to confirm P lipase data on oils containing predominantly 18:1 as the unsaturated fatty acid. More information is needed concerning the specificity of GC lipase, especially as to the relative rates of lipolysis of TG's containing other *cis* unsaturated acids and of TGs containing 18:1 in different positions. However, present evidence indicates that GC lipase is highly specific for *cis* 18:1 and that in conjunction with other methods the enzyme can be used to study the structure of TG's.

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Application of Computer Methods to the Calculation of Triglyceride Structure[1]

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Abstract

A digital computer method for the calculation of triglyceride structure using a FORTRAN program has been developed. The results of several methods of calculation and hypothesis of glyceride structure were compared with values determined experimentally. The comparison obtained with the random, restricted random, 1,3-random-2-random distribution hypotheses, as well as other proposed hypotheses, indicated that the 1,3-random-2-random hypothesis best approximated the values obtained experimentally by other investigators.

Introduction

EXPERIMENTS DESIGNED to examine the effect of dietary fat on the glyceride structure of carcass or depot fat yield large amounts of data which must be transformed into a form suitable for interpretation. Computer methods of rapid calculation become especially desirable where a long or relatively complex series of calculations must be performed repeatedly on a small amount of data. Such is the case in many of the methods for the estimation of triglyceride structure, especially when comparisons between theories of triglyceride distributions are to be made. In this report we wish to describe a FORTRAN program for the calculation of triglyceride distribution.

Procedure

A digital computer program (FORTRAN) may be written for the purpose of performing straightforward calculations since the FORTRAN machine language allows algebraic formulas to be represented in familiar form. A computer program translates the formulas, when punched in correct form, into the actual computer instructions which govern the calculations.

The FORTRAN method provides for five basic operations: Each of these operations is represented by a distinct symbol (1):

Addition	+
Subtraction	−
Multiplication	*
Exponentiation	**
Division	/

In addition, provisions are also made for certain mathematical functions. Every function has a preassigned name. In order to make use of any function (square root or exponentiation), it is only necessary to write the name of the function followed with an expression enclosed in parenthesis. The computer will then carry out the named operation.

Several versions of FORTRAN are available. The version employed in the work reported used the Control Data Corporation 1604 computer (which is capable of storing about 32,000 items).

Before one can solve a problem using this type of computer it must be outlined using a number of statements. These statements control and outline the necessary arithmetic operations, the input of data, and the final printing out of results according to a predetermined format. Statements concerning the order of execution and statements which provide additional information about the problem are included. This information in FORTRAN language is then punched out on tape using the FORTRAN 60 compiler. The program, which is now on punched tape, contains the complete instructions necessary for the solution of the problem. Sub-programs containing instructions not in the main program for the detailed solutions of the glyceride distribution equations are then written.[2] The main program is reproduced in Table I.

Mathematical equations[3] for the calculation of a random distribution are shown in Table II. The

[1] Presented at the AOCS meeting, April 1965, Houston.

[2] A very limited number of the complete computer programs are available from the authors.

[3] S = saturated fatty acids; U = unsaturated fatty acids. S_3, S_2U, SU_2 and U_3 represent the four possible types of glycerides in terms of their S and U content without regard to position. SUS, SSU, USU and UUS represent structurally the varieties possible in terms of S and U content when the position is indicated in the sequence. SUS and SSU are therefore isomers comprising S_2U.

TABLE I

Fortran Computer Program

```
      PROGRAM FATS
      DIMENSION DENT (71), EXPER (8), RAND (8), RSTRAN
     (8), 1 GUN (8), OTTOLD (8), OTTFCA (8), OTTFCB (8),
     OTTFCC (8), 2 OTTCUB (8)
   50 PRINT 1000
      READ 98, DENT
      PRINT 100, DENT
      N = 1
      PRINT 110
      IF (SENSE SWITCH 2) 12, 9
    9 IF (SENSE SWITCH 1) 10, 11
   10 N = 8
      GO TO 12
   11 N = 4
   12 READ 99, S, S2, (EXPER (I), I = 1, N)

    C IF NEITHER SWITCH ONE NOR SWITCH TWO IS SET
      DATA IS EXPECTED IN THE FOLLOWING FORM—
        LINE OR CARD 1—SPACE, 71 CHARACTER
        IDENTIFICATION
        LINE OR CARD 2—S, THEN S2 (FORMAT 2F6.2),
        IN PERCENT
        LINE OR CARD 3—GS3, GS2U, GSU2, GU3 IN
        PERCENT (FORMAT 4F6.2).

          IF SWITCH ONE IS SET, CARD OR LINE 3 MUST
          ALSO CONTAIN SUS, SSU, USU AND UUS IN
          PERCENT, THUS LINE OR CARD 3 IS READ WITH
          FORMAT 8F6.2.

          IF SWITCH TWO IS SET, THE SECOND CARD OR
          LINE IS EXPECTED TO CONTAIN SIX BLANKS,
          SIGNIFYING NON-AVAILABILITY OF DATA.

            IF S2 IS KNOWN TO BE HIGHER THAN THE
            PREDICTED RANDOM VALUE, AS IN PIG FAT,
            BUT THE EXACT VALUE IS NOT KNOWN, USE
            —1.0 AS THE VALUE FOR S2.

      INSERT G83 APPROXIMATION HERE—IF (EXPER (1))
     23, 23, 24
   24 IF (S2) 19, 21, 20
   21 CALL EVALST (S, EXPER (1), OTTCUB, ES23)
      CALL EVALS2 (S, ES23, OTTOLD)
      STWO = ES23
      GO TO 22
   20 CALL EVALST (S, EXPER (1), OTTCUB, ES23)
      CALL EVALS2 (S, S2, OTTOLD)
      STWO = S2
      GO TO 22
   19 CALL EVALST (S, EXPER (1), OTTCUB, ES23)
      STWO = 2.★S − ES23
      CALL EVALST (S, STOW, OTTOLD)
      GO TO 22
   22 CALL RANDOM (S, RAND)
      CALL EVALRR (S, EXPER (1), RSTRAN)
      CALL EVAGUN (S, GUN)
      CALL EVALST (S, RAND (1), OTTFCA, ES21)
      CALL EVALST (S, GUN (1), OTTFCB, ES22)
      CALL EVALST (S, OTTOLD (1), OTTFCC, ES24)
      PRINT 101, S, S2, (EXPER (I), I = 1, N)
      PRINT 102, S, RAND
      PRINT 103, S, RSTRAN
      CALL FULERR (RSTRAN, EXPER, RAND, N)
      PRINT 104, S, GUN
      CALL FULERR (GUN, EXPER, RAND, N)
      PRINT 105, S, STWO, OTTOLD
      CALL FULERR (OTTOLD, EXPER, RAND, N)
      PRINT 106, S, ES21, OTTFCA
      CALL FULERR (OTTFCA, EXPER, RAND, N)
      PRINT 107, S, ES22, OTTFCB
      CALL FULERR (OTTFCB, EXPER, RAND, N)
      PRINT 108, S, ES24, OTTFCC
      CALL FULERR (OTTFCC, EXPER, RAND, N)
      PRINT 109, S, ES23, OTTCUB
      CALL FULERR (OTTCUB, EXPER, RAND, N)
      GO TO 50
   98 FORMAT (1X, 71A1)
   99 FORMAT (2F6.2, /8F6.2)
  100 FORMAT (/1X, 71A1//)
  101 FORMAT (20H EXPERIMENTAL VALUES 10(2X, F6.2,
     2X))
  102 FORMAT (20H COMPLETE RANDOM 2X, F6.2, 9X,
     1H − 2X, 18(2X, F6.2, 2X)/)
  103 FORMAT (/20H RESTRICTED RANDOM 2H ★F6.2,
     1H★8X, 3H − 11X, 1H★F6.2, 1H★1X, 7(2X, F6.2, 2X)/)
  104 FORMAT (/20H GUNSTONE, THEORY 1 2H ★F6.2, 2H★
     7X, 13H − 8(2X, F6.2, 2X)/)
  105 FORMAT (/20H 1–3 RAN., 2 RANDOM 2(2H ★F6.2, 2H★)
     18(2X, F6.2, 2X)/)
  106 FORMAT (/20H CUBIC, FULL RANDOM 2H ★F6.2, 2H★
     2X, 1F6.2, 2X, 8(2X, F6.2, 2X)/)
  107 FORMAT (/20H CUBIC, GUNSTONE 1 2H ★F6.2, 2H★
     2X, 1F6.2, 2X, 8(2X, F6.2, 2X)/)
  108 FORMAT (/20H CUBIC, OLD 1–3, 2 RAN 2H ★F6.2, 2H★
     2X, 1F6.2, 2X, 8(2X, F6.2, 2X)/)
  109 FORMAT (/20H 1–3, 2 RANDOM CUBIC 2H ★F6.2, 4H★
     F6.2, 13X, 1H★F6.2, 1H★1X, 7(2X, F6.2, 2X)/)
  110 FORMAT (6X, 8H★METHOD★6X, 4X, 3H★S★7X,
     4H★S2★6X, 3HGS3 17X, 4HGS2U6X, 4HGSU26X,
     3HGU37X, 3HSUS7X, 3HSSU7X, 3HUSU27X, 3HUUS//)
 1000 FORMAT (1H137X, 43H★DISTRIBUTION OF FATTY
     ACIDS IN GLYCERIDES★38X)
      END
      RETURN
      END
      FUNCTION SCALE (BLANK)
      SCALE = 0.01
      RETURN
      END
      SUBROUTINE APPROX (A, B, FAKEF, X, SCALE)[a]
      X = 0.U
      MARK = 0
      DIF1 = ABSF (FAKEF (A, B, X))
      IF (DIF1) 1, 300, 1
    1 X = X + SCALE
      DIF2 = ABSF (FAKEF (A, B, X))
      IF (DIF1 − DIF2) 3, 301, 2
    2 X = X + SCALE
      IF (100. − ABSF (X)) 10, 10, 11
   10 IF (MARK) 13, 13, 12
   12 X = 1000.
      RETURN
   13 MARK − 1
      X= 0.0
      DIF2 = ABSF (FAKEF (A, B, X))
      GO TO 3
   11 DIF1 = DIF2
      DIF2 = ABSF (FAKEF (A, B, X))
      IF (DIF1 − DIF2) 201, 301, 2
    3 SCALE = −SCALE
      GO TO 2
  201 X = X − SCALE
  300 RETURN
  301 PAUSE 1
      IF (SENSE SWITCH 1) 3, 2
    C SET SWITCH ONE IF THE NEGATIVE SOLUTION IS
      DESIRED
      RETURN
      END
      END
    X
REMARK, DATA
    ..
    ..
```

[a] Calculation of X and estimation of S-2.

FORTRAN language employed in the sub-program is very similar to that used in the standard mathematical expression (Table II).

A calculation of restricted random distribution may be carried out using the method of Kartha (2) or the modification of Kartha's method as published by Hammond and Jones (3). This calculation is represented in Table III. The sub-program written in FORTRAN language is quite similar and is illustrated in Table III.

VanderWal (4), Richardson (5) and Coleman (6) have proposed another distribution theory, known as the 1,3-random-2-random distribution. This theory states that: the acyl groups occupying the C-2 hydroxyl of the glycerol moiety are distributed therein at random; the 1- and 3-positions of the glycerol moiety are identical and are occupied by identical kinds and proportions of fatty acids distributed within these groups at random.

Still another version of this distribution was later presented by Gunstone (7); this version stated that the C-2 hydroxyl group is preferentially acylated by C_{18} unsaturated acids; the C-1,3 hydroxyl groups are subsequently acylated by all remaining acids and by any C_{18} unsaturated acid not required at C-2. Within these limits, the distribution at each position is statistical. The mathematical expression of the equations required for the calculation of glyceride composition using this theory as well as the FORTRAN sub-program employed is given in Table IV.

The 1,3-random-2-random theory has repeatedly received experimental confirmation (8–10) when applied to animal and plant depot fats. A computer sub-program for the calculation of this distribution would be especially useful. The equations which describe one version of this distribution ("X-cubic"), as given by VanderWal (11), are illustrated in Table V. These formulas may be translated into a FORTRAN sub-program (Table V) and the values for X calculated by solution of the cubic equation by the special sub-program included at the end of Table I. Another method of calculation for the 1,3-random-2-random hypotheses originally published by

TABLE II

Calculation of Random Distribution

Mathematical notation	Fortran sub-program
$S_3 = S^3/10{,}000$ $S_2U = 2\,S^2U/10{,}000$ $S_2U\ (SUS) = S^2U/10{,}000$ $SU_2 = 2SU^2/10{,}000$ $SU_2\ (USU) = SU^2/10{,}000$ $U_3 = U^3/10{,}000$	Subroutine random (S,Ran) Dimension ran(8) U = 100. − S Ran (1) = S*S*S/10,000 Ran (4) = U*U*U/10,000 Ran (5) = S*S*U/10,000 Ran (6) = Ran(5)*2 Ran (7) = U*U*S/10,000 Ran (8) = Ran(7)*2 Ran (2) = Ran(5) + Ran(6) Ran (3) = Ran(7) + Ran(8) Return End

TABLE III

Calculation of Restricted Random Distribution

Mathematical notation (3)	Fortran sub-program
GS_3,GS_2U,GSU_2, and GU_3 = a–d respectively S = Mol porportion of saturated acids and a + b + c + d = 1 In the equilibrium: $GU_3 + GS_2U = 2GSU_2$; $db/c^2 = K$ K = ⅓ when the distribution is not restricted therefore GS_2U = b = 3/2 (1 + S − 2a) − 3/2 GSU_2 = c = 3S − 2b − 3a GU_3 = d = 1 + 2a + b − 3S	Subroutine Eval RR(S,A,G) Dimension G(8) SS = S/100 AA = A/100 Arg = (SS − 1)*(4.*AA − 3.*SS − 1.) if(arg) 251,250,250. G(2) = ((3/2) + (1 + SS − (250) G(1) = A 2*AA) − SQRTF(ARG))) * 100 G(3) = (3 * S − 2 * G (2) − 3. *A G(4) = 100. + 2. + * A + G(2) − 3.*S G(5) = G(2)/3 G(6) = G(5) 2 G(7) = G(3)/3 G(8) = G(7) 2 Return (251) ARG = −Arg Print 252

VanderWal (4) similar to that shown in Table V is illustrated in Table VI.

The values for varying percentages of glyceride types and isomeric forms of triglycerides predicted by any particular theory can thus be readily calculated. These values and their deviations from both the values obtained experimentally, and from those predicted by random distribution can then be printed in an easily readable format on a line printer attached to the computer. Using manual methods, the calculation of five different distributions for any given fat required a considerable amount of time; using the FORTRAN methods described above, the time

TABLE IV

Calculation of Gunstone's Distribution (Theory 1)

	Mathematical notation (7)			
%S	S_3	S_2U	SU_2	U_3
<33⅓ 33⅓–66⅔	0	$\left(\frac{3S}{20}\right)^2$	$\frac{3S(3U-100)}{200}$	$\left(\frac{3U-100}{20}\right)^2$
>66⅔	100–3U	3U	0	0

Fortran sub-program

```
Subroutine Evagun (S,Gun)
Dimension Gun (8)
If (S − 200./3.)150,150,151
(150) U = 100. − S
Gun (1) = 0.0
Gun (2) = (3.*S/20.)**2
Gun (3) = 3.*S*(3.*U−100.)/200.
Gun (4) = ((3.*U − 100.)/20.)**2
(152) Gun (5) = Gun (2)
Gun (6) = 0.0
Gun (7) = 0.0
Gun (8) = Gun (3)
Return
(151) U = 100. − S
Gun (1) = 100. − 3.*U
Gun (2) = 3.*U
Gun (3) = 0.0
Gun (4) = 0.0
Go to 152
End
```

TABLE V

Calculation of 1,3-Random-2-Random Distribution ("X-Cubic" method)

Mathematical notation (11)	Fortran sub-program
R_1 = (S + X/2) (S − X) (S + X/2)/10,000	X2 = X/2
$R_2 = R_5 + R_6$	UU = 100 − S
$R_3 = R_7 + R_8$	R(1) = (S + X2)*(S − X)*(S + X2)/10,000
R_4 = (UU − X/2)(UU + X) (UU + X/2)/10,000	R(7) = (UU − X2)*(S − X)*(UU − X2)/10,000
R_5 = (S + X/2)(UU + X) (S + X/2)/10,000	R(6) = 2.*(S + X2)*(S − X)*(UU − X2)/10,000
R_6 = (2)(S + X/2)(S − X) (UU − X/2)/10,000	R(5) = (S + X2)*(UU − X)*(S + X2)/10,000
R_7 = (UU − X/2)(S − X) (UU − X/2)/10,000	R(8) = 2.*(UU − X2)*(UU + X)*(S + X2)/10,000
R_8 = (2)(UU − X/2)(UU + X) (S + X/2)/10,000	R(4) = (UU − X2)*(UU + X) *(UU − X2)/10,000
R_1 = R_8 are S_3, S_2U, SU_2, U_3, SUS, SSU and UUS	R(2) = R(5) + R(6)
S = %S in triglycerides (Total S)	R(3) = R(7) + R(8)
UU is the % U in the triglyceride (Total U)	S and B are read into the computer as data:
X is found from (S + X/2)(S − X)(S + X/2)/10,000 − B = 0 where B is the %S_3 found.	X was calculated from the cubic equation by a separate sub-program.

required for the computation was about 7 seconds; approximately 30 seconds were required to print out the data in tabular form.

Results and Discussion

A digital computer program was developed from the existing mathematical methods for the calculation of the triglyceride distribution within a fat. The results obtained were then compared with those obtained experimentally on fats having differing percentages of saturated fatty acids. The following theories were compared:

A) Complete random distribution (Table II).
B) Restricted random distribution (Table III).
C) The theory which has been presented by Gunstone (7) as a modification of restricted random distribution (Table IV).
D) The method of calculation for the 1,3-random-2-random hypothesis originally presented by VanderWal (4) (Table V).
E) This method yielded the same results as method (D) but employed the program outlined in Table VI derived from the "X cubic" set of equations. The values for the percentages of S_3 and S as found by experiment are required and the amount of S in the 2 position of the glycerol moiety is estimated. If the amount of S in the 2 position obtained by estimate agrees with that determined experimentally, then the final percentages of glycerides are in agreement with those calculated by method D. If the estimate differs then somewhat different results are obtained.

TABLE VI

Calculation of 1,3-Random-2-Random Distribution (Original method)

Mathematical notation (4)	Fortran sub-program
a = %S (total) b = %S in 2 position c = b(100)/3a d = 100 − e e = (d)(a)/100 f = 1.5 e g = 100 − b h = f^2/100 i = g^2/100 j = 100 − (h + i) S_3 = (b)(h)/100 USU = (b)(i)/100 USS = (b)(j)/100 UUU = (100 − b)(i)/100 UUS = (100 − b)(j)/100	Subroutine Eval S2 (A,B,Q) Dimension Q (8) C = R*100./(3.*A) D = 100. − C E = D*A/100. F = 3.*E/2. G = 100. − F H = F*F/100. AI = G*G/100. AJ = 100. − H − AI Q(1) = B*H/100. Q(7) = B*AI/100. Q(6) = B*AJ/100. Q(5) = (100. − B)*H/100. Q(8) = (100. − B)*AJ/100. Q(4) = (100. − B)*AI/100. Q(2) = Q(5) + Q(6) Q(3) = Q(7) + Q(8) Return End

TABLE VII

Distribution of Triglycerides in Chicken Fat

Method	S	S2 [a]	S_3	S_2U	SU_2	U_3	SUS	SSU	USU	UUS
Experimental Values (8)	31.30	.....	3.00	19.00	50.00	28.00	10.00	9.00	12.00	38.00
Complete Random	31.30	.....	3.07	20.19	44.32	32.42	6.73	13.46	14.77	29.55
Restricted Random	31.30	.....	3.00	20.27	44.36	32.37	6.76	13.51	14.79	29.58
Gunstone, Theory 1	31.30	.....	0.00	22.04	49.81	28.14	22.04	0.00	0.00	49.81
1-,3-Random,-2-Random (Original method)	31.30	26.12	3.00	20.19	44.52	32.29	8.49	11.70	11.42	33.11
1-,3-Random,-2-Random ("X-Cubic" method)	31.30	26.12	3.00	20.19	44.52	32.29	8.49	11.70	11.42	33.11

[a] %S in the 2 position of the glycerol moiety.

TABLE VIII

Distribution of Triglycerides in Linseed Oil

Method	S	S2 [a]	S_3	S_2U	SU_2	U_3	SUS	SSU	USU	UUS
Experimental Values (8)	7.80	0.00	0.00	0.00	26.00	74.00	0.00	0.00	4.00	22.00
Complete Random	7.80	.....	0.05	1.68	19.89	78.38	0.56	1.12	6.63	13.26
Restricted Random	7.80	.....	0.00	1.70	20.00	78.30	0.57	1.13	6.67	13.33
Gunstone, Theory 1	7.80	.....	0.00	1.37	20.66	77.97	1.37	0.00	0.00	20.66
1-,3-Random,-2-Random (Original method)	7.80	0.00	0.00	1.37	20.66	77.97	1.37	0.00	0.00	20.66
1-,3-Random,-2-Random ("X-Cubic" method)	7.80	0.00	0.00	1.37	20.66	77.97	1.37	0.00	0.00	20.66

[a] %S in the 2 position of the glycerol moiety.

A series of calculations were made on several representative fats containing varying amounts of saturated fatty acids. The computer program was applied to the calculation of the glyceride distribution of 70 different fats representing 116 analyses which have been published by other investigators. The results of two such comparisons (chicken fat and linseed oil) are illustrated in Tables VII and VIII. The comparisons obtained indicated that the 1,3-random-2-random hypothesis of VanderWal (4) and Coleman (6) best approximated the values obtained experimentally by other investigators. The suggestions put forth by Gunstone (7) also yielded good approximations of the actual results when glyceride types were considered, but diverged when values for isomeric forms were compared. Although complete data are not available for most fats reported in the literature (in most cases only the percentages of glyceride types are available), this agreement was found to be generally true for those fats where complete data were available as well as for those where only the percentages of glyceride types has been reported.

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Structure of the Intestinal Mucosa and Lymph Glycerides of Rats after Absorption of Fats Containing Elaidic Acid[1]

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Abstract

Elaidic acid was given to rats either as free acid or triglyceride (trielaidin or mixed glycerides transesterified with elaidic acid). The intestinal mucosa and lymph triglycerides were isolated and their structure determined by pancreatic lipase. The elaidic acid level was determined by GLC using capillary columns.

Results showed a marked tendency for elaidic acid to be located at the external positions of the triglyceride molecule beginning with the lymph. The results are discussed in relation to the absorption process and triglyceride synthesis.

[1] Presented at the AOCS meeting in Houston, Texas, 1965.

Introduction

IN PREVIOUS WORK made in cooperation with Raulin (6, 7), we have demonstrated that when rats and pigs are fed rations containing elaidinized peanut oil, the *trans* fatty acids are found predominantly in the external positions of the depot triglycerides (TG). This positional specificity occurs in spite of the fact that the distribution of saturated and unsaturated acids between the internal and external positions is different in these two species.

It seemed worthwhile, therefore, to study the location of elaidic acid in the TG molecule throughout the digestive process. This paper describes experiments in which the mode of incorporation into lymph triglycerides of different forms of dietary elaidic acid was determined. The relative degree of incorporation