Summary

The fatty acids of alga *Chlorella* have been examined. The cells used were grown in high concentrations of fixed nitrogen and therefore contained only 12.4% lipid on a dry weight basis. The lipid contained only some 50% fatty acids, about 80% of these containing unsaturation. All double bond systems appear to be of the all-cis, singly methylene interrupted type. The C_{18} diene and triene appear to be largely normal linoleic and linolenic acids. Perhaps the most probable structure for the unusual hexadecatetraene is 4,7,10,13-cis,cis,cis,cis-hexadecatetraenoic acid. The estimated composition of the fatty acids of *Chlorella* is:

Below C_{16}	less than 2%
Saturated	17%
C_{16} monoene	4%
C_{16} diene	6%
C_{18} triene	12%
C_{16} tetraene	3%
C_{18} monoene	7%
C_{18} diene	11%
C_{18} triene	34%
C_{18} tetraene	1%
Above C_{18}	less than 4%

REFERENCES

1. Milner, H. W., J. Biol. Chem., *176*, 813 (1948).
2. Milner, H. W., J. Amer. Oil. Chem. Soc., *28*, 363 (1951).
3. Heyes, J. K., and Shorland, F. B., Biochem. J., *49*, 503 (1951).
4. Toyama, Y., and Tsuchiya, T., Bull. Chem. Soc. Japan, *10*, 192 (1935).
5. Tutiya, T., J. Chem. Soc. Japan, *61*, 1188 (1940), C.A., *37*, 1382 (1943).
6. Tutiya, T., *ibid.*, *62*, 10 (1941), C.A., *37*, 1383 (1943).
7. Farmer, E. H., Trans. Faraday Soc., *38*, 356 (1942).
8. Toyama, Y., and Tsuchiya, T., Bull. Chem. Soc. Japan, *10*, 232 (1935).
9. Ralston, A. W., "Fatty Acids and Their Derivatives," p. 145, John Wiley and Sons Inc., New York (1948).
10. Shorland, F. B., Nature, *170*, 924 (1952).
11. Lovern, J. A., Dept. Sci. Ind. Research (Brit.), Rept. Food Invest. Board *1936*, 96 (1937), C.A. *32*, 5651 (1938).
12. Lovern, J. A., *ibid.*, *1935*, 75 (1936), C.A., *32*, 2380 (1938).
13. Lovern, J. A., Biochem. J., *32*, 1214 (1938).
14. Cartter, J. L., and Hopper, T. H., U. S. Dept. Agr. Tech. Bull., *787*, 66 pp. (1942).
15. Stansbury, M. F., Hoffpauir, C. L., and Hopper, T. H., J. Amer. Oil Chem. Soc., *30*, 120 (1953).
16. Mitchell, J. H., Kraybill, H. R., and Zscheile, F. P., Ind. Eng. Chem. Anal. Ed., *15*, 1 (1943).
17. Brice, B. A., Swain, M. L., Herb, S. F., Nichols, P. L., and Riemenschneider, R. W., J. Am. Oil. Chem. Soc., *29*, 279 (1952).
18. Jackson, J. E., Paschke, R. F., Tolberg, W., Boyd, H. M., and Wheeler, D. H., J. Am. Oil Chem. Soc., *29*, 229 (1952).
19. Brice, B. A., and Swain, M. L., J. Opt. Soc. Am., *35*, 532 (1945).
20. Celmer, W. D., and Solomons, I. A., J. Am. Chem. Soc., *74*, 3838 (1952).
21. White, M. F., and Brown, J. B., J. Am. Oil Chem. Soc., *26*, 133 (1949).
22. Paschke, R. F., and Wheeler, D. H., J. Am. Oil Chem. Soc., *27*, 9 (1950)
23. White, M. F., Orians, B. M., and Brown, J. B., J. Am. Oil Chem. Soc., *26*, 85 (1949).
24. Chapman, V. J., "An Introduction to the Study of Algae," p. 4, The Macmillan Company, New York (1941).
25. Montfort, C., Jahrb. wiss. Botan., *79*, 493 (1934).
26. Hoffman, H. D., and Green, C. E., Oil and Soap, *16*, 236 (1939).
27. Earle, F. R., and Milner, R. T., Oil and Soap, *17*, 106 (1940).
28. Levin, E., and Lerman, F., J. Am. Oil Chem. Soc., *28*, 441 (1951).

[Received May 26, 1953]

The Glyceride Structure of Natural Fats. III. Factors Governing the Content of Fully Saturated Glycerides

A. R. S. KARTHA,[1,2] Maharaja's College, Ernakulam, India

IN part I of this series (1) a procedure was described for the quantitative determination in fats of the four glyceride types GS_3, GS_2U, GSU_2, and GU_3.[3]

In part II (2) a rule was stated for calculating the glyceride type distribution in natural fats. It was based on the assumption that there is a maximum proportion of GS_3 which may be present in each species of fat. This limit varies, according to circumstances, up to the proportion of GS_3 which may be produced by random or chance distribution of the component fatty acids among the glyceryl radicals. When the proportion of GS_3 which can exist is less than that which could be synthesized by chance distribution of the saturated fatty acids, the excess S is distributed according to chance among the remaining glyceryl radicals without formation of any more GS_3. In the present contribution some factors governing the quantity of GS_3 which may be present in a fat will be discussed.

Any hypothesis accounting for glyceride type distribution must explain the operation, with remarkable accuracy, of the rule for calculation of the glyceride type distribution in natural fats. It must also show how restriction of chance distribution, when this occurs, is effected. It must agree with what appears to be the fact that fatty acid synthesis and esterification take place in all vegetable and animal fat depots (3).

It must also agree with certain evidence that in adipose tissues as well as in mammary glands of animals fat can be deposited from ingested foods without affecting the normal glyceride type distribution. Kartha and Menon (4) have shown that in buffalo and cow milk fats, and in ox depot fat, the ratio of GS_3 actual to GS_3 chance retains a value of very nearly unity in spite of variations in saturated acid content from about 50% to about 70% due to variations in food fat, etc. Kartha (4) has shown that samples of the same depot fats from different sources, varying in saturated acid content possibly because of differences in diet, all give analytical results in agreement with those obtained by application of the rule for calculation of glyceride type distribution.

Furthermore in any hypothesis the mechanism of esterification postulated must agree with the known specificities of the lipases effecting the esterification under normal *in vivo* conditions (5). It must also agree with the fact that lipolytic esterification is reversible and proceeds according to the law of mass action (6).

It is well known that the melting ranges of refined fats vary. This variation may be due to several causes, such as the relative proportions of saturated

[1] Present address, Indian Agricultural Research Institute, New Delhi, India.

[2] Edited and revised by R. J. Vander Wal, Armour and Company, Research Division, Chicago, Ill.

[3] The following abbreviations will be used in the text:
G = Glyceryl radical.
S = Saturated fatty acid(s) or saturated fatty acid group(s) according to context.
U = Unsaturated fatty acid(s) or unsaturated fatty acid group(s) according to context.

and unsaturated acids, the chain length of the saturated acids, and the positions of the acyl groups on the glyceryl radical. The solubilities of constituent triglycerides in the substrate will be factors governing the melting range of the whole mixture. A fat containing a high proportion of GS_3 will remain partly solid at a higher temperature than a comparable fat containing much less GS_3.

The composition and melting ranges of cacao butter and mutton tallow are shown in Table I.

TABLE I

Composition and Melting Ranges of Cacao Butter and Mutton Tallow (7)

Fat	Component saturated acids			% Saturated acids in total acids	% GS_3	M.R.
	C_{14}	C_{16}	C_{18}			
Cacao butter	....	24.3	35.5	59.8	2.5	30-34°C.
Mutton tallow	5.5	26.2	29.3	61.0	26.6	44-51°C.

Each of these fats contains about 60% of saturated acids, and both contain more C_{18} than C_{14}-C_{16} acids. In spite of the fact that mutton tallow contains less C_{18} and more of the lower melting C_{14}-C_{16} acids, its melting range is greatly above that of cacao butter. There is, however, more than 10 times as much GS_3 in mutton tallow than in cacao butter which fact probably accounts, at least in part, for the higher melting range.

The maximum points of the melting ranges (melting points) of refined fats are not necessarily the same as those of the same fats *in vivo*. Although animal tallows often show melting ranges the upper limits of which are 10-15°C. above the depot temperatures, the fat is always present in the liquid state in living adipose tissue (8). It is evident that, in the adipose tissues of higher land animals, biologic factors are present whereby the fat is kept fluid at temperatures sometimes 10-15° C. below its normal melting point. If these factors are present in the higher land animals, they are likely to be present in lower animal tissue and in vegetable tissue as well.

It seems to be a reasonable hypothesis, in view of the preceding observations, that GS_3 can be synthesized in the depots only to the extent that it can remain in a fluid state *in vivo*. If so, the upper limit of fluidity is a variable limiting the synthesis of GS_3 in many natural fats to less than chance values.

If the hypothesis is correct and GS_3 will never be produced *in vivo* in excess of the amount that will remain fluid, then a GS_3 which possesses characteristics favoring its existence in a fluid state should appear in fats in greater quantity than a GS_3 which does not have these fluidizing properties to the same degree.

Kartha (9) has shown that the maximum limit of the melting range of a mixture of a particular GS_3 in a particular non-GS_3 substrate is a function of the GS_3 content. He has defined the "melting point constant" as the difference in "melting point" (upper limit of the melting range) produced by decreasing the concentration of GS_3 by half. The melting point constant for a number of GS_3 mixtures comprised of varying amounts of C_{16} and C_{18} acids in a substrate of peanut oil was found to be 5.8-6.0°F. The constants for Actinodaphne Hookeri fat and coconut oil, which consist mainly of trilaurin, in a substrate of peanut oil are 7 and 8°F., respectively.

Maximum limits of the melting ranges (melting points) of the mixtures decrease as the concentration of GS_3 decreases, and the experiments demonstrate that solid GS_3 becomes liquid by solution in the liquid substrate rather than by actual melting. It also appears that a decrease in the mean chain length of the GS_3 results in increased solubility in the substrate in addition to that due to the decreased melting point (9), since the melting point constants increase in value. The mean chain length of the GS_3 in refined fats governs therefore to a large extent the amount which can liquefy at a given temperature in the non-GS_3 substrate. The same relationship should apply in general to fats *in vivo*.

The data in Table II show that, in general, the shorter the mean chain length, the more GS_3 is found in proportion to the total quantity of saturated acids in natural fats of approximately the same melting ranges.

The data in the table were mostly derived from those given by Hilditch (7) except for the melting ranges, many of which were taken from those recorded by Jamieson (7). Others were obtained from the data supplied by the American Oil Chemists' Society (7). Although some data may not be strictly accurate (1, 2) and the melting ranges may not be those of the samples actually examined, the error involved is sufficiently small to permit use of the assembled values.

It will be obvious that as the proportions of C_{18} or higher acids in the total saturated acids (and consequently in the GS_3) decrease to be replaced by C_{16} acids, and these in turn are replaced by C_{14} or lower acids, there is, in general, an increase in the amount of GS_3 found by experiment. There is also some tendency for the refined fat to melt over a lower range, but the melting ranges of refined fats are not a reliable criterion of the melting ranges *in vivo*, which are the only ones that are significant. As has been stated, fats are often fluid *in vivo* at temperatures several degrees below the maximum limits of their melting ranges outside the living organism.

The series comprising the tropical vegetable fats illustrate the relationship especially well. The component saturated fatty acids of rambutan, pulasan, *Garcinia indica*, and *Allanblackia stuhlmannii* fat contain very high proportions of stearic or higher acids and only traces of GS_3. Borneo tallow contains a smaller proportion of C_{18} or higher acids and more C_{16}. The GS_3 content increases correspondingly. The same changing relationships are evident in phulwara butter, palm oil, and stillingia tallow. Babassu oil, palm kernel oil, nutmeg butter, dika fat, and coconut fat all contain high percentages of C_{14}, or lower, acids among the saturated acids, and these fats contain exceedingly high proportions of GS_3.

The hypothesis that the quantity of GS_3 which can be produced in natural fats is restricted to the quantity which can remain fluid *in vivo* is thus supported by a considerable amount of experimental evidence.

Kartha, in part II of this series (2), has demonstrated that the proportions of GS_3, GS_2U, GSU_2, and GU_3 in a natural fat can be calculated according to the rules of probability even when the quantity of GS_3 which may be present is limited to less than chance values. Limitation of the formation of GS_3 in

TABLE II

The Saturated Components of Some Natural Fats

Fat	Type	Component S Fatty Acids (Approx.)			% S (mol.)	% GS_3 actual (mol.)	M.R. fat	% GS_3 chance
		% C_{18} or higher	% C_{16}	% C_{14} or lower				
Rambutan tallow	Tropical vegetable	96	4	....	49.0	1.4	40-46°C.	11.76
Pulasan fat	Tropical vegetable	95	5	....	55.3	1.5	40-42°C.	16.91
Garcinia indica	Tropical vegetable	95	5	....	59	1.5	40-43°C.	20.5
Allanblackia stuhlmannii	Tropical vegetable	94	6	....	56	1.5	43-46°C.	17.6
Chicken fat	Bird fat	20	79.71	0.29	34.0	2.5	32-34°C.	3.93
Garcinia morella	Tropical vegetable	85	14	1	50	2.7	34-37°C.	12.5
Borneo tallow	Tropical vegetable	63	35	2	63	4.5	34-39°C.	25.0
Phulwara butter	Tropical vegetable	5.4	92	2.6	62	8.0	39-51°C.	23.8
Palm oil (Belgian Congo)	Tropical vegetable	11	86	3	51	10.3	27-50°C.	13.3
Hog fat (perinephric)	Swine fat	37.31	58.42	4.26	46.9	13.2	33-46°C.	10.3
Beef tallow (N. American)	Bovine fat	26.8	58.2	15.0	50.0	13.9	40-48°C.	12.5
Camel milk fat	Ruminant milk fat	14.6	50.0	35.4	62.6	25.6	35.3 SP	24.5
Mutton tallow	Sheep fat	48.03	42.95	9.02	61.0	26.6	44-51°C.	22.7
Cow milk fat (English) (Spring pasture 1929)	Ruminant milk fat	14.9	39.5	45.6	61.9	27.2	28-35°C.	23.7
Stillingia tallow U. S. (Florida)	Tropical vegetable	9.8	83.0	7.2	72.5	28.4	48.2 SP	38.1
Babassu	Tropical vegetable	1	9	90	86.7	67.3	24-26°C.	65
Palm kernel	Tropical vegetable	4	6	90	85.3	66	24-26°C.	62.1
Nutmeg butter	Tropical vegetable	....	6.7	93.3	90.2	73	42-45°C.	73.4
Dika fat	Tropical vegetable	....	....	100	91.7	81	41-42°C.	77.1
Coconut fat	Tropical vegetable	2	11	87	93.9	86	23-26°C.	82.8

natural fats to the quantity which can be present in the fluid state must therefore result from circumstances which permit an otherwise random distribution of the fatty acid groups.

The action of the lipase, both plant and animal, which effects esterification of the fatty acids and glycerol is reversible and proceeds according to the law of mass action (6). In this respect it acts like an ordinary esterification catalyst. Hence when a depot fat is in the presence of active lipase and water, a dynamic equilibrium will eventually exist similar to that existing in esterification reactions outside living tissue. The alpha and beta positions of the glyceryl radical show no specificity toward any fatty acid (10). Therefore in a reaction *in vivo* unless the enzyme itself exerts some specificity of action, complete random distribution of the fatty acids at equilibrium will result.

It was suggested earlier that deviations from the completely random pattern may be due to inability of the plant or animal to produce GS_3 in excess of the amount which can be present in the fluid state *in vivo*. With this restriction random distribution of all the fatty acids appears to occur under the influence of the lipase. The inability of living tissue to synthesize GS_3 in excess of the quantity which may be present in the fluid state may well be due to a specific quality of the lipolytic enzymes which makes it impossible for them to synthesize triglycerides which are solid *in vivo*. If they cannot synthesize solid triglycerides, then obviously they can synthesize GS_3 up to the exact amount which can remain fluid and then can produce no more since any increment will be solid. With this limitation these enzymes should be able to promote random distribution of the fatty acids among the glyceryl molecules provided the latter are in a liquid state.

What the property of lipolytic enzymes may be which prevents them from synthesizing solid GS_3 is now only a field for speculation. If however it be assumed that they are colloidal bodies existing as a separate solid phase and that they effect esterification by formation of stable intermediate complexes with the reactants which rearrange to form stable intermediate complexes of the enzymes with the reaction products, there is a more or less plausible explanation.

In order to form the stable enzyme-reactants complex, the reactants must, according to the theory, be in a state to be adsorbed as a continuous film on the colloidal enzyme. This will be possible only if they are liquids or in solution. If one of the reactants is a solid, it cannot be adsorbed on the enzyme surface and the complex cannot form. The absence of reaction between lipase and solid triglycerides has been demonstrated by Willstätter and Waldschmidt-Leitz (11). These investigators showed that ricinus or pancreas lipase can be adsorbed on tristearine crystals and can be recovered unchanged and active by elution, leaving the tristearine entirely unaffected.

Collins (12) has confirmed this by showing that lipolytic bacteria do not hydrolyze solid tristearine. Tofte (13) showed that the rate of hydrolysis of hydrogenated fats by ricinus or pancreas lipase at fixed temperatures decreases with increasing melting point of the fat and that for fats of the same melting points rates of hydrolysis increase with increasing temperature. Evidently the higher the proportion of solid triglycerides, the less rapid is the reaction.

It may be true that the enzymes cannot react with solid phase triglycerides because the solid glycerides cannot form a continuous adsorbed film on colloidal enzyme. If a glyceride-enzyme-water complex cannot be formed, it obviously cannot rearrange to produce a fatty acid-glycerol-enzyme complex and no hydrolysis will occur.

The converse of this may be equally true and may be the explanation for any inability of enzymes to synthesize solid GS_3. Perhaps enzymes cannot synthesize solid fats because the fatty acid-glycerol-enzyme complex, which can exist because all reactants are liquid, cannot rearrange to form a solid glyceride-enzyme-water complex because this cannot exist. The same glyceride can however be synthesized to the extent that the glyceride-enzyme-water complex remains liquid.

Norris and Mattil (14) refer to enzymatic action in the synthesis of fats and to interesterification and ester-interchange during metabolism. The principles discussed in preceding paragraphs can apply also to ester-interchange reactions.

The limitation of chance distribution observed in many natural fats may then be due to the inability of lipase to synthesize solid phase glycerides because

these are incapable of forming adsorbed films on the enzyme surface.

Summary

Experimental evidence from the literature is given to support the hypothesis that the GS_3 which may be present in natural fats cannot be in excess of the quantity which can exist in the fluid state *in vivo*. It is suggested that solid fat is not produced *in vivo* because the mode of action of lipolytic enzymes allows them to form only liquid fats.

REFERENCES

1. Kartha, A. R. S., J. Am. Oil Chem. Soc., *30*, 280 (1953).
2. Kartha, A. R. S., J. Am. Oil Chem. Soc., *30*, 326 (1953).
3. Kartha, A. R. S., "Studies on the Natural Fats", Vol. 1, pp. 84-87, published by the author. The subject is discussed and references are made to original work.
4. Discussed with original references by: Kartha, A. R. S. and Menon, K. N., Proc. Indian Acad. Sci., *27A*, 279 (1948); Kartha, A. R. S., J. Am. Oil Chem. Soc., *29*, 109 (1952).
5. Velluz, L., Bull. soc. chim. biol., *16*, 909 (1934); Ono, T., J. Agr. Chem. Soc., Japan, *16*, 43 (1940); Longenecker, H. E. and Haley, D. E., J. Am. Chem. Soc., *57*, 2019 (1935); Loury, M. and Becker, Y., Bull. matieres grasses inst. colonial Marseille, *29*, 5 (1945); Morel, L. and Terroine, E., Compt. rend., *149*, 236 (1909); Weinstein, S. S. and Wynne, A. M., J. Biol. Chem., *112*, 641 (1936); Quagliariello, G. and Cedrangolo, F., Enzymologia, *4*, 73 (1937); Cedrangolo, F., Enzymologia *5*, 1 (1938); Balls, A. K., Matlack, M. B., and Tucker, I. W., J. Biol. Chem., *122*, 125 (1937); Balls, A. K. and Matlack, M. B., J. Biol. Chem., *123*, 679 (1938); Kraut, H., Weischer, A. and Hügel, R., Biochem. Z., *316*, 96 (1943); also references under (10).
6. Armstrong, H. E. and Gosney, H. W., Proc. Roy. Soc. London (B), *88*, 176 (1914); Groen, J., Arch. néerl. physiol., *11*, 169 (1926); Velluz, L., Bull. soc. chim. biol., *10*, 1213 (1928); Sym, E. A., Biochem. Z., *262*, 406 (1933); Fabisch, W., Biochem. Z., *259*, 420 (1933); Benigno, P., Enzymologia, *5*, 293 (1938).
7. Hilditch, T. P., "The Chemical Constitution of Natural Fats", 2nd ed., Chapman and Hall Ltd., London, 1947; Jamieson, G. S., "Vegetable Fats and Oils", 2nd ed., Reinhold Publishing Corporation, New York, N. Y., 1943; "Official and Tentative Methods of the American Oil Chemists' Society", Chicago, Ill.
8. Hammond, J., Chem. Ind., *52*, 637 (1933); "Encyclopedia Brittanica", 14th ed., Vol. 1, p. 166 (1939); Bloor, W. R., "Biochemistry of the Fatty Acids", 1st ed., pp. 240-244, Reinhold Publishing Corporation, New York 1943; Starling, E. H. and Evans, C. L., "Principles of Human Physiology", 9th ed., pp. 28, 905, Churchill, London, 1947.
9. Kartha, A. R. S., J. Sci. Ind. Research (India), *11A*, 354 (1952).
10. Weber, H. H. R., and King, C. G., J. Biol. Chem., *108*, 131 (1935); Holwerda, K., Verkade, P. E. and de Willigen, A. H. A., Rec. trav. chim., *56*, 382 (1937); Artom, C. and Zummo, C., Enzymologia, *3*, 231 (1937); Mazza, F. P. and Malaguzzi Valeri, C., Arch. sci. biol. (Italy), *25*, 270 (1939).
11. Willstätter, R. and Waldschmidt-Leitz, E., Z. physiol. Chem., *126*, 132 (1923).
12. Collins, M. A., Iowa State Coll. J. Sci., *8*, 187 (1933).
13. Tofte, F., Biochem. Z., *272*, 308 (1934).
14. Norris, F. A. and Mattil, K. F., J. Am. Oil Chem. Soc., *24*, 274 (1947); J. Am. Oil Chemists' Soc., *23*, 289 (1946).

[Received April 22, 1953]

Estimating Carbonyl Compounds in Rancid Fats and Foods[1]

A. S. HENICK, M. F. BENCA,[2] and J. H. MITCHELL JR., Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois

THE need for an objective test which will correlate with flavor changes in fats and foods containing fats is apparent to all workers in the field. The only test now in general use, that for peroxide value, is somewhat less than satisfactory especially since the hydroperoxides of fats are generally without flavor or odor. A useful test to correlate with flavor changes, then, should measure some substance, formed during the autoxidation of fat, which is inherently flavored or odorous. Among the several substances in this category, carbonyl compounds seem particularly promising.

Several recent publications (2, 4, 6) report finding carbonyl compounds in the volatile products of autoxidized fats and oils. Although some of these compounds have been qualitatively identified, no quantitative method of universal applicability has been published. The use of 2,4-dinitrophenyl hydrazine derivatives in the colorimetric determination of mono-functional aldehydes and ketones was proposed by Pool and Klose (5), who used a chromatographic separation to eliminate the excess reagents and the derivatives of polycarbonyl compounds. Lappin and Clark (3) described a procedure which also employs the 2,4-dinitrophenyl hydrazones for determining traces of carbonyl compounds in aqueous and alcoholic solutions. Although the latter method was generally inapplicable to fats because the components did not remain in solution throughout the reaction, it was repeatedly observed to give much higher values than did the selective and more reproducible monocarbonyl method of Pool and Klose (5). It was of interest therefore to see whether or not the method of Lappin and Clark could be adapted to use with fats and fat containing foods. The modifications described here permit the quantitative determination of carbonyl compounds in fats and fats contained in foods. The first requirement, a solvent for the fats and the reagents, was fulfilled by benzene. The second requirement, a catalyst which unlike hydrochloric acid usually used would not precipitate as a potassium salt during color development, was met with trichloroacetic acid, the salt of which is soluble in benzene. These modifications and the restandardization necessitated by them gave a procedure universally applicable to fats.

Experimental Procedure

REAGENTS

Carbonyl Free Benzene. Analytical reagent grade benzene is usually sufficiently carbonyl-free as received, but if the blank has an absorbency greater than 0.35 against water at 430 mμ, the benzene can be purified as follows: to one liter of benzene add 5 g. of 2,4-dinitrophenyl hydrazine and 1 g. of trichloroacetic acid; reflux for one hour and then distill through a short Vigreux column.

Carbonyl Free Ethanol. To one liter of ethyl alcohol add 5-10 g. of aluminum granules and 8-10 g. KOH and reflux the mixture for 1 hour. On distilling, discard the first 50 ml. of distillate, and stop the distillation before the last 50 ml. has distilled.

0.05% 2,4-Dinitrophenyl Hydrazine Solution. Dissolve 0.5 g. 2,4-dinitrophenyl hydrazine twice recrystallized from carbonyl-free methanol (which can be prepared in same manner as carbonyl-free ethanol)

[1] This paper reports research undertaken at the Quartermaster Food and Container Institute for the Armed Forces and has been assigned No. 428 in the series of papers approved. The views or conclusions contained in this paper are those of the authors; they are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

[2] Present address: Custom Chemical Laboratories, Chicago, Ill.