CC. SPECTROSCOPIC CHANGES IN FATTY ACIDS

IV. ACIDS FROM SPECIMENS OF BUTTER FAT FROM COWS UNDER DIFFERENT NUTRITIONAL TREATMENTS

BY JAMES HOUSTON

*From the Queen's University, Belfast, and the National Institute
for Research in Dairying, University of Reading*

ALBERT GEORGE COTTON AND STANISŁAW KAZIMIERZ KON

*From the National Institute for Research in Dairying,
University of Reading*

AND THOMAS MOORE

*From the Nutritional Laboratory, University of Cambridge
and Medical Research Council*

(Received 20 August 1939)

THE presence in butter fat of acids showing spectroscopic absorption in the ultra-violet region was established by Gillam *et al.* [1931]. Booth *et al.* [1935] showed that the absorptive acids were present in greater concentration in summer than in winter butter fat, and that the absorption could not be due to oleic acid, or to polyethylenic acids in their usual form as present in seed fats. Dann *et al.* [1935] found that when fats containing polyethylenic acids are given to the cows they are converted from the spectroscopically "pro-absorptive"¹ into the "absorptive" state. This phenomenon was similar to that found to occur *in vitro* on the prolonged refluxing of cod liver oil with alcoholic KOH [Dann & Moore, 1933]. The purpose of these notes is to record data obtained upon three specimens of butter obtained from cows under different nutritional treatments.

EXPERIMENTAL

Fractionation of butter fat from cows at pasture. Spectroscopic data on the mixed butter acids from cows at pasture has already been given [Booth *et al.* 1935]. Through the kindness of Prof. T. P. Hilditch a series of fractions from the mixed liquid esters was made available (for details of the separation see Hilditch & Thompson [1936]). The spectroscopic absorption of the acids in the ultra-violet, as determined with a Hilger H 290 spectrophotometer, are given in Table I. A rapid increase of absorption with increasing I_2 values is evident.

¹ For definition see Dann *et al.* [1935], footnote, p. 139.

Table I. *Spectroscopic absorption of fractions of the mixed liquid esters of butter fat from cows at pasture*

No. of fraction	Wt. of fraction	Sap. equiv. (Hilditch)	i.v. (Hilditch)	$E_{1\text{ cm.}}^{1\%}$ at 230 m μ
1	31.23	227.6	33.5	5
2	6.37	280.3	71.8	9.7
3	8.99	286.1	80.1	12
4	10.46	291.7	87.1	13
5	11.14	293.4	91.9	20
6	8.85	296.0	94.0	21
7	10.6	297.6	96.3	23
8	6.19	298.7	96.9	38
Residue	6.46	325.5	99.7	84

Fractionation of butter fat from cows receiving cod liver oil. Spectroscopic data on the mixed butter acids from cows receiving cod liver oil have already been given by Dann *et al.* [1935]. Fractions from the mixed liquid esters of cows given cod liver oil were also made available by Prof. Hilditch. (See Hilditch & Thompson [1936].)

Table II. *Spectroscopic absorption of fractions of the mixed liquid esters of butter from cows receiving cod liver oil*

No. of fraction	Wt. of fraction	Sap. equiv. (Hilditch)	i.v. (Hilditch)	$E_{1\text{ cm.}}^{1\%}$ at 230 m μ
1	45.44	251.7	53.5	29
2	12.00	290.0	89.0	85
3	12.58	290.2	93.7	81
4	13.38	296.4	96.9	97
5	10.99	296.8	99.6	130
6	15.91	298.5	102.3	130
7	7.56	299.7	103.2	120
8	7.46	303.3	105.5	180
9	7.42	306.4	106.9	170
10	6.02	317.3	113.5	180
Residue	8.16	375.8	118.5	110

The maximum for absorption was always at about 230 m μ , with no band at 270 m μ . Further data were obtained on specimens which had been obtained by the refractionation of the fraction of lowest i.v. (53.5) in Table II. The usual association of intensity of absorption with i.v. is apparent in both primary and secondary fractionations.

Table III. *Refractionation of fraction 1 (Table II)*

No. of fraction	Wt. of fraction	Sap. equiv. (Hilditch)	i.v. (Hilditch)	$E_{1\text{ cm.}}^{1\%}$ at 230 m μ
1	3.04	182.7	10.5	2.1
2	4.37	215.5	17.2	2.6
3	5.45	238.0	33.3	3.3
4	5.89	252.5	37.1	6.7
5	7.85	265.9	55.9	20
6	5.36	280.4	77.1	40
7	4.97	288.9	89.7	65
8	4.57	296.9	99.3	86

Butter fat from cows receiving tung oil. Tung oil is unique among those fats already investigated in containing as its main constituent an acid (elaostearic)

which shows intense spectroscopic absorption in the untreated oil. The absorption maximum is at about 270 $m\mu$.

Four cows which had been kept on a winter diet (hay 5 lb., mangels 36 lb., silage 18 lb., cubes¹ 4 lb. for each gallon of milk over 2 gallons) were divided into two pairs, the milk from each pair being combined. After a pre-experimental period of 8 days tung oil was added to the diet of one pair at the rate of 2 oz. twice daily, the other pair serving as controls. The tung oil was not readily ingested, even when mixed with bran and treacle. It was frequently refused at the morning feed, and was therefore given as a drench. It caused a decline in the yield of milk.

The spectroscopic changes produced are shown in Table IV. The feeding of tung oil caused a marked increase in absorption at 270 $m\mu$. Absorption at 230 $m\mu$ was not increased beyond the range of variation shown by the control group.

Table IV. *Effect of tung oil on the spectroscopic absorption of butter fat acids*

Date of beginning collection of milk	Valven and Valencia (controls)		Remarks	Pax and Orange (tung oil)		Remarks
	$E_{1\text{ cm.}}^{1\%}$			$E_{1\text{ cm.}}^{1\%}$		
	230 $m\mu$	270 $m\mu$		230 $m\mu$	270 $m\mu$	
14 Feb.	5.5	0.47	Pre-experimental period	7.9	0.39	Pre-experimental period
16 "	5.2	0.44	"	7.0	<0.3	"
18 "	8.3	0.32	"	4.7	0.55	"
20 "	3.8	0.29	"	6.3	0.28	"
22 "	8.2	<0.2	Controls	5.8	0.32	4 oz. tung oil daily
23 "	9.8	<0.2	"	8.1	1.24	"
24 "	8.5	<0.2	"	12	1.55	Oil partially refused
25 "	10	0.32	"	9.2	1.41	Drenched
1 Mar.	12	0.54	"	14	6.9	6 oz. tung oil daily
4 "	5.2	0.3	"	9.8	6.3	Tung oil stopped on 3rd
8 "	8.9	0.29	"	7.0	0.53	Cows recovered
$E_{1\text{ cm.}}^{1\%}$ at 270 $m\mu$ of the tung oil used = 1800						

$E_{1\text{ cm.}}^{1\%}$ at 270 $m\mu$ of the tung oil used = 1800

DISCUSSION

The above results confirm the general principle that spectroscopic absorption, actual or potential, is concentrated in the more highly unsaturated fractions. They also support the previous finding that in butter fat the acids are present in the "absorptive" state. Attention may be drawn to the following additional points of interest.

Butter from cows at pasture. The presence of absorptive acids may be considered in relation to the evidence given in Part VI of this series [Moore, 1939, 1] that fatty acids of dried grass are present in the "pro-absorptive" state. A change in spectroscopic properties would therefore appear to take place in the cow, as previously found for fatty acids added to the diet [Dann *et al.* 1935].

Marine acids. The acids of cod liver oil, in common with those of most marine oils so far investigated, have the property of showing absorption at 270 $m\mu$ even more intensely than at 230 $m\mu$ as the result of a comparatively short (48 hr.) treatment with alcoholic KOH. The acids responsible for this absorption do not appear to be solid, as in the case of elaeostearic acid or of the acid absorbing in the same position which may be obtained from linseed oil by more prolonged treatment with KOH. It is remarkable that in the butter

¹ The cubes were composed of oil 4.1 %, albumin 16.4 %, carbohydrate 74.9 %, fibre 4.6 %.

fat the increased absorption caused by giving cod liver oil to the cow is situated at 230 $m\mu$ only, without any sign of a maximum at 270 $m\mu$, either before or after treatment of the butter acids with KOH. This must either imply that the acids absorbing at 270 $m\mu$ are not included in the butter fat, either through failure of absorption or rapid catabolism, or that they are in some way converted into acids absorbing at 230 $m\mu$.

Elaeostearic acid. The effect on the butter fat of giving tung oil was relatively small, suggesting as the most probable explanation that this acid is not well absorbed from the intestinal tract by the cow. The highest concentration reached in the butter fat as the result of feeding 6 oz. daily was about 0.5%. The acid was apparently included in an unchanged state, and could be detected with certainty even in this low concentration through its very intense absorption. It is noteworthy that there was no change in the position of absorption maximum to 230 $m\mu$, as found when tung oil is fed to hens (Part V) [Moore, 1939, 2] or rats [Miller & Burr, 1937].

The cause of the failure of the acids of butter fat to form crystalline bromides. Green & Hilditch [1935] have found that the linoleic acid of butter fat does not yield a crystalline tetrabromide. Presumably the acid is present in a different isomeric form from that found in seed fats. This view is supported by the results obtained in the present work, since the linoleic acid of butter fat is spectroscopically in the "absorptive" state, which would not be expected to give crystalline bromides. The form present in butter, however, would appear to be different from that present in grass, having undergone change to the absorptive form in the cow, as stated above.

SUMMARY

1. Fractions of the mixed liquid fatty acids obtained by high vacuum distillation of the butter fat of cows at pasture showed spectroscopic absorption at 230 $m\mu$. The intensity of this absorption increased rapidly with the ascending i.v. of the fractions, in agreement with the conclusion that spectroscopic absorption is attributable to polyethylenic acids.

2. Similar results were obtained for the fractions of the liquid acids from the butter of cows given cod liver oil. According to the i.v., the content of polyethylenic acids was greater than that in the fractions from the pasture butters, and the spectroscopic absorption at 230 $m\mu$ was relatively more intense. No evidence was obtained of the transference to the milk of the acids present in cod liver oil "pro-absorptive" at 270 $m\mu$.

3. Tung oil was poorly tolerated by cows, but small doses resulted in the appearance of spectroscopic absorption at 270 $m\mu$ in the butter acids at an intensity suggesting the inclusion of about 0.5% of unchanged elaeostearic acid. There was no evidence of the increased absorption at 230 $m\mu$ reported in the hen and rat when given tung oil.

REFERENCES

- Booth, Kon, Dann & Moore (1935). *Biochem. J.* **29**, 133.
Dann & Moore (1933). *Biochem. J.* **27**, 1166.
——— Booth, Golding & Kon (1935). *Biochem. J.* **29**, 138.
Gillam, Heilbron, Hilditch & Morton (1931). *Biochem. J.* **25**, 30.
Green & Hilditch (1935). *Biochem. J.* **29**, 1564.
Hilditch & Thompson (1936). *Biochem. J.* **30**, 677.
Miller & Burr (1937). *Proc. Soc. exp. Biol., N.Y.*, **36**, 726.
Moore (1939, 1). *Biochem. J.* **33**, 1635.
——— (1932, 2). *Biochem. J.* **33**, 1630.