XVIII. A NEW SPECTROSCOPIC PHENOMENON IN FATTY ACID METABOLISM. THE CON- VERSION OF "PRO-ABSORPTIVE" TO "ABSORPTIVE" ACIDS IN THE COW.

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IN an accompanying communication [Booth *et al.*, 1935] we have reported that when the cow is turned out to pasture after the winter the fatty acids obtained from the butter fat show greatly increased absorption for radiation of wave length $230m\mu$. The work described in the present paper owes its origin to an attempt to correlate this increased absorption with the marked fall in the amount of fat in the milk which, as is already known, also frequently occurs when the cow first obtains access to green pasture. Now past work has shown that the feeding of cod-liver oil likewise causes a marked diminution in the amount of butter fat in the milk [Drummond *et al.*, 1924]. Further, cod-liver oil was reputed to yield acids having very high absorption for radiation of wave length $230m\mu$ [Gillam *et al.*, 1931]. It seemed possible, therefore, that the depression of the fat might be linked up with the presence in it of highly absorbing¹ acids, derived either from grass or from cod-liver oil.

This explanation in its simplest form was ruled out when it was found by Dann and Moore [1933] that the high absorption of the fatty acids of cod-liver oil, recorded by Gillam *et al.*, is not observed if the acids are prepared by a brief saponification, but only after the acids have been submitted to prolonged treatment with the hot alkali. It remained possible, nevertheless, that acids which resembled those of cod-liver oil in showing increased absorption after suitable treatment might exert a similar depressing influence on the amount of fat in milk. Support for this view was afforded by early experiments in which we found that the absorption of the butter fat was indeed greatly increased when the relatively non-absorbing cod-liver oil was fed to the cows.

Further experiments have shown that fats capable of depressing the milk fat certainly do as a general rule possess unusual potency in raising the absorption at $230m\mu$. The same property, however, is shared, if in a less degree, by other fats possessing no noticeable depressing action. The increase of absorption would indeed appear to be of a general character. It may occur in two ways, either in

¹ The word "absorption" is used throughout the present communication to designate the extinction coefficient at the head of an absorption band at $230m\mu$. Unless specifically excepted in the text, all figures for the extinction coefficient $E_{1\text{ cm.}}^{1\%}$ at $230m\mu$ (shortened for brevity to $E/230$) refer to the head of a band.

vitro as the result of chemical treatment, or *in vivo* in the course of the absorption of the fats by the cow and their secretion among the constituents of milk fat. The present communication is devoted to the description of this phenomenon. The possible bearing of our results on the nature of the substances responsible for the depression of the milk fat is being postponed for discussion elsewhere.

EXPERIMENTAL.

The conversion of "pro-absorptive" into "absorptive" acids by chemical means¹.

Experiments in vitro. Data relating to the absorptive properties and iodine values of the mixed acids obtained from the five oils used in our feeding experiments (two specimens of cod-liver oil, sardine oil, linseed oil, rape oil), both as prepared by a short saponification and after prolonged treatment of the acids by boiling with alcoholic potash, are given in Table I. The acids were prepared by the method described in the accompanying paper [Booth *et al.*, 1935]. Prolonged treatment with alkali was applied to the acids after their preliminary preparation and separation from unsaponifiable matter by the routine 2 min. saponification. Any complication arising through the possible action of the alkali on the unsaponifiable matter was thereby avoided.

It will be seen that for each oil the acids prepared by a short saponification showed only low absorption at 230 $m\mu$. As the result of prolonged treatment with alkali there was in every instance at least a tenfold increase in absorption. In regard to the iodine values slight falls were observed in the acids from crude cod-liver oil (8 % decrease), medicinal cod-liver oil (10 %) and linseed oil (7 %). There was no change in the iodine values of acids from sardine oil and rape oil.

Table I. *Influence of prolonged treatment with alcoholic potash on the absorption of various fatty acids.*

Mixed acids from	Time of treatment	<i>E</i> /230	I.V.
Sardine oil (crude)	2 mins.	7.7	168
	48 hrs.	185	167
Cod-liver oil (crude)	2 mins.	7.9	166
	48 hrs.	196	153
Cod-liver oil (Engelberg's medicinal)	2 mins.	8.6	185
	37 hrs.	121	166
Linseed oil	2 mins.	6.3	188
	48 hrs.	67.9	181
Rape oil (deodorised)	2 mins.	5.0	114
	48 hrs.	65.8	115

The conversion of "pro-absorptive" acids into "absorptive" acids in vivo.

Feeding experiments. The feeding experiments extended over the late winter and early spring seasons of 1933 and 1934, the absorption of the butter acids on each occasion having reached its minimum level as a result of the fact that the cows had subsisted on stall feeding since the previous autumn. The oils, or oil

¹ A "pro-absorptive" fat or fatty acid is one which itself possesses little specific absorption but is capable of being converted into one which possesses such absorption, either *in vitro* or *in vivo*.

fractions, were usually fed twice daily. The butters were prepared, the fats rendered and the fatty acids prepared according to routine methods¹.

Experiments of Series I (1933). *The effect of feeding crude cod-liver oil and crude sardine oil on the absorptive properties of the mixed butter acids*. Two cows (Lena 3 and Welcome 7) were given 2 ozs. of crude cod-liver oil twice daily on March 14th, 3 ozs. on March 17th and 4 ozs. on March 20th, from which date 8 ozs. per day was continued in two doses till the end of the experiment. Two other (Flora 6 and Winsome 2) were given similar doses of crude sardine oil. The butter fats of Lena 3 and Welcome 7 were compounded, but since Winsome 2 took her sardine oil incompletely her butter was not compounded with that of Flora 6. By an oversight no samples of butter were taken from any of these cows to establish that the absorption of the butter acids had actually reached the typical winter level before the commencement of dosing. The results obtained during oil feeding may be compared however with those obtained upon butter given by the same cows after the cessation of dosing, or upon the butter given by the typical herd receiving the basal ration only during the period of dosing. The results obtained are given in Table II. It will be seen that as the result of feeding either cod-liver oil or sardine oil the absorption of the butter acids was greatly increased

Table II. *Exp. 1. Crude cod-liver oil and crude sardine oil feeding.*

Cows	Date of collection of milk	Remarks	E/230 of mixed acids
Typical herd	March 31–April 4	No oil given	13.1
Lena 3 and Welcome 7	March 30–April 3	4 ozs. cod-liver oil twice daily	48.4
Lena 3 and Welcome 7	April 20	After discontinuation of cod-liver oil feeding	18.0
Flora 6	March 30–April 3	4 ozs. sardine oil twice daily	42.8
Winsome 2	March 30–April 3	4 ozs. sardine oil twice daily, badly consumed	28.6
Flora 6 and Winsome 2	April 20	After discontinuation of sardine oil feeding	12.0

Experiments of Series II (1934). *Feeding experiments with linseed oil and rape oil*. Two cows (Flora 16 and Flora 20) were first given twice daily 2 ozs. of deodorised rape oil on February 19th, on March 6th this was increased to 3 ozs. and on March 9th to 4 ozs. On March 14th the rape oil feeding was discontinued and 3 ozs. of linseed oil were given twice daily to each cow. This was increased to 4 ozs. on March 16th. Since the sample taken during the feeding of linseed oil was prepared from milk collected 13 days after the discontinuation of the rape oil, there would appear to be little danger of the previous diet seriously affecting the result obtained. In this experiment also control samples were unfortunately not taken before the commencement of dosing, but the average value for specimens collected daily between April 7th and April 12th (about 1 week after the discontinuation of linseed oil feeding) may be accepted as a normal control.

The value $E/230 = 14.4$ obtained for the butter acids during the rape oil period was only slightly higher than the value observed for acids obtained from the same cows after the discontinuation of dosing, and it is doubtful whether it

¹ Since our experiments were not all carried out with the same immediate object in view it is unavoidable that there should be some lack of uniformity in the collection and presentation of results. Thus in some experiments the effect of feeding oil has been examined only by taking single specimens of butter fat at arbitrary periods after the commencement of oil feeding, in other experiments specimens have been collected daily over considerable periods.

was significantly higher than values around 10 which we would consider typical for winter butter fat. The value (20.0) obtained for the acids during the feeding of linseed oil showed a definite increase well beyond the range of experimental error.

Table III.

Cows	Inclusive dates of collection of milk	Remarks	$E/230$ of mixed acids
Flora 16 and Flora 20	p.m. March 13–a.m. March 14	4 ozs. of rape oil twice daily	14.4
Flora 16 and Flora 20	p.m. March 27–p.m. March 28	4 ozs. of linseed oil twice daily	20.0
Flora 16 and Flora 20	April 7–April 12	Average value over the period mentioned. Linseed oil feeding discontinued on March 30, p.m.	11.8

Fig. 1. Welcome 7. (1) Cod-liver oil feeding; (4) absorptive acid feeding.

Experiments of Series III (1934). *The feeding of medicinal cod-liver oil (Engelberg's)*. In this series of experiments specimens of the butter acids were prepared daily. It has therefore been possible to study the time which elapses before foreign acids appear in the butter fat and before their disappearance

after the cessation of dosing. The results obtained in the various experiments are shown graphically in Figs. 1 and 2.

(1) *Whole oil feeding* (Welcome 7, Fig. 1). Dosing was commenced at the rate of 2 ozs. twice daily, subsequently raised to 3 ozs. twice daily. The feeding of the cod-liver oil resulted in an extremely rapid rise in the absorption of the mixed butter acids. From an average value of 8.4 on the 3 days preceding dosing, $E/230$ for the mixed acids rose to 15.4 on the third day of dosing and to 35.2 on the seventh day of dosing. At this point a maximum value for the particular level of dosing appeared to have been reached. After 11 days on cod-liver oil,

Fig. 2. Lottie 5. (2) Pro-absorptive acid feeding; (3) non-saponifiable matter feeding.

dosing was discontinued, with the result that the absorption of the acids fell almost as rapidly as it had risen. It may be noted, however, that the absorption did not quite return to the level observed before dosing began and also showed a number of minor fluctuations during the post-experimental period. Iodine value determinations on selected specimens of butter acids taken just before dosing and at the point when the absorption of the fatty acids had reached its maximum gave values of 37.6 and 50 respectively, the former value being about the usual level for winter acids.

(2) "*Pro-absorptive*" acid feeding (Lottie 5, Fig. 2). The acids were prepared in bulk by a technique essentially similar to our usual routine method, the time taken over the saponification being kept short by ensuring that the alcoholic potash was at boiling-point before the addition of the cod-liver oil. Previous to dosing, $E/230$ for the mixed acids had shown a value not greater than 10 for 11

days. The cod-liver oil acids were given over a period of 3 days, in doses as follows:

p.m. 8. iii. 34	...	...	2 ozs.
a.m. 9. iii. 34	...	...	2 ozs.
p.m. 9. iii. 34	...	...	3 ozs.
a.m. 10. iii. 34	...	...	3 ozs.
p.m. 10. iii. 34	...	...	3 ozs.
a.m. 11. iii. 34	...	...	1.5 ozs.
Total ...			14.5 ozs.

On the second day of dosing $E/230$ had risen to 20.0. The maximum value: 33.3, was reached one day after the cessation of dosing, after which the absorption fell rapidly. As in the previous experiments there were several minor fluctuations in the absorption during the post-experimental period. Iodine value before dosing 40, at point of maximum absorption 52.3.

(3) *Feeding of the non-saponifiable residue* (Lottie 5, Fig. 2). The crude unsaponifiable matter obtained by evaporation of the ether extracts of the soaps during the preparation of the acids was fed in doses calculated to correspond to the amounts of acids previously given. The period of dosing happened to correspond with a minor upward fluctuation in the "absorptive" power, probably related to previous acid feeding, otherwise the values obtained showed no divergence from the normal winter level.

(4) *Feeding experiments with "absorptive" acids* (Welcome 7, Fig. 1). The "pro-absorptive" acids ($E/230=8.6$), after preparation in the manner described above, were converted into the "absorptive" acids ($E/230=121$) by boiling for 37 hours with alcoholic potash. The doses given were the same as the doses of "pro-absorptive" acids described in (2), and 14.5 ozs. were fed over the period p.m. 23. iii. 34—a.m. 26. iii. 34.

The absorption of the butter acids rose rapidly, reaching a maximum value ($E/230=42.4$) 4 days after the commencement, and 2 days after the cessation, of dosing. The fall of the absorption from the maximum value was checked by a considerable upward fluctuation of several days' duration. Iodine values—before dosing 41.1, at point of maximum absorption 51.1.

The behaviour of the butter acids on treatment in vitro by boiling with alcoholic potash. In order to gain some insight into the proportion of "pro-absorptive" acids secreted in the butter fat, selected specimens from the group of butters described above were submitted to prolonged treatment with alcoholic potash, which was calculated to increase the absorption by significant amounts if "pro-absorptive" acids were present in quantity. The results obtained are given in Table IV. It will be seen that in no case was there more than a slight rise in the absorption as a result of this treatment. The behaviour of the acids was thus the same as that of the acids from normal winter or summer butter [Booth *et al.*, 1935].

Table IV.

Material given to cows	Butter fat acids		
	Time of treatment	$E/230$	I.V.
"Pro-absorptive" acids of cod-liver oil	2 mins.	33.3	52.3
	48 hrs.	26.5	46.5
Linseed oil	2 mins.	20	49.6
	48 hrs.	23.4	51
Rape oil	2 mins.	14.4	49.5
	48 hrs.	17.9	49.1

Differentiation between the "absorptive acids" and the "inhibitory substance" of the antimony trichloride test. Experiments, which need not be described in detail, have demonstrated that while the absorption of acids from butter fats of cows fed on linseed-oil, cod-liver oil or sardine oil might be equal to or greatly exceed that of normal summer acids, the inhibitory power was invariably much lower.

DISCUSSION.

The conversion of "pro-absorptive" into "absorptive" acids. The above experiments demonstrate that certain fatty acids characterised by a slight absorption in the far ultra-violet ("pro-absorptive acids") can be converted *in vivo* and *in vitro* into acids having a much stronger absorption in the same region. The change can be brought about either by feeding the fatty acids (or the fat) to the cow or by treating the acids for long periods with caustic potash. For the present it must remain doubtful whether the changes occurring *in vivo* and *in vitro* are identical in all respects, but as a working hypothesis it will be assumed that the two processes are similar. Since the absorption of the butter acids from cows receiving ordinary diets or dosed with various oils shows no significant increase after prolonged treatment with potash, it seems probable that almost the whole of the ingested acids capable of transformation into the strongly absorbing substance either undergo the transformation or are not secreted into the milk.

Quantitative aspects of the transformation. The increase in the absorption of the fatty acids from the various oils brought about by treatment with caustic potash is self evident. On the other hand, the increased absorption of the fatty acids of the butter following the ingestion of the oils might seem to be explicable on other grounds than the transformation of "pro-absorptive" into "absorptive" acids. Two such alternative explanations may be advanced, but both can be excluded by examining the data quantitatively. In the first place it might be considered that the increased absorption was essentially due to the depression in the actual amount of butter fat secreted, on the assumption that the depression might take place at the expense of the non-absorbing constituents of the butter acids. Since however the depression seldom amounted to more than 30 %, while the absorption was sometimes increased 4-5 fold, it is obvious that this explanation would be inadequate. Secondly it might be argued that the increased absorption of the mixed butter acids over that of the ingested fat might be due not to an actual increase in the specific absorption of the ingested acids, but to the concentration in the milk fat of highly absorbing components present in low concentration in the ingested acids. This explanation also fails, however, since the increased absorption of the butter acids, calculated on weight basis, was always much greater than the absorption present in the ingested acids. To illustrate this point we may take the figures for Lottie 5, which was given a preparation of the acids of cod-liver oil in the "pro-absorptive" state. If we study the "absorption units" secreted in the butter acids during a 12-day period commencing with the 3 days during which the acids were administered, we may draw up the following balance sheet:

	<i>E</i> /230 × g. units
Absorption units secreted in butter acids. (Calculated by multiplying the daily butter acid yield in g. by the daily value for <i>E</i> /230.)	82,000
Allowance for basic absorption due to butter on winter scale (calculated by multiplying the total butter acid yield over the 12 days (5050 g.) by the average value for <i>E</i> /230 over 11 days preceding dosing (9.2))	46,000
Increase due to cod-liver oil acid feeding	= 36,000
Absorption present in cod-liver oil acids as ingested (411 g. having <i>E</i> /230 = 8.6)	= 3,500

The increase in absorption of the mixed acids of the butter fat was therefore about 10 times greater than the absorption of the ingested acids.

The nature of the change involved. At the present stage little can be said about the nature of the acids capable of showing increased absorption. Judging however from the limited experience of Dann and Moore [1933] it appears probable that the property is confined to acids having two or more unsaturated linkages. As to the cause of increased absorption, it appears probable that intramolecular changes take place. The possibility that oxidative changes might be involved appears to be ruled out by (1) iodine value determinations. In the *in vitro* experiments, the absorption rose to high values with little and in some cases no fall in the iodine value. This would indicate that only small amounts of oxidation products can have been formed. If these were responsible for the increased absorption, then the specific absorption must have been of an unusually high order. (2) Preliminary experiments have indicated that the "absorptive" acids are themselves unstable to oxidation and are therefore unlikely to be the end-products of an oxidative reaction. Thus when the mixed acids of cod-liver oil were first converted into the "absorptive" state by prolonged treatment with potash and then aerated for 24 hours at 95°, $E/230$ fell from 121 to 20.6. (3) Oxidative treatment of the "pro-absorptive" acids from cod-liver oil increases slightly the extinction coefficient at 230 $m\mu$, but the characteristic band at that wave length is no longer evident, and the absorption is non-specific end absorption. The above evidence, of course, does not rule out the possibility of oxidative changes of the peroxide formation type, if those changes can be visualised as occurring without affecting the iodine-absorbing capacity of the unsaturated linkages.

The rapidity of transference of exogenous fats to the milk fat. As a point of secondary interest the above experiments provide a good illustration of the great rapidity with which foreign fats may be secreted in the milk fat, since when cod-liver oil, or preparations of its acid fraction, were administered, a distinct rise in the absorption of the butter acids could always be detected on the following day. The presence of the cod-liver oil acids in the butter fat could be readily observed by the formation of dark brown soaps on treatment of the fat with alcoholic potash. This is a property of the cod-liver oil acids, whereas the acids of butter fat form colourless soaps when obtained from cows subsisting under normal nutritional conditions.

SUMMARY.

1. In the mixed acids prepared from cod-liver oil, sardine oil, rape oil and linseed oil the absorption of radiation at wave length 230 $m\mu$ is small. When the oils were fed to the cow the acids secreted in the butter fat possessed much greater absorption in this region.
2. A similar increase in absorption could be brought about by the prolonged boiling of the "pro-absorptive" acids with alcoholic potash.
3. The change from slightly absorbing ("pro-absorptive") acids to strongly absorbing acids is not an oxidative one; it is postulated that a molecular rearrangement occurs with production of an isomeric form.

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APPENDIX.

Samples of two of the butters examined in the above experiments, and also two other samples not examined were sent to Prof. T. P. Hilditch who has kindly sent the following analyses: (Letter dated May 11th, 1934.)

Cow or cows	...	Gertie 3	Flora 16 Flora 20	Flora 16 Flora 20	Portia 3
Oil fed	...	None (control)	Rape oil	Linseed oil	Cod-liver oil
Milk collected from		p.m. 14. iii. 34	p.m. 13. iii. 34	p.m. 27. iii. 34	a.m. 17. iii. 34
to, inclusive		a.m. 16. iii. 34	a.m. 14. iii. 34	p.m. 28. iii. 34	p.m. 18. iii. 34
Reichert-Meissl value		33.6	28.5	28.9	16.0
Polenske value		3.3	1.8	2.1	1.4
Kirschner value		24.3	21.7	22.4	13.9
Saponification equivalent		239.2	251.2	249.3	264.2
Iodine value		34.5	44.5	46.0	51.7

Prof. Hilditch quoted these figures at the Meeting of the British Association at Aberdeen. He said, "These results indicate sufficiently clearly that intake of fatty oil by the cows may cause considerable modification of the milk fat; the influence of cod-liver oil on the character of the butter fat is especially marked in its diminution of the most volatile acids and the 50 % increase in mean unsaturation as compared with the control."

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