml for use as a blank. Take 10 ml of each germanium distillate and of the blank, and add reagents to develop the colour as when testing for residual hydrofluoric acid as above. After 15 min. compare the test solns. with the blank in a filter photometer (absorptiometer) using a red filter. Calibrate with suitable standards prepared from standard germanium soln., adding 10 ml of water, 1·5 ml of 1:1 hydrochloric acid and 2 ml of sodium hydroxide soln. and diluting to 25 ml. Take 10 ml and proceed as with the test solns. The blue colour produced obeys Beer's Law up to a conc. of 1·5μg per ml. The arsenic content of igneous rocks (usually less than 0·001%) should not interfere. A sample with 0·01% of added arsenic showed an increase from 0·00026 to 0·00030% in apparent germanium content, a change within the experimental error. The effect of selenium was not investigated, as it is normally present in negligible amounts. A trace of chloride in the sample does not cause significant loss of germanium during the original decomposition. L. A. D.

Volumetric Determination of Iron and Titanium. W. M. McNabb and H. Skolnik. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 711.)—The volumetric estimation of iron and titanium generally used in the titanium pigment industry involves reduction of soln. (zinc-amalgam reductor), titration of the combined iron and titanium with standardised potassium permanganate and separate titration of titanium with standardised ferric ammonium sulphate. A method of air-oxidation for titanium

alone, following the reduction of both metals, makes it possible to titrate the reduced iron and determine the titanium by difference. In the presence of mercuric chloride air-oxidation of tervalent titanium can be completed in about 20 min., and the method requires only one standardised soln. *Method.*—Adjust the acidity of the soln. containing iron and titanium to 4 *N* in sulphuric acid, reduce with zinc, pour through a reductor with moderate suction, and receive in ferric alum soln. Titrate ferrous iron with standard permanganate, thus finding the equivalent of the total iron and titanium in terms of permanganate. Repeat the reduction (on a separate portion) and pour the reduced iron and titanium through the reductor into 4 *N* sulphuric acid. Add 50 ml of a sat. soln. of mercuric chloride and bubble air through until the violet colour is discharged and for 5 min. longer. Titrate the ferrous iron with permanganate and calculate as ferric oxide. Calculate titanium dioxide from the difference between the two permanganate readings. C. F. P.

Determination of Aluminium in Manganese and Aluminium Bronzes. A. C. Holler and J. P. Yeager. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 719–720.)—Interfering elements are removed in a mercury-cathode electrolysis cell, using an acetic acid electrolyte and the determination is completed by pptn. of the aluminium with ammonia or 8-hydroxyquinoline. Separation from iron, manganese, lead, zinc, nickel and copper is complete. *Procedure.*—Heat 1 g of the sample with 20 ml of conc. nitric acid, add 100 ml of hot water, boil, and digest almost at b.p. for 1 hr. or more. Filter off, wash and discard the tin precipitate. Add 10 ml of conc. sulphuric acid and evaporate until copious fumes are evolved. Cool, add 50 ml of water, boil and filter off the lead sulphate. Neutralise the filtrate with 10% sodium hydroxide soln. and add 5 ml excess; then neutralise with 1 : 3 acetic acid, heat to boiling and add 5 ml of glacial acetic acid. Cool, dilute to 350 ml and add 2 or 3 drops of octyl alcohol or proprietary anti-foaming agent. Transfer to a Melaven electrolytic cell (*id.*, 1930, **2**, 180), and pass 1 to 2 amps. for 3 hrs., using a rotating stirring platinum anode. Test for the removal of copper and iron with potassium ferrocyanide and ferricyanide respectively. Remove the electrolyte, filter off any ppt. of manganese dioxide and complete the determination in the customary manner. The neutralisation and electrolysis may also be applied to the soln. remaining after the successive determination of tin, lead and copper by A.S.T.M. methods. (*"Tentative Methods of Chemical Analysis of Manganese Bronze,"* 1939, B 27; 36T, pp. 113, 129.) L. A. D.

Solubility of Strontium Chromate and the Detection of Strontium. T. W. Davis. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 709.)—The solubility of strontium chromate in alcoholic soln. has been examined and a modification of the Noyes procedure for the analysis of the alkaline earth group (*"Qualitative Analysis,"* A. A. Noyes and E. H. Swift, 10th Ed.) is suggested. The preparation of the salt and method of determining the solubility by iodimetric titration of chromate have been described previously (Davis and Ricci, *J. Amer. Chem. Soc.*, 1939, **61**, 746). The concns. of alcohol in mixed solvents have been determined by density measurements. The solubility determinations were complicated by the following factors:—(1) saturation is approached extremely slowly and probably takes years, but for practical purposes is sufficiently complete after 1 month; (2) the solid phase slowly takes up water to form a hydrate; (3) the solid is slowly contaminated by glass from containing vessels.

Solubility of Strontium Chromate

Medium	25° C. g/100 g	50° C. g/100 g	75° C. g/100 g
Water ..	0·096	0·090	0·080
19·7% alcohol	0·012	0·012	0·008
41·8% ,,	0·0004	0·0009	0·0003
92·5% ,,	∿0·00005	<0·00005	<0·00005

These results suggest that better separations in a shorter time should be achieved if the Noyes procedure is carried out at 75° C. instead of at room temp. This conclusion was tested in the following manner:—To 25 ml of a soln. containing 25 mg of strontium ion were added 1 ml of dil. acetic acid, 2 ml of dil. ammonia, 2 ml of 3 *N* potassium chromate and varying amounts of 95% alcohol. Pptn. was carried out at various temps.

Qualitative Removal of Strontium as Chromate

Temp., °C.	Alcohol added ml	Time of shaking min.	Time for filtering min.	Strontium in ppt. mg	Strontium in soln. mg
25	30	15	22	23·9	1·3
50	20	6	6	24·2	0·6
75	20	0	6	24·4	0·5

This procedure may be useful for the quantitative separation of strontium from calcium. It should be reliable and faster than the nitrate procedure and would avoid the use of expensive anhydrous reagents. C. F. P.

Improvements in the Colorimetric Microdetermination of Phosphorus. C. P. Sideris. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 762).—Details are given of modifications to the Berenblum–Chain method for the determination of phosphorus (*Biochem. J.*, 1938, **32**, 287); the procedure for organic samples is as follows:—Dissolve the ash from 1 or 2 g of dry sample in 10 ml of 5 *N* hydrochloric acid, add 20 ml of water and warm; make up to 100 ml and filter. Transfer 10 ml of the filtrate to a 200 × 25 mm Pyrex ignition tube, neutralise with 5 *N* sodium hydroxide, using litmus paper indicator, add 2 ml of 2 *N* sulphuric acid and 5 ml of ammonium molybdate (*vide infra*) and heat on a water-bath for 10 min. Pour the mixture into a 250-ml Squibb separating funnel, rinse the tube with 5 ml of hot 0·5 *N* sulphuric acid and transfer the washings to the funnel. Add 10 ml of *n*-butyl alcohol, close with a glass stopper and shake for 30 sec. Allow the upper yellowish phosphomolybdate layer to separate from the lower aqueous acidified layer. Break up any butyl alcohol and water emulsion by adding 5 ml or more of 0·5 *N* sulphuric acid. Discard the aqueous layer, add 10 ml of the reagent of tin and hydrochloric acid in 0·5 *N* sulphuric acid (*vide infra*) to the butyl alcohol layer, and shake vigorously. Allow time for separation and discard the lower aqueous layer. If the phosphomolybdate compound shows a greenish-blue tinge, add more of the tin and hydrochloric acid reagent. Transfer the blue butyl alcohol layer to a 15-ml graduated centrifuge tube, adding any further washings of butyl alcohol that have been used to remove all traces of blue compound from the funnel. Add further butyl alcohol as necessary to the contents of the centrifuge tube to adjust the vol. Then

add a further 2 to 5 ml of the tin and hydrochloric acid reagent to stabilise and retard the fading of the blue colour. If this causes emulsification, break up by centrifuging for 5 to 10 min. The butyl alcohol containing the phosphomolybdate compound should now occupy the upper layer of the tube. Transfer approx. 8 ml by pipette to a glass cell or tube and determine the transmission factor, using a photoelectric absorptiometer or similar instrument. A light-filter having transmission limits of 565 and 630mμ is recommended. Calibration is effected with samples of known phosphorus content. *Reagents.—Ammonium molybdate.*—Dissolve 40 g of ammonium molybdate in 1 litre of water and filter. *Tin and hydrochloric acid.*—Place 0·5 g of metallic tin in a 50-ml flask containing 40 ml of 0·5% hydrochloric acid. *Tin and hydrochloric acid in 0·5 N sulphuric acid.*—Add 10 ml of the previous reagent to 50 ml of 2 *N* sulphuric acid in a 200-ml flask and make up to the mark with water. B. S. C.

Colorimetric Determination of Fluorine. P. Urech. (*Helv. chim. Acta.*, 1942, **25**, 1115–1125.)—Fahey's colorimetric determination of fluorine with "ferron" (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 362) has been adapted to the photometric determination of fluorine in the distillate of hydrofluosilicic acid obtained by Willard and Winter's method (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 7). Ferron, 7-iodo-8-hydroxyquinoline-7-sulphonic acid, gives with ferric iron a green colour which is changed towards yellow by fluoride; the solution shows decreased light absorption according to the amount of fluoride present. With amounts of fluorine below 3 mg, the results were independent of whether the fluorine was added as fluoride or as fluosilicic ions, but they diverged with larger amounts. To prepare the reagent, dissolve 75 mg of ferron in water at 50° C., cool, and dilute to 50 ml; to 45 ml of the soln. add 50 ml of water and 5 ml of a 0·1 *N* ferric chloride soln. in 2 *N* hydrochloric acid. *Method.*—Place the soln. (about 30 ml), obtained if necessary by preliminary fusion of the sample with sodium carbonate, in a Claisen distilling flask (a distilling flask with a second neck carrying the side tube) and add 35 ml of conc. sulphuric acid and 0·5 g of calcined silica. Distil the liquid until its temp. reaches 165° C.; then continue the distillation in a current of steam with the liquid at 165° C. until a total of 400 ml of distillate is collected. Make the distillate alkaline to phenolphthalein and evaporate to 25 ml. Distil in a similar apparatus with addition of 25 ml of perchloric acid instead of sulphuric acid, begin steam distillation at 135° C., and continue at that temp. until 400 ml of distillate have been collected. Treat an aliquot part of the distillate containing up to 5 mg of fluorine with 5 ml of the ferric-ferron reagent, dilute to 100 ml and measure the % light absorption in a photoelectric colorimeter (one of the tpye described by R. Lange "*Kolorimetrsiche Analyse mit Besonderer Berücksichtigung der Lichtelectrischen Kolorimetrie*," Berlin, 1941, is recommended, a red filter, RG2, being used, with a 15-watt lamp). Read the result from a standardisation curve obtained from determinations with known amounts of fluorine. S. G. C.

Physical Methods, Apparatus, etc.

Analysis of Hydrocarbon Mixtures by means of Raman Spectra. D. H. Rank, R. W. Scott and M. R. Fenske. (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 816.)—Where the determination of infrared absorption spectra is too complicated, qualitative and quantitative analysis of hydrocarbon mixtures by means of their Raman spectra can often be substituted. These spectra are excited by the 4358 A radiation from a mercury arc. Shorter wavelengths are removed by absorption in a sodium nitrite filter (incidentally this also reduces the fluorescence of any fluorescent impurities present), whilst a praseodymium ammonium nitrate filter removes a large proportion of the continuous mercury spectrum on the high wavelength side of 4358 A. The 3-prism glass spectrograph used had an f. 4·5 objective and gave a dispersion of 32 A per mm at 4500 A. All samples examined had 7·4% by vol. (2 ml added to 25 ml of unknown) of carbon tetrachloride added as an internal standard. The "scattering coefficient," defined as the ratio of the intensity of a particular Raman line of some hydrocarbon under investigation to that of the 459 cm^{-1} Raman line of carbon tetrachloride, has been determined for each of a number of lines of 27 different hydrocarbons, and all these values are tabulated. It is pointed out, *e.g.*, that, although the b.p. and n_D of 2,2,3-trimethylbutane are matched very closely by a mixture containing 8% of cyclohexane and 92% of 2,2-dimethylpentane, the Raman procedure readily distinguishes these materials. B. S. C.

Comparison of the Capillary Flow and Falling Sphere Methods of Determining the Viscosity of Cellulose in Cuprammonium Solutions. R. S. Hatch, R. N. Hammond and J. J. McNair. (*Paper Ind.*, 1942, **24**, 436, 438, 440.)—The results obtained by the capillary flow method (Clibbens and Geake, ANALYST, 1928, **53**, 306) are not readily reproducible with samples which do not dissolve easily in the solvent (*e.g.*, partly bleached and unbleached pulps), owing to clogging of the viscometer orifice. This may be partly overcome by closer attention to the tolerances for the composition of the cuprammonium soln.; concns. of copper, ammonia, sucrose and nitrates of 15 ± 0·1, 200 ± 0·1, 1 and less than 1 g per litre are now recommended. The soln. is prepared from bright copper turnings in a clean copper tube surrounded by a freezing-mixture, by passing in air for 1·5–3 hr. at 30 litres per hr. This soln. is used for a modified form of the standard falling sphere method of the Cellulose Division of the American Chemical Society (*Ind. Eng. Chem.*, 1929, **21**, 49). Dissolve strips of the sample (⅛ × 3 in.) in sufficient of the soln. to obtain a rate of fall of the sphere of 5–30 sec. (allow for the moisture content of the sample), by placing them in the viscometer tube (diam., 1 cm.), used for the capillary method (*loc. cit.*) with an aluminium ball (diam., 1/16 in., *e.g.*, a ball-bearing) and filling the tube from the base by nitrogen pressure. Clamp the tube on to a rotating wheel (4 r.p.m.), and after 16 hr. in the dark in absence of light, transfer the tube to a const. temp. jacket. Determine the rate of fall of the sphere against a fluorescent light background between 2 lines etched on the tube 15 cm. apart. Advantages over the A.C.S. method are the use of a smaller wt. of sample and smaller vol. of a stabler solvent, and a lighter ball (enabling satisfactory results to be obtained with 1% solns. of medium-viscosity pulps); elimination of the necessity to transfer the soln. from the dissolving tube to the viscometer tube, and of contamination by oxygen; rapidity, and a sensitiveness of 1%. J. G.

Reviews

MODERN SYNTHETIC RUBBERS. By H. BARRON, Ph.D., A.I.C., A.I.R.I. Pp. viii + 274. London: Chapman & Hall, Ltd. 1942. Price 25s.

It seems a pity that the generic name of "synthetic rubbers" should be perpetuated in the title of a book which devotes a chapter to the nomenclature of these materials. Many take exception to it, and Dr. Barron himself advocates a justifiable alternative, although his suggestion of "elastics" has associations with haberdashery.

The first part of the book discusses the present position and prospects of the synthetic rubber industry, the economic view-point being strongly emphasised. Although he may be too enthusiastic over the newer materials, the author must be credited with the more cautious statement that it is "most unwise to be pontifical or dogmatic about the prospects of synthetic rubber." We must agree with him that the future will undoubtedly witness a great expansion in this field and that this country does not yet show signs of being in the forefront. A historical account of the subject is followed by a discussion of the necessary raw materials and their availability. This section closes with a full and critical description of the various methods used for the polymerisation of these substances. The author's interest here lies in the industrial aspect of the processes, but he also presents the current views on the chemistry and structure of the polymers. The third part of the book (comprising nearly half) is devoted to the technology and uses of the various materials (including "rubbers," "ebonites," "latices," solutions and reclaims), about a hundred of which are given in the comprehensive subject-index. Not only does the author make the familiar superiority claims, but he also shows the disadvantages of the products.

In such an all-embracing survey as the present, one might expect to find analysis mentioned on more than two pages. This, however, is scarcely the fault of the author, since a scheme has yet to be devised for systematic analysis of these materials. Complications arise with commercial products from the incorporation of fillers, accelerators, antioxidants, softeners or plasticisers, and polymerisation catalysts. Moreover, the rubber-like material may not be simple, but two monomers may be co-polymerised or raw synthetic rubbers may be compounded together. The sulphur, nitrogen, and chlorine of certain polymers lend themselves to attack and the greatest analytical difficulty is to be anticipated among the purely hydrocarbon products (including natural rubber). The present book will help the analyst in that he may see the exact nature of the problem confronting him; he will find in it the compositions and chemical properties of the materials insofar as they have been published. One feels, however, that the future will see still further applications of physical and performance tests for routine purposes, particularly because variation in manufacturing technique may greatly affect the physical properties of the product without appreciably changing its composition.

In spite of evidence of careless proof reading and some loose writing, the book can be thoroughly recommended to all interested in the subject. G. H. WYATT

BIOCHEMISTRY AND MORPHOGENESIS. By JOSEPH NEEDHAM, F.R.S. Pp. xvi + 787. Cambridge University Press. 1942. Price 52s. 6d.

The subject-matter of Dr. Needham's book is the problem of why organisms develop the way they do; and one of the most powerful methods of attacking it is by examining the consequences of their developing in some other way. It is possible, for example, to cut small pieces off the frog's embryo and graft them in new positions; or to cut the embryo into two halves in different planes to find out what, if anything, each half develops into. This method of approach (the art of making real some of those "possible frogs" of which Nature has given us only one—no doubt the best) led, in 1924, to the discovery of the *organising centre* of the amphibian embryo; that part which is sufficient and necessary to cause the tissues in its neighbourhood to turn into the nerve tube, skeletal rod and muscle blocks of the embryonic axis. Of all that has happened in developmental physiology since then, "*Biochemistry and Morphogenesis*" is an authoritative synthesis and review.

There are three key-problems in developmental physiology. One is the problem of what growth is—how it is that viruses and genes, as well as ordinary tissue cells, can synthesise their raw materials in the harmonious way that enables a replica of themselves

to be formed. The second is, how this growing is accompanied, as in development it is, by a progressive diversification; so that cells become recognisable first as "inside" cells, then as cells of the primitive gut, and then as cells of the true gut, liver or pancreas as the case may be. The third problem is, what part hereditary factors play in this process of diversification. Anyone who has thought about these problems will think more clearly after reading what Needham has to say. His analysis of the second problem is masterly. The process of diversification is one of threefold specificity: of the chemical substance, which is now known to initiate it; of the tissue, which must be competent to respond; and a specificity of time and place, the most miraculous of all, which ensures that the reaction between morphogenetic hormone and responding tissue shall take place at the time and in the pattern that makes for what—wise after the event—we call "normal" development. This analysis is logical; but Needham shows that it is biological too, and that these several categories reflect the action of distinguishable form-determining processes.

This is only the backbone of the book. Of the rest, little or nothing of direct or indirect relevance to morphogenesis escapes review and judgment. There are, in fact, some 6000 references, and it is at least in part as a reference book that many biologists will use it. Many people believe that encyclopaedic works like this can be written by anyone with an orderly mind and with access to a large library. No such work would deceive a scientist for a moment. Needham's book has the special quality of expertness that comes only from practical experience and long reflection on the problems with which it deals.

The book itself is well presented. The print is excellent, and the paper almost white, though we have no right in wartime to expect anything better than wholemeal. And, too, it is well written. It must be very difficult to write a book of this length in such a way that the reader is not tired and irritated by the recurrence of particular constructions, such as a favourite way of handling subordinate clauses. Yet Needham's book is vigorously written throughout. P. B. MEDAWAR

THE THEORY OF EMULSIONS AND THEIR TECHNICAL TREATMENT. By WILLIAM CLAYTON. Fourth Edition. Pp. 492, 103 illustrations. J. & A. Churchill, Ltd. Price 42s.

Much new material is incorporated in most sections of the present edition, but especially in those dealing with agricultural sprays, waxing of fruit and vegetables, cutting oils, fire-fighting, fuel and pharmaceutical emulsions, soil stabilisation, churning and emulsification by ultrasonics and by solid emulsifiers. The selected list of patents is modernised. Its value has been enhanced by the inclusion of much new work on the factors involved in emulsification, and progress is most noticeable in the application of surface and interfacial film studies to three-phase emulsions and in the electrokinetic studies of two-phase emulsions. The introduction of the new material has led to a re-arrangement of the first three chapters, which is a marked improvement on earlier editions. A still more drastic re-organisation might be advantageous, since the argument is often interrupted by inclusions. The stability of emulsions forms one of the most important topics, and the subject is exhaustively treated and critically presented. The discussion is, however, scattered throughout the book, which makes it difficult to arrive at a clear analysis of the many factors involved.

The success of this book has been firmly established by its previous editions, and this should be maintained by the present volume. The subject is intricate and, although there is a broad background of theory in many branches, there is, in addition, a wealth of detail of industrial and manufacturing technique not closely assimilated with the theoretical aspects. A comprehensive survey has been provided, which is of very great value to research workers on emulsions and also to specialists who use it as a reference book.

The binding and paper are uniform with the third edition. Wartime conditions probably account for lack of thoroughness in proof reading. W. E. GARNER

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