however, concludes that in man dietary cholesterol has but minor influence on plasma cholesterol levels (7).

Trace Substances in milk fat, hitherto not recognized, are being identified by the newly perfected methods now available to the laboratories. For example, Hansen, Shorland, and Cooke have identified fatty acids in the range of C_{20} to C_{26} which are believed to result from the direct assimilation of dietary lipides by the cow (*15*).

Conclusions

Despite innumerable studies comparing the nutritional value of fats, there are many unanswered questions. For instance, with certain dietary regimens while milk fat induces a more rapid growth than most fats in young animals, longevity sometimes is significantly reduced (*29*). In practical infant nutrition, however, a parallel manifestation would seem unlikely. Current pediatric literature emphasizes the importance of providing the infant and child with a favorable nutritional environment as an essential condition for optimal health in later decades.

Literature Cited

(1) Ahrens, E. H., Hirsch, J., Insull, W., Peterson, M. L., "Chemistry of Lipides as Related to Atherosclerosis, A Symposium," p. 225, C. C Thomas, Springfield, Ill., 1958.
(2) Am. Med. Assoc., "Handbook of Nutrition," Chap. 18, p. 383, Blakiston, New York, 1951.
(3) Barboriak, J. J., Krehl, W. A., Cowgill, G. R., Whedon, A. D., *J. Nutrition* **64,** 241 (1958).
(4) Barki, V. H., Collins, R. A., Elvehjem, C. A., Hart, E. B., *Ibid.*, **40,** 383 (1950).
(5) Bosshardt, D. K., Kryvokulsky, M., Howe, E. E., *Ibid.*, **69,** 185 (1959).
(6) Boutwell, R. K., Geyer, R. P., Elvehjem, C. A., Hart, E. B., *Ibid.*, **26,** 601 (1943).
(7) Committee on Fats in Human Nutrition, "Role of Dietary Fat in Human Health," National Research Council, Washington, D. C., Pub. **575,** 7, 10 (1958).
(8) Cox, W. M., *J. Biol. Chem.* **103,** 777 (1933).
(9) De, H. N., Karkun, J. N., *Indian J. Dairy Sci.* **2,** 114 (1949); *Nutrition Abstr. and Revs.* **19,** 942 (1950).
(10) Deuel, H. J., "The Lipids," Vol. **II,** p. 174, Interscience, New York, 1955.
(11) Deuel, H. J., Alfin-Slater, R. B., Wells, A. F., Kryder, G. D., Aftergood, L., *J. Nutrition* **55,** 337 (1955).
(12) Dryden, L. P., Gleis, P. F., Hartman, A. M., *Ibid.*, **58,** 335 (1956).
(13) Freeman, S., Ivy, A. C., *J. Dairy Sci.* **25,** 877 (1942).
(14) Gullickson, T. W., Fountaine, F. C., Fitch, J. B., *Ibid.*, **25,** 117 (1942).
(15) Hansen, R. P., Shorland, F. B., Cooke, N. J., *J. Dairy Research* **26,** 190 (1959).
(16) Hegsted, D. M., Gotsis, A., Stare, F. J., *J. Nutrition* **70,** 119 (1960).
(17) Hilditch, T. P., "The Chemical Constitution of Natural Fats," 3rd ed., Wiley, New York, 1956.
(18) Hopkins, C. Y., Murray, T. K., Campbell, J. A., *Can. J. Biochem. and Physiol.* **33,** 1047 (1955).
(19) Insull, W., Ahrens, E. H., *Biochem. J.* **72,** 27 (1959).
(20) Insull, W., Hirsch, J., James, T., Ahrens, E. H., *J. Clin. Invest.* **38,** 443 (1959).
(21) Jack, E. L., Smith, L. M., *J. Dairy Sci.* **39,** 1 (1956).
(22) Keane, K. W., Cohn, E. M., Johnson, B. C., *J. Nutrition* **45,** 275 (1951).
(23) Kehar, N. D., *et al.*, "Studies on Fats, Oils and Vanaspatis," Monograph, Indian Council of Agricultural Research, New Delhi, India, 1956.
(24) Kesten, H. D., Salcedo, J., Stetten, D. W., *J. Nutrition* **29,** 171 (1945).
(25) McGillivray, W. A., *J. Dairy Research* **25,** 344 (1958).
(26) Macy, I. C., *et al.*, "The Composition of Milks," National Research Council, Washington, D. C., Publ. **254** (1953).
(27) Mead, J. F., *Am. J. Clin. Nutrition* **6,** 606 (1958).
(28) Morrison, L. M., *Geriatrics* **13,** 12 (1958).
(29) *Nutrition Revs.* **13,** 278 (1955); **14,** 305; 349 (1956); **15,** 39 (1957); **16,** 350 (1958).
(30) *Ibid.*, **17,** 102 (1959).
(31) Peterson, D. W., *Am. J. Clin. Nutrition* **6,** 644 (1958).
(32) Rouser, G., *Ibid.*, **6,** 681 (1958).
(33) Schantz, E. J., Elvehjem, C. A., Hart, E. B., *J. Dairy Sci.* **23,** 181 (1940).
(34) Schön, H., Gey, F., Strecker, F. J., Weitzel, G., *Nutrition Abstr. and Revs.* **26,** 138 (1956).
(35) Snyderman, S. E., Morales, S., Holt, L. E., *Arch. Disease Childhood* **30,** 83 (1955).
(36) Stetten, D. W., Salcedo, J., *J. Nutrition* **29,** 167 (1945).
(37) Strach, Erich, Loeschke, Adalbert, Blum, Karl, *Ber. Verhardl. Sächs. Akad. Wiss. Leipsig. Math.-phys. Klasse* **84,** 129–208 (1932).
(38) Tange, U., *J. Agr. Chem. Soc. Japan* **11,** 533 (1935).
(39) Thomasson, H. J., *J. Nutrition* **56,** 455 (1955).
(40) U. S. Dept. Agr., Human Research Division, "Fatty Acids in Animal and Plant Products," May 1959.
(41) Viswanatha, T., Gander, J. E., Liener, T. E., *J. Nutrition* **52,** 613 (1954).

Received for review February 29, 1960. Accepted August 8, 1960.

BEEF AROMA

Some Volatile Constituents of Cooked Beef

M. H. YUEH and F. M. STRONG

Department of Biochemistry, University of Wisconsin, Madison 6, Wis.

This study was undertaken to identify as many as possible of the volatile compounds responsible for the characteristic odor of cooked beef. While it is a common observation that this aroma develops only after heating, the literature affords relatively little information as to the nature of the compounds responsible.

Experimental Procedures and Results

Beef Broth. Fresh lean beef (round steak, U. S. Choice or U. S. Good) was trimmed free of fat, passed through a meat grinder, and refluxed with an equal weight of water for 3 hours. Two drops of Dow-Corning antifoam A were added before refluxing to prevent excessive foaming. After cooling and filtering, the broth was distilled to one third to one half of the original volume at atmospheric pressure either at its natural pH of 5 to 6 or after pH adjustment. Most of the characteristic odor was present in the distillate. Unless otherwise specified, only the distillate was used for chemical studies.

Basic Fraction. When the broth was to be examined for volatile basic compounds the distillate was collected in 2*N* acid, either sulfuric or hydrochloric, or the distillate was allowed to pass directly into a 2.6 × 28 cm. Dowex 50 (H^+) column and the column was subsequently eluted with 1*N* hydrochloric acid. The acidic solution obtained by either of these

The volatile fraction from lean beef cooked in boiling water was shown to contain hydrogen sulfide, ammonia, acetaldehyde, acetone, and diacetyl. In addition the presence of formic, acetic, propionic, butyric, and isobutyric acids, and of dimethyl sulfide was tentatively established. Volatile alcohols and esters were absent. The amount of hydrogen sulfide obtained after 3 hours of boiling was 6 to 8 mg. per kg. of beef, but much larger amounts were evolved after boiling for 7 days.

Table I. Volatile Carbonyl Compounds from Beef Broth

Compound, DNP Derivative	Melting Points, ° C. Isolated	Authentic	Mixed	Additional Evidence
Acetone	121–3	124–5	123–4	Paper chromatography, infrared spectrum, x-ray diffraction
Acetaldehyde	155–7	156–7	155–7	Same as for acetone
Diacetyl	d. >300	d. >300	d. >300	Infrared spectrum, ultimate analysis

methods was evaporated to dryness, the residue made alkaline with sodium hydroxide, and the mixture then redistilled into redistilled aqueous hydrochloric acid. After vacuum evaporation of the resulting solution, a white salt was obtained in a yield of approximately 0.10 gram per kg. of fresh lean beef.

This product gave a negative test for carbon by the method of Pepkowitz (*8*). Upon addition of sodium hydroxide, it liberated a volatile base which turned red litmus paper blue and gave the odor of ammonia. It was, therefore, concluded that this salt was ammonium chloride. This conclusion was confirmed by decomposing a portion of the salt with sodium hydroxide and aerating the volatile base into a solution of phenylisothiocyanate. A solid derivative formed almost immediately which, after washing with Skellysolve B and 50% ethyl alcohol and recrystallizing from 95% ethyl alcohol, melted at 157°–158° C. This product showed no melting point depression when mixed with known phenylthiourea.

Carbonyl Compounds. Fresh broth prepared as described above was distilled into a flask which was immersed in an ice bath and contained a 0.2% solution of 2,4-dinitrophenylhydrazine (DNP) in 2*N* hydrochloric acid. The precipitate of crude dinitrophenylhydrazones was filtered off, dried, and weighed. The yield was approximately 0.09 gram per kg. of fresh beef.

The crude mixture of DNP derivatives so obtained was stirred up repeatedly with 30- to 40-ml. portions of boiling ethyl alcohol and the insoluble portions were filtered off. About 0.04 gram of this insoluble fraction, presumably consisting of polycarbonyl derivatives, was obtained per kg. of beef. The ethyl alcohol solution containing the monocarbonyl derivatives was evaporated to dryness, the residue dissolved in warm 1 to 1 benzene to heptane, and the solution poured onto the top of a 2.2 × 10 cm. column of powdered anhydrous magnesium sulfate. The column was developed with the same solvent mixture which was passed through under pressure, until the carbonyl derivatives were eluted and the effluent became almost colorless. Under these conditions the unreacted dinitrophenylhydrazine reagent remained at the top of the column.

The effluent was evaporated to a small volume and a convenient amount chromatographed on Whatman No. 1 paper according to the method of Huelin (*5*). The developed chromatogram showed two yellow spots with R_f values of 0.26 and 0.40 which corresponded to those of the DNP derivatives of acetone and acetaldehyde, respectively. Judging from the intensities of the spots on the chromatograms, the acetaldehyde derivative was the major component.

To obtain sufficient material for identification, the concentrated effluent from the magnesium sulfate column was applied to a number of papers in a narrow, intensely colored band. After development of the chromatograms the bands corresponding to the above two spots were separately eluted with 95% ethyl alcohol and the eluates pooled and evaporated to dryness. The derivatives were recrystallized from 95% ethyl alcohol to a constant melting point. As shown in Table I these two products proved to be identical with the derivatives of acetone and acetaldehyde, respectively. In addition to melting point and paper chromatographic data, infrared tracings and x-ray diffraction patterns were obtained for each of the dinitrophenylhydrazone derivatives. In all cases the results with the isolated and known compounds were identical.

The ethyl alcohol–insoluble polycarbonyl fraction was recrystallized from nitrobenzene without previous passage through the magnesium sulfate column. Comparison of the melting point and infrared spectrum of these crystals with those of an authentic sample of diacetyl - *bis*- 2,4 - dinitrophenylhydrazone showed that they were identical (Table I).

Analysis. Calculated for $C_{16}H_{14}O_8N_8$: C, 43.05; H, 3.16; N, 25.11. Found: C, 43.20; H, 3.33; N, 25.04.

Fatty Acids. Fresh broth was acidified with sulfuric acid to pH 1 and distilled at atmospheric pressure to two thirds of the original volume. The distillate was collected in a receiver cooled in an ice bath and was titrated to a phenolphthalein end point with sodium hydroxide. Approximately 1 meq. of acids was obtained per kg. of fresh meat.

The neutralized distillate was evaporated to dryness in vacuum and the salts decomposed with 6*N* sulfuric acid. The free fatty acids were taken up in ether and a solution of diazomethane in ether was added in small portions until a faint yellow color persisted. Most of the solvent was evaporated from the resulting solution of methyl esters by placing the mixture in an open Erlenmeyer flask and warming gently. No effort was made to remove the last traces of ether for fear that volatile esters might also be evaporated. The remaining solution was then subjected to gas chromatography on a Model 154 Perkin-Elmer Vapor Fractometer. The column used was 5 mm. in internal diameter and 2 meters long, and was filled with one part of diisodecylphthalate on four parts of 60- to 80-mesh Celite 505 (Johns-Manville Co., filter aid) support. The conditions and results are shown in Figure 1.

In addition to peaks caused by air and ether, five more peaks corresponding to the methyl esters of formic, acetic, propionic, isobutyric, and butyric acids were observed. The retention times of the esters checked very closely with those of the known samples. However, no additional evidence was obtained to verify the tentative identification of the esters as listed above.

Sulfur Compounds. In order to look for volatile sulfur compounds the vapor arising from the simmering meat was carried by a stream of high purity nitrogen gas through a reflux condenser, then through a wash bottle containing 20 ml. of 2*N* sulfuric acid for removal of ammonia, and then into various trapping solutions. When either aqueous lead acetate or mercuric cyanide solutions were used as trapping agents, black precipitates formed. The black precipitate in the lead acetate trap was

filtered off, washed, and dried, and subsequently found to give a negative test for carbon by the method of Pepkowitz (*8*). The black precipitate obtained in the mercuric cyanide trap gave no odor when warmed with dilute hydrochloric or sulfuric acids. These results indicate the presence of hydrogen sulfide and the absence of volatile mercaptans, as mercaptans are released when their mercuric salts are warmed with dilute mineral acid (*7*). It is possible that mercaptans might have been oxidized to the corresponding disulfides during passage of the vapors through the 2.N sulfuric acid trap and the other compartments of the absorption train (*3*). However, no disulfides were detected either, hence no evidence was obtained in the present work that the vapors contained mercaptans.

A quantitative determination of the hydrogen sulfide released from the cooked beef was carried out by the methylene blue method as described by Sands and coworkers (*9*) and by Marbach and Doty (*7*). The vapor above the simmering beef was aerated with high purity nitrogen into 2% zinc acetate as the trapping solution. This was replaced by fresh solution as soon as any visible turbidity developed. After 3 hours the turbid mixtures were pooled and diluted to 500 ml. with 2% zinc acetate, and the color was developed as previously described (*9*). Absorbancy readings were obtained on a Bausch and Lomb Spectronic No. 20 colorimeter at 665 mμ. The readings were converted into equivalent hydrogen sulfide values by interpolation on a standard curve prepared with known quantities of sodium sulfide (*7*). In two determinations the amount of hydrogen sulfide found varied from 6 to 8 mg. per kg. of fresh beef.

When the vapors from the cooking meat were passed first through a sulfuric acid trap and then into a mercuric chloride trap a white precipitate was obtained in the latter. This product showed no crystalline form when examined under the microscope and began to decompose at 250° C. On treatment with 6.N hydrochloric acid no odor was detected. This result indicated that disulfides were absent. However, the precipitate gave rise to an odor very similar to that of dimethyl sulfide when treated with 10% sodium hydroxide. This behavior is characteristic of volatile thioethers (*1*).

The quantities of volatile thioether obtained were so small that positive identification by classical methods was practically impossible. The vapor obtained from 1.35 kg. of beef after decomposition of the mercuric chloride salt with sodium hydroxide was passed through the Perkin-Elmer gas chromatography apparatus, but failed to result in a detectable peak. Subsequently, the gas chromatographic analysis of a similar thioether preparation was repeated on a Barber-Colman gas-liquid chromatograph Model 10 with the very sensitive strontium-90 detector (*6*). The column employed was 8 feet long and was filled with five parts of silicone oil on 100 parts of Chromosorb W support (Murray and Baker Co., Dagenham, England). In addition to peaks due to air and water, a small peak corresponding in retention time to authentic dimethyl sulfide was obtained.

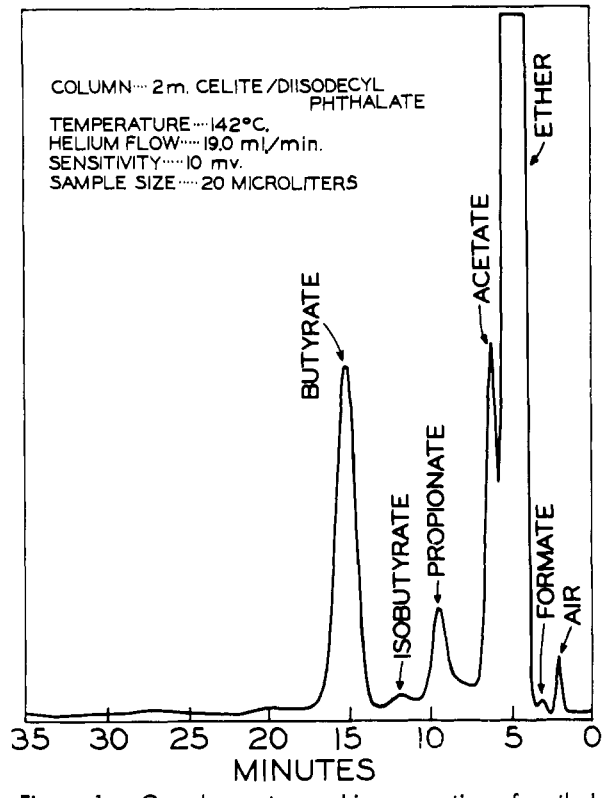

Figure 1. Gas chromatographic separation of methyl esters of volatile fatty acids of cooked beef

Discussion

The results obtained in the present study establish the presence of a number of compounds in the volatile fraction of cooked beef in addition to hydrogen sulfide, previously reported by Crocker (*2*). The compounds which have been firmly identified include ammonia, acetaldehyde, acetone, and diacetyl. In addition, reasonably good evidence for the presence of formic, acetic, propionic, isobutyric, and butyric acids and of dimethyl sulfide was obtained. An attempt to find volatile alcohols in the distillate from the broth by preparation of 3,5-dinitrobenzoyl esters was unsuccessful. Similarly, a qualitative test for esters by means of alkaline hydroxylamine followed by ferric chloride was negative. It seems probable, therefore, that volatile alcohols and esters were not present in appreciable amounts in the beef broth.

While the present report was in preparation, a paper by Hornstein *et al.* (*4*) appeared which described the identification of small amounts of carbonyl compounds, ammonia, and hydrogen sulfide in the volatile fraction of heated beef extract. Their results agree well with those of the present study.

Hydrogen sulfide, ammonia, diacetyl, and acetaldehyde appear to be some of the major components responsible for the characteristic aroma of cooked beef. All of these substances seem to be liberated during cooking from essentially odorless precursors. Whether the ammonia and hydrogen sulfide are derived from the amino and sulfhydryl groups of the meat proteins has not, to the authors' knowledge, been established. It was noted in the present study that greatly increased amounts of hydrogen sulfide were evolved from the meat if the heating period were prolonged. For example, the white precipitate in the mercuric chloride trap (presumably mostly $HgCl_2.2HgS$) obtained after boiling and aerating continuously for 3 hours and, in another experiment, for 7 days weighed 0.04 and 1.34 gram per kg. of beef, respectively.

Literature Cited

(1) Challenger, F., "Aspects of the Organic Chemistry of Sulfur," p. 18, Academic Press, New York, 1959.
(2) Crocker, E. C., *Food Research* **13,** 179 (1948).
(3) Doty, D. M., American Meat Institute Foundation, Chicago Ill., personal communication, June 17, 1960.

(4) Hornstein, I., Crowe, P. F., Sulzbacher, W. L., J. AGR. FOOD CHEM. **8,** 65 (1960).
(5) Huelin, F. E., *Australian J. Sci. Research Ser. B*, **5,** 328 (1952).
(6) Lovelock, J. E., *J. Chromatog.* **1,** 35 (1958).
(7) Marbach, E. P., Doty, D. M., J. AGR. FOOD CHEM. **4,** 881 (1956).
(8) Pepkowitz, L. P., *Anal. Chem.* **23,** 1716 (1951).
(9) Sands, A. E., Grafius, M. A., Wainwright, H. W., Wilson, M. W., U. S. Bur. Mines, Rept. Invest. **4547** (1949).

Received for review March 14, 1960. Accepted June 27, 1960. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Campbell Soup Co. Presented in part at Division of Agricultural and Food Chemistry, 135th Meeting, ACS, Boston, Mass., April 1959.

MEAT FLAVOR CHEMISTRY

Flavor Studies on Beef and Pork

IRWIN HORNSTEIN and PATRICK F. CROWE

Meat Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

The odor responses, and the chemical compounds isolated, from the volatile pyrolysis products of lyophilized cold water extracts of lean beef and lean pork were found to be similar. The flavor precursors in lean meat are low molecular weight compounds present in the dialyzable portion of the cold water extracts of the raw lean meats. Beef and pork fat when heated produced dissimilar aromas. Free fatty acids and carbonyls were determined in these fats before and after heating. The results suggest that, on heating, the lean portions of pork and beef contribute an identical meaty flavor to these meats, while the characteristic flavor differences in pork and beef reside in the fat.

PRIMARY EMPHASIS has been placed on the odor constituents of meats. The authors previously studied lean beef and found the flavor precursors present in the raw meat to be cold water–extractable. This extract was lyophilized, and the resulting powder heated under vacuum and fractionated into two major portions. The more volatile fraction has been studied (*7*). Investigation of the less volatile fraction (fraction I) is reported in this paper. Lean pork has also been subjected to the techniques previously applied to lean beef and its volatile constituents have been examined. In addition, beef and pork fat have been analyzed for free fatty acids and monocarbonyl compounds, and possible flavor precursor systems have been studied.

Experimental

Lean Beef, Fraction I. The powder obtained by the lyophilization of a cold water extract of raw, lean beef contains flavor precursors of cooked beef. The total volatiles produced by pyrolysis of this powder at 100° C. are trapped at liquid nitrogen temperatures and fractionated at room temperature under vacuum into two major fractions. The less volatile of these two fractions, fraction I, is a viscous residue of meaty aroma (*7*).

INITIAL OBSERVATIONS. Approximately 100 mg. of fraction I were obtained for every 30-gram batch of dried powder pyrolyzed. The pH of a water solution of 10 mg. of I per ml. varied from 3.5 to 4.0. The infrared spectrum of a film of I on rock salt plates was obtained on a Perkin-Elmer Model 137 Infracord spectrophotometer. Major peaks were recorded at 3.15, 5.81, 6.32, 7.12, 8.9, 9.6, and 11.7 microns (Figure 1). The ultraviolet spectrum of a water solution of 2 mg. of I per ml. was obtained on a Beckman DU spectrophotometer; a maximum was observed at 290 to 295 mμ. Elemental analysis of I was: C, 34.21%; H, 7.69%; N, 5.46%; S and P, absent; and O (by difference), 52.64%. The neutral equivalent of I was 216.

PAPER CHROMATOGRAPHY OF I. Fraction I was best separated on paper by developing the chromatogram with butyl alcohol saturated with water. The ascending chromatographic technique and apparatus described by Mitchell (*12*) were used to separate I on a milligram scale. Two Whatman No. 1 sheets, 8 × 8 inches, were streaked across the paper 1 inch from the bottom with 2 ml. of methanol containing 37.5 mg. of I, and the chromatogram was developed until the solvent front was 1 inch from the top. The papers were hung in a well ventilated hood to dry, and then a 0.5-inch strip was cut from the edge of the sheet and sprayed with 1% permanganate. Three bands appeared at R_f 0.82, 0.50, and 0.25. The remainder of each of these fractions was eluted with methanol, concentrated on a rotary evaporator, rechromatographed, and again eluted with methanol. An appropriate amount of each solution was placed on a rock salt plate and the solvent volatilized by heat. The infrared spectra for the fractions at R_f 0.82 and 0.25 were obtained. An insufficient amount of material of the components at R_f 0.50 was recovered to obtain an infrared curve. Ultraviolet spectra of water solutions containing 2 mg. per ml. of the fractions recovered at R_f 0.82 and 0.25, respectively, gave no characteristic peaks; when 3 ml. of water were added to the residue from the fraction at R_f 0.50, a slight increase in absorption at 290 to 295 mμ was observed.

TITRATION CURVES. Seven milliliters of the solution to be titrated, containing 77.0 mg. of fraction I, were placed in a small constant temperature cell equipped with electrodes attached to a line-operated pH meter. The solution was stirred magnetically and blanketed by nitrogen. A calibrated micropipet, made from a glass capillary and a vernier micrometer, was used to add small increments of alkali or acid. After each addition, the pH of the solution was recorded.

LABILE NITROGEN. Labile nitrogen in I was determined by comparing Kjeldahl nitrogen results with those obtained by the following modified Kjeldahl procedure. Twenty-five milligrams of I in 3 ml. of water were placed in a test tube ending in a standard-taper 24/40 joint connected to an adapter, through which 5 ml. of 30% sodium hydroxide were added and through which nitrogen gas was admitted. The nitrogen passed slowly over the magnet-