CXV. SOME FURTHER OBSERVATIONS ON FACTORS WHICH INFLUENCE THE COMPONENT FATTY ACIDS OF BUTTER.

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ATTENTION was drawn in a former communication [Hilditch and Sleightholme, 1930] to the somewhat abrupt increase in iodine value and diminution in Reichert-Meissl value of butter-fats from cows when the latter had returned from winter conditions to spring pastures; the detailed analyses then available showed, correspondingly, that the butter-fat from cows on spring pasture contained considerably more oleic acid and somewhat less butyric, caproic and stearic acids than that from the same animals a few months earlier (when they were stall-fed).

The animals upon which these observations were based were from the typical herd at the National Institute for Research in Dairying, Shinfield. We have since been supplied with further samples of butter-fat from the milk of members of the same herd at intervals during the seasons 1930-32 by the courtesy of Captain Golding, who kindly informed us that in the spring season of 1932 attention was being focused at the Institute on the changes which occur when the cows go out to grass and suggested that a favourable opportunity offered for further detailed analyses of the butter-fat component acids during the transition from indoor to pasture conditions. In the course of the work it was found that, apart from the seasonal change under particular observation, the component acids of all three of the 1932 fats studied contained only 22-23 % (wt.) of palmitic acid as compared with 26-29 % (wt.) in the case of milk-fats from members of the same herd in 1928-29. This led us to examine the palmitic acid contents of the butter-fats in the intervening years, and the present communication accordingly deals with (i) the increase in the unsaturated character of butter-fat following the removal of the cows from stall to pasture in spring, and (ii) changes in the palmitic and oleic acid contents of milk-fats which are apparently connected rather with the age of the cows than with seasonal or similar influences.

The change in composition of butter-fatty acids following the transition of cows from stall to pasture feeding.

The iodine and Reichert-Meissl values of fats from the milk of cows from the typical herd taken at intervals from the autumn pasture season of 1928 to the early summer pasture conditions of 1932 establish clearly the presence of more unsaturated components in the butters from pasture-fed than stall-fed animals and, perhaps slightly less definitely, a correspondingly lower Reichert-Meissl

figure, indicating somewhat less of the volatile (low molecular weight) fatty acids in the butters from pasture-fed cows. The butter-fats were not derived in every case from the milk of the same individuals, although these all belonged to the typical herd; in order to make the data more complete, therefore, it is convenient to refer to the individuals by letter and, in the first place, to give a list of the animals concerned (Table I).

Table I. *Cows supplying milk-fats dealt with in this paper.*

Reference letter		Date born	Date of first calf	Number of calves to	
				May 1929	May 1932
A	Doll II	5. xi. 21	3. x. 24	5	7
B	Flora I	28. xi. 21	19. xi. 24	5	8
C	Pearl	17. iii. 23	28. xi. 25	4	6
D	Rosebud	3. xii. 23	4. xii. 26	3	6
E	White Rose	4. i. 24	29. viii. 26	3	4
F	Winsome III	14. x. 24	3. x. 27	2	5
G	Lottie II	2. iii. 25	16. ii. 28	2	5*
H	Peach II	3. iv. 25	25. xii. 27	2	5
I	Firefly III	24. ix. 25	21. viii. 28	1	4
J	Lucy III	9. iv. 27	7. xi. 29	—	1†
K	Lottie IV	17. iii. 29	21. xii. 31	—	1

* Including one on May 27, 1932.

† Lucy III sold fat after first lactation.

The complete series of iodine values and Reichert-Meissl values which have been determined on winter and summer butter-fats from these cows between 1928 and 1932 are given in Table II.

Table II.

Milk collected	Conditions	Cows supplying the milk	Butter-fats	
			Iodine value	R.-M. value
28-30. x. 28	Late autumn pasture + stall	A, D, E, G, H, I	41.3	29.6
12-15. iii. 29	Indoor winter feed	*	34.8	32.3
26-27. v. 29	Early summer pasture	A, B, C, D, E, F, G, H, I	41.6	25.7
20-21. xi. 29	Indoor winter feed	B, E, I	42.2	27.0
18-19. xii. 29	"	B, D, E, I, J	38.3	29.3
4-5. ii. 30	"	B, D, E, I	38.9	27.3
24-25. ii. 30	"	B, C, D, E, I	36.5	29.7
14-15. iii. 30	"	B, C, D, E, F, I	37.3	30.6
25-26. iv. 30	Spring pasture	B, C, D, E, F, I	43.3	28.4
2-3. vi. 30	Early summer pasture	B, C, D, E, F, I	44.2	24.6
26-27. iii. 31	Indoor winter feed	F	38.9	—
10-11. v. 31	Spring pasture	B	37.7	—
"	"	F	44.6	—
"	"	B	44.4	—
"	"	D	40.7	—
21-22. iii. 32	Indoor winter feed	B, D, F, I, K	40.5	29.6
30. iv. and 1. v. 32	Stall + early spring pasture	B, D, F, I, K	45.9	25.7
27-28. v. 32	Early summer pasture	B, D, F, I, K	45.0	24.7

* Special feed including soya-bean cake, to 2 cows from the Shinfield herd not included in the list in Table I.

This group of general characteristics (for unsaturation and volatile fatty acid content) covers milk-fats taken over a considerable length of time from animals from the same herd and to a large extent from the same in-

dividuals. It is therefore probably safe to draw the following conclusions from the observations.

(1) The proportion of unsaturated acids is higher in the milk-fats of pastured cattle than in those of the same cows under indoor winter conditions. The seasonal rise in iodine value of the fats is rather abrupt, the change being completed within 2 or 3 weeks (or perhaps even less) after the cows have been put out to grass.

(2) At the same time, there is a definite tendency for the iodine values of the pasture milk-fats to rise slightly but consistently throughout the series. Since the greater part of each sample included composite milk-fats from animals born in 1921 (B), 1923 (C, D), 1924 (E, F) and 1925 (I), there is reason to think that this slow increase in the maximum iodine values in succeeding years is a function of the age of the animal or (what is much the same thing) of the number of calves borne.

(3) The minima in iodine values for the winter milk-fats do not form so regular a series. Those for the winters 1929-30 and 1931-32 are 4-5 units below the preceding summer maxima, whereas those for the winters 1928-29 and 1930-31 show corresponding differences of nearly 7 units. It is probably significant that the smaller differences correspond with very mild winters and the larger ones with the much colder conditions of the early months of 1929 and 1931, a relation which was, indeed, pointed out in the previous communication [1930]. The degree of unsaturation of the winter fats may well be governed by at least three factors, namely (a) an increase in saturation, relative to that of summer pasture milk-fats, caused by either the indoor life or the indoor diet (or both), (b) the slow increase in unsaturated acid content suggested by the outdoor data to be connected with increasing age of the animals, and (c) a seasonal variation dependent upon the average temperature of a particular winter.

(4) Throughout the series the Reichert-Meissl values of the milk-fats tend to vary inversely with their iodine values, but they do not alter so regularly and abruptly as the latter. Moreover, it appears from the early spring pasture samples of April 1930 and April-May 1932 that the characteristic, relatively high iodine value of the pasture fats has already been attained, but the Reichert-Meissl value is intermediate between the higher values for milk-fats of winter, stall-fed cattle and the lower values finally reached when the cows have been at pasture for some time.

The foregoing review, based upon the average characteristics as defined by iodine and Reichert-Meissl values, has been supplemented by full analyses of the component acids present in the butter-fats of March, April and May, 1932. As already mentioned, Hilditch and Sleightholme [1930] pointed out apparent differences in the proportions of some of the individual fatty acids from analyses of the mixed fatty acids of autumn pasture 1928, winter feed 1929 and early summer pasture 1929 butters. The data for the winter-fed sample were primarily collected, however, in an attempt to study the effect of added soya-bean and coconut oil-cakes in the diet, and the simple winter-summer variation in composition was thus to some extent obscured; also, the two cows used for the soya-bean cake experiment were unfortunately not amongst those listed in Table I, from some or all of which all the remaining milk-fats dealt with in Table II emanated. In the present instance, 4 of the 5 cows concerned had been used frequently in providing specimens earlier in the series, and the stall diet in the winter of 1931-32 (*cf.* Table III) was normal and contained no special additions of fatty matter. The fresh detailed analyses therefore bear more directly on the

point under discussion, although, as already stated, the difference in character (e.g. iodine values 40.5 and 45.9) of the 1932 stall-fed and pasture butters is unfortunately not so extreme as in some of the former years.

Full details of the cows contributing the composite samples of butter studied, and of their daily rations throughout the period concerned, are given in Table III.

Table III. *Details of cows used for production of butter-fat in winter to summer change of food, 1932.*

		Individual cows used for composite sample					Total lbs. milk Fat* %	
		Flora I	Rose- bud	Winsome III	Firefly III	Lottie IV		
Date of birth of cow		28. xi. 21	3. xii. 23	14. x. 24	24. ix. 25	17. iii. 29		
No. of calves before 20. iii. 32		8	6	5	4	1		
Date of last calving		7. xi. 31	8. i. 32	20. ii. 32	12. i. 32	21. xii. 31		
		lbs.	lbs.	lbs.	lbs.	lbs.		
Milk yields	21. iii. 32 p.m.	21	20	19	12½	11	83½	4.10
	22. iii. 32 a.m.	27	27½	29½	22½	18½	124½	3.25
	30. iv. 32 p.m.	19	20	21	13½	11	84½	4.78
	1. v. 32 a.m.	27½	29	30	22½	19½	128½	3.05
	27. v. 32 p.m.	21	16	22	12½	10½	82	4.3
	28. v. 32 a.m.	29	23	30½	19	18½	120½	2.88
Daily rations:								
Winter period	Meadow hay	14	14	14	14	14		
up to April 25th	Mangels							
	(Red Inter- mediates)	50	50	50	50	50		
	Concentrate mixture†	21	21	18	15	14		
Intermediate	Hay	7	7	7	7	7		
period April	Mangels	25	25	25	25	25		
26th to May 1st	Concentrate mixture	14	15	15	9	9		
	Grass		8.30 a.m. to 3 p.m.					
Summer period	Grass		Day and night					
May 3rd on- wards	Concentrate mixture	11	11	12	7	6		

* These fat percentages were on the composite sample from the whole typical herd and not of the 5 cows.

† Concentrate mixture: Dried grains 2 parts; Maize germ meal 2 parts; Soya bean meal 1 part.

Prior to investigation the butters were melted, settled and decanted and filtered through paper in a steam-oven. Their general analytical characteristics were as follows.

Table IV.

Butter-fat from milk of	21-22. iii. 32	30. iv. and 1. v. 32	27-28. v. 32
Sap. equiv.	248.8	255.4	254.8
Iodine value	40.5	45.9	45.0
Reichert-Meissl value	29.6	25.7	24.7
Polenske value	1.7	1.5	1.5
Kirschner value	24.0	20.7	20.4
Refractive index n_D^{60}	1.4473	1.4485	1.4482

The determination of component fatty acids was carried out as described in former communications [Hilditch and Sleightholme, 1930; Hilditch and Jones,

1929] and, since the full fractionation and analytical details are extremely lengthy, only the final summaries of the data for each group of acids (steam-volatile, "solid" and "liquid" non-volatile in steam) are given here (Table V):

Table V. *Summarised experimental data for component fatty acids of the 1932 butter-fats.*

Acid	Volatile acids g.	Acids non-volatile in steam		Total	% (wt.)	% (mol.)
		Solid acids S g.	Liquid acids L g.		(Excluding unsaponifiable matter)	
Butter-fat from milk of 21-22. iii. 32 (indoor, winter ration).						
	25.92	200.6	213.4	439.92		
Butyric	17.19	—	—	17.19	3.9	10.6
Caproic	6.72	—	—	6.72	1.5	3.2
Caprylic	0.75	—	2.42	3.17	0.7	1.2
Capric	0.93	—	7.42	8.35	1.9	2.6
Lauric	—	1.05	15.31	16.36	3.7	4.4
Myristic	—	18.77	18.20	36.97	8.4	8.8
Palmitic	—	96.30	0.17	96.47	22.0	20.5
Stearic	—	65.65	—	65.65	15.0	12.6
Arachidic	—	2.93	—	2.93	0.7	0.5
Oleic	0.33	15.80	152.66	168.79	38.5	32.5
Linoleic	—	—	16.02	16.02	3.7	3.1
Unsaponifiable	—	0.10	1.20	1.30	—	—
Butter-fat from milk of 30. iv. and 1. v. 32 (intermediate period).						
	23.53	186.0	227.0	436.53		
Butyric	14.13	—	—	14.13	3.3	8.9
Caproic	7.31	—	—	7.31	1.7	3.5
Caprylic	0.73	—	2.32	3.05	0.7	1.2
Capric	1.11	—	6.89	8.00	1.8	2.6
Lauric	—	—	9.86	9.86	2.3	2.7
Myristic	—	15.73	22.72	38.45	8.8	9.4
Palmitic	—	92.16	2.90	95.06	21.8	20.6
Stearic	—	55.05	—	55.05	12.7	10.8
Arachidic	—	1.82	—	1.82	0.4	0.3
Oleic	0.25	21.14	155.89	177.28	40.7	35.0
Linoleic	—	—	25.19	25.19	5.8	5.0
Unsaponifiable	—	0.10	1.23	1.33	—	—
Butter-fat from milk of 27-28. v. 32 (early summer pasture).						
	23.09	182.8	225.2	431.09		
Butyric	13.23	—	—	13.23	3.1	8.5
Caproic	7.21	—	—	7.21	1.7	3.5
Caprylic	1.56	—	1.52	3.08	0.7	1.2
Capric	0.67	—	7.22	7.89	1.8	2.6
Lauric	—	—	13.56	13.56	3.2	3.8
Myristic	—	12.87	17.51	30.38	7.1	7.5
Palmitic	—	93.66	4.32	97.98	22.8	21.6
Stearic	—	53.60	—	53.60	12.5	10.7
Arachidic	—	3.17	—	3.17	0.7	0.6
Oleic	0.42	19.36	157.95	177.73	41.3	35.6
Linoleic	—	—	21.92	21.92	5.1	4.4
Unsaponifiable	—	0.14	1.20	1.34	—	—

We shall, as usual, discuss these figures mainly from the point of view of the molar distribution of the fatty acids present, and, for convenience, these are placed side by side in Table VI, together with the molar amounts of the following groups of acids: butyric-lauric, myristic-palmitic, C_{18} acids, oleic-linoleic [*cf.* Hilditch and Sleightholme, 1931].

Table VI. *Molar distribution of the component fatty acids in the 1932 butter-fats.*

Fat from milk of	21-22. iii. 32	30. iv. and 1. v. 32	27-28. v. 32
Acid	(Indoor, winter ration)	(Intermediate period)	(Early summer pasture)
Butyric	10.6	8.9	8.5
Caproic	3.2	3.5	3.5
Caprylic	1.2	1.2	1.2
Capric	2.6	2.6	2.6
Lauric	4.4	2.7	3.8
Myristic	8.8	9.4	7.5
Palmitic	20.5	20.6	21.6
Stearic	12.6	10.8	10.7
Arachidic	0.5	0.3	0.6
Oleic	32.5	35.0	35.6
Linoleic	3.1	5.0	4.4
Butyric-lauric	22.0	18.9	19.6
Myristic-palmitic	29.3	30.0	29.1
C ₁₈ (+ arachidic)	48.7	51.1	51.3
Oleic-linoleic	35.6	40.0	40.0

The fats from the "intermediate period" and the early summer pasture were closely similar, judged by their general characteristics (Table IV), and this is borne out to a remarkable extent by the component fatty acid figures in Table VI. Clearly, an increase of about 4 (mols.) % has occurred in the amount of unsaturated acids as a result of the change from indoor to pasture conditions, whilst the palmitic acid content has perhaps also increased slightly. The compensating fall in molar amount of saturated acids appears to be confined mainly, and in about equal proportions, to butyric and stearic acids; the individual figures for the other acids show no significant change (although those for lauric and myristic acids are less regular than the rest). In these complex mixtures, of course, too much reliance must not be placed on fractional percentages; but we believe that the close accordance between the observed compositions for the second and third samples justifies the general conclusions which have just been given.

The totals of the molar contents of butyric-lauric acids, the "volatile" acid group, range from 18.9 to 22.0 % in all three butters, and thus remain almost within the limits suggested by Hilditch and Sleightholme [1931] as characteristic for normal butters: "this figure appears to tend to a constant value of about 21-22, four of the six fats giving values between 20.7 and 21.6" (p. 518). The corresponding totals for the myristic-palmitic and C₁₈ groups are, however, distinctly different from those given previously (C₁₄-C₁₆, 29-30 instead of 34-36, and C₁₈ group, 48.7-51.1 instead of 43-44); comparison of the individual component acids of Hilditch and Sleightholme's Shinfield pasture-fed butters [Nos. I and IV, 1930] with the present series will show that this difference is mainly accounted for by a general fall of several mols. % in palmitic acid and an increase in both stearic and unsaturated acids. This change appears to be unconnected with the seasonal alteration in the composition of the milk-fat and to be a function of the age or progressive development of the cows concerned (*cf.* below).

Progressive changes in the composition of butter-fats apparently connected with increasing age of the cow.

We believe that the data presented in this series of observations show that, over a period of years, the milk-fat of a given cow tends to increase in its content of C₁₈ acids and to diminish in that of palmitic acid. We have been able to obtain

additional evidence on this point by a partial analysis of samples of 1930 and 1931 butter-fats from members of the Shinfield herd sent to us at those dates for determination of their general characteristics. Since in these cases a detailed component fatty acid analysis was not made at the time of receipt, and in view of the fact that (owing to the onset of slight rancidity) determination of the volatile and of the unsaturated acids at the time the present experiments were undertaken could not be relied upon to yield trustworthy results, a modified form of analysis only was made of the 1930-31 samples for the present purpose. The mixed acids from a known weight of butter-fat were at once submitted to the lead salt-alcohol separation (from which our customary addition of 0.5-1 % of acetic acid was omitted, since this procedure, which leads to more complete removal of oleic acid from the precipitated lead salts, also tends to take slight amounts of palmitic acid into the "liquid" acids); the "solid" acids obtained after recrystallisation of the deposited lead salts were converted into methyl esters and fractionally distilled in the usual manner. Using the modified lead salt method indicated, there is little chance of any measurable amount of palmitic acid escaping as dissolved lead salt, and it was thus possible to determine the palmitic and stearic acid contents of the 1930 and 1931 specimens.

In order to eliminate as far as possible any seasonal factors, the present comparison is based only upon the fats from pasture-fed animals of the seasons 1928-32 (in all but the first-named, the specimens were from early summer pasture feeding of about the same date in each case). Since it was only possible to obtain proportions by weight in the partially-analysed fats of 1930 and 1931, we must revert here to the somewhat less satisfactory comparison by weight, instead of by molar, percentages. The complete series of data is given in Table VII.

Table VII. *Weight-percentages of palmitic, stearic, oleic and linoleic acids in butter-fats from pasture-feeding, 1928-32.*

Milk collected	Pasture-feeding	Cows used	Av. age (years)	Component fatty acids % (wt.)			
				Pal-mitic	Stearic	Oleic	Lin-oleic
28-30. x. 28	Autumn	A, D, E, G, H, I	4.5	29.0	7.6	40.1	3.6
26-27. v. 29	Early summer	A, B, C, D, E, F, G, H, I	5.4	26.1	6.5	40.9	4.1
2-3. vi. 30	"	B, C, D, E, F, I	6.5	23.6	8.3	Not determined	
10-11. v. 31	Spring	B	9.5	24.4	7.5	"	
10-11. v. 31	"	F	6.6	21.8	7.6	"	
27-28. v. 32	Early summer	B, D, F, I, K	7.3	22.8	12.5	41.3	5.1

The cows contributing to the 1928 specimen were respectively seven (1), five (2), and three (3) years old; the 1929 sample included milk-fats from the same six animals and from three others then aged approximately eight (1), six (1) and five (1) years; whilst in 1932 four of the cows were amongst those used in 1928-29, but the fifth was a younger animal, then aged three years (the latter, however, only contributed about 14 % of the milk used to prepare the butter, 86 % coming from the four older animals). On the whole, and bearing in mind that it has not been possible to study the milk-fat produced by a single individual throughout the whole period of years, it seems fairly certain that the proportion of palmitic acid present declines with the age of the cows.

Certain well-defined similarities have been shown to exist [Hilditch and Sleightholme, 1931] between the milk and depôt fats of the cow, including not only a somewhat similar ratio in the amounts of palmitic as compared with the sum of the remaining acids, but also in some features of their glyceride structure, notably the relationship between the proportion of fully-saturated glycerides and of total saturated acids in the whole fats and a tendency towards identity in composition of the fully-saturated components within each type of fat. It may therefore be of some significance that Banks and Hilditch [1931] observed two classes of beef tallows, in one of which the molar content of C_{18} acids was about 68–70 %, while in the other it was about 61–63 %. The remaining acids, in this case, are palmitic with small amounts of myristic, so that these range from about 30 to 39 % (mols.); correspondingly, the weight-percentages of palmitic acid in the mixed fatty acids of the beef tallows examined by these authors and also by Armstrong and Allan [1924] vary over the range 25 to 33 %. In the light of the present results on milk-fats, it seems quite possible that the variation in palmitic acid content of beef tallows may, at least in part, depend upon the age of the animals when slaughtered. (In all the analyses referred to, the specimens were market tallows and there is thus unfortunately no information available as to the age of the animals which furnished them.)

SUMMARY.

A series of butter-fats, from milk of various (and frequently the same) members of the typical herd at the National Institute for Research in Dairying, Shinfield, at intervals from 1928 to 1932, has been studied by means of the usual analytical characteristics, supplemented by several detailed analyses of the component fatty acids. The earlier data appeared in a former paper, and the complete set affords an opportunity for detecting two characteristic changes in the composition of the butter fatty acids.

One change is seasonal and consists in an abrupt increase in the proportion of oleic (and linoleic) acids, with a parallel diminution in butyric and stearic acids, which is shown to have occurred in each of the four spring seasons when the cows return to pasture from indoor winter conditions. The increase in unsaturated acids amounts to about 4 % (mols.), and detailed analyses made on butters of the 1932 spring season show that this takes place within a very short time of the cows' return to pasture; the compensating diminution in saturated acids appears to be shared almost wholly between butyric and stearic acids.

The other change, which is a function of the age of the cow and not seasonal in character, consists in a gradual augmentation of the amount of unsaturated components of the fat over the whole period of years, mainly at the expense of palmitic acid, which, in the samples under review, declined from 29 to 22–23 % (wt.) of the mixed butter fatty acids between 1928 and 1932; the average age of the cows contributing the butter was 4.5 years in 1928 and 7.3 years in 1932, and the figures quoted refer in all cases to butters from pasture-fed cows (usually late in May or in early June).

The similar range of variation observed by Banks and Hilditch [1931] in the respective palmitic and C_{18} acid contents of beef tallows may therefore, it is suggested, also be connected with the age of the animal from which the depôt fat is obtained.

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butter and for providing us with full details of the individual cows used in each case; and to the Department of Scientific and Industrial Research for a grant to one of us (H. K. D.) during part of the time in which this work was in progress.

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