CCI. SPECTROSCOPIC CHANGES IN FATTY ACIDS

V. THE EFFECT OF THE DIETARY FAT ON THE BODY FAT AND EGG FAT OF THE HEN

By ETHEL MARGARET CRUICKSHANK

From the School of Agriculture, University of Cambridge

JAMES HOUSTON

*From the Queen's University, Belfast, and 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)

IN Part I of this series [Moore, 1937] it was shown that while the polyethylenic acids of seed fats are usually in the spectroscopically "pro-absorptive" state, those present in animal fats may be either "pro-absorptive" or "absorptive". Thus in fishes and marine animals "pro-absorptive" polyethylenic acids have been found, whereas in land mammals, a part, at least, has been found in the "absorptive" state. The one bird fat investigated (duck) was found to contain "pro-absorptive" acids. It will be shown below that this finding also applies to the hen under normal conditions of nutrition. The results furnish evidence that divergences may exist between different species in their treatment of fatty acids.

EXPERIMENTAL

Eggs were first collected by trap-nesting from hens which had been maintained since hatching on the same diet, which was designed to have a low content of fat (de-germed maize 40 parts, bran 15, yeast 5, dried skimmed milk 15, alfalfa 5, polished rice 20, mineral mixture 2.5). The egg fat was obtained by grinding up the yolks with anhydrous Na_2SO_4 and extracting for prolonged periods with ether. The acids were prepared by a brief saponification with alcoholic KOH, followed by the removal of unsaponifiable matter and acidification in the usual way. They were examined spectroscopically as so obtained and after refluxing for 48 hr. with alcoholic KOH.

Selected hens were then given the same diet but with the addition of sperm oil, linseed oil or tung oil. Eggs were collected and examined as before. The effect of the oils on the spectroscopic absorption of the egg and abdominal fats is shown in Tables I-V.

For convenience results are given to two figures, although the second is of doubtful significance if the first is high. Efforts were made to keep the conditions of refluxing constant but large differences were often observed between values which would be expected to be closely similar. The tendency of the added oils to increase absorption was, however, evident in all instances.

*Sperm oil.*¹ After eggs had been collected from a hen receiving the "low fat" diet an addition of 10 % of sperm oil was made, over a period of 4 weeks. Eggs were collected at intervals.

Table I. *Spectroscopic absorption of the fatty acids of the eggs of hens receiving sperm oil*

Hen no.	Date	$E_{1\text{ cm.}}^{1\%}$ Short saponification (3 min.)		$E_{1\text{ cm.}}^{1\%}$ Long saponification (48 hr.)		Remarks
		230 $m\mu$	270 $m\mu$	230 $m\mu$	270 $m\mu$	
125	22. xi. 36	2.7	0.3	17	11	"Low fat" diet
	23. xi. 36	3.2	0.6	12	6.5	
	26. xi. 36	2.9	0.36	19	18	
	Means	2.9	0.42	16	12	
	17. i. 37	2.7	0.41	25	22	Sperm oil (10%) added to diet
	4. ii. 37	2.4	0.37	33	33	
	5. ii. 37	4.9	0.84	17	29	
	8. ii. 37	5.1	0.72	22	28	
	Means	3.8	0.59	24	28	
		$E_{1\text{ cm.}}^{1\%}$ for fatty acids of sperm oil				
		10	<1	55	47	

The absorption of the acids was low when prepared by a short saponification. Refluxing with KOH increased the absorption, the intensity at 270 $m\mu$ having a mean value slightly greater than at 230 $m\mu$.

Linseed oil. The experiment was conducted as above but with the addition to the diet of 10% of linseed oil for 11 days.

Table II. *Spectroscopic absorption of the fatty acids of the eggs of hens receiving linseed oil*

Hen no.	Date	$E_{1\text{ cm.}}^{1\%}$ Short saponification (3 min.)		$E_{1\text{ cm.}}^{1\%}$ Long saponification (48 hr.)		Remarks
		230 $m\mu$	270 $m\mu$	230 $m\mu$	270 $m\mu$	
375	2. ii. 37	2.1	0.30	26	20	"Low fat" diet
	4. ii. 37	3.3	0.39	23	18	
	5. ii. 37	2.1	0.51	29	25	
	Means	2.5	0.40	26	21	
	12. ii. 37	2.6	1.0	62	43	Linseed oil (10%) added to diet
	15. ii. 37	2.9	0.36	96	63	
	17. ii. 37	3.5	0.45	55	38	
	Means	3.0	0.60	71	48	
		$E_{1\text{ cm.}}^{1\%}$ for fatty acids of linseed oil				
		2.5	—	250	90	

The absorption was again low when the acids were prepared by a short saponification, and was increased by refluxing with KOH.

¹ The oil was ordered as "whale oil", a description which is properly restricted to the oils of whales belonging to the *Balaena* and *Balaenoptera* genera. On examination after the experiment, it was found to have an unsaponifiable matter content of 35%. This high value is typical of sperm oil, i.e. the oil obtained from the sperm whale, *Physeter macrocephalus*, which contains a large proportion of the sperm wax.

Tung oil. Tung oil (5 %) was added to the diet for 6 weeks.

Table III. *Spectroscopic absorption of the fatty acids of the eggs of hens receiving tung oil*

Hen no.	Date	$E_{1\text{ cm.}}^{1\%}$ Short saponification (3 min.)		$E_{1\text{ cm.}}^{1\%}$ Long saponification (48 hr.)		Remarks
		230 μ	270 μ	230 μ	270 μ	
1	22. xi. 36	2.5	0.19	33	20	"Low fat" diet
	25. xi. 36	2.9	0.21	11	4.9	
	Means	2.7	0.20	22	12	
	13. ii. 37	67	11	110	29	Tung oil (5%) added to diet
	14. ii. 37	52	8.5	120	49	
	21. ii. 37	90	15	120	32	
	Means	70	12	120	37	
43	19. ii. 37	89	19	113	37	Tung oil (5%) added to diet
		$E_{1\text{ cm.}}^{1\%}$ for fatty acids of tung oil				
		100	1500	—	—	

Fatty acids from the eggs obtained after giving tung oil differed from all others in showing intense preformed spectroscopic absorption. In surprising contrast with the position of absorption in the tung oil ($E_{1\text{ cm.}}^{1\%}$ at 270 m μ = 1650) the absorption maximum in the egg fats was at 230 m μ . Absorption at 270 m μ was only about one-seventh to one-fifth as intense as at 230 m μ .

The intensity of absorption at both positions was increased by refluxing with KOH. The amount of this increase was greater than that shown when the hen was not receiving tung oil (see Table V) suggesting that the tung oil contained small amounts of "pro-absorptive" acids in addition to the absorptive elaeo-stearic acid, the absorption of which has been found in previous experiments to be unaffected by refluxing.

In view of the unexpected nature of the result an egg from another hen receiving tung oil was also examined. Similar values were obtained.

Abdominal fats. After the collection of eggs the hens were killed, together with an additional hen still receiving the "low fat" diet; and specimens of the abdominal fat were taken. The experimental procedure was the same as that employed for the egg fats.

Table IV. *Spectroscopic absorption of acids from the abdominal fats of hens receiving a diet with and without added fats*

Hen no.	Diet	$E_{1\text{ cm.}}^{1\%}$ 2 min. saponification		$E_{1\text{ cm.}}^{1\%}$ 48 hr. refluxing with KOH	
		230 m μ	270 m μ	230 m μ	270 m μ
26	Free from added oil	2.5	<0.4	22	2.8
125	Sperm oil	4.1	<0.41	24	3.5
375	Linseed oil	4.8	<0.48	110	75
1	Tung oil	90	17	—	—

The changes in spectroscopic properties were generally similar to those observed in the egg fats. With fats from hens on the "low fat" and sperm oil diets, however, the absorption at 270 m μ after refluxing was much lower than in the egg fats.

DISCUSSION

The above results indicate that the hen normally has its polyethylenic acids present in the egg fat and abdominal fat in the spectroscopically "pro-absorptive" form. An exception to this rule occurs when tung oil has been given. The data on egg fats are summarized with the inclusion of I_2 values in Table V.

Table V. *Summary of data on the spectroscopic absorption of the eggs of hens on a diet with and without added fats*

Hen no.	Diet	I.V. of ingested oil	I.V. of egg fat acids	$E_{1\text{ cm.}}^{1\%}$ 2 min. KOH		$E_{1\text{ cm.}}^{1\%}$ 48 hr. KOH	
				230 $m\mu$	270 $m\mu$	230 $m\mu$	270 $m\mu$
125	Fat-free	—	80	2.9	0.42	16	12
375	"	—	—	2.5	0.40	26	21
1	"	—	—	2.7	0.20	22	12
			Means	2.7	0.34	21	15
125	Sperm oil	91	83	3.8	0.59	24	28
375	Linseed oil	179	106	3.0	0.60	71	48
1	Tung oil	169	84	70	12	120	37

Eggs from hens receiving sperm oil or linseed oil. The marine oils, as a class, contain fatty acids which have the property of showing absorption at 270 $m\mu$ of intensity equal to or greater than that at 230 $m\mu$ as the result of relatively short treatment with KOH. This property was found in the eggs of the hen receiving sperm oil. When linseed oil had been given the intensity of absorption after refluxing was considerably greater at 230 $m\mu$ than at 270 $m\mu$. In agreement with the greater degree of unsaturation of linseed oil the increases in absorption after refluxing were much greater in both positions than with sperm oil.

Eggs from hens receiving tung oil. The effect of feeding tung oil on the spectroscopic absorption of the acids of the egg and abdominal fats was striking. The same phenomenon of the change in absorption from 270 to 230 $m\mu$ has recently been reported by Miller & Burr [1937] who found that the feeding of tung oil to the rat caused the development of absorption at 230 $m\mu$ in the fat throughout the body. The most probable cause of the change is the reduction of the number of conjugated unsaturated linkages of elaeostearic acid from 3 to 2, either by hydrogenation or less probably by oxidation with loss of part of the carbon chain. As shown in Part VI [Moore, 1939] catalytic hydrogenation causes a rapid decrease in absorption at 270 $m\mu$ accompanied by an increase at 230 $m\mu$, and partial saturation is therefore a plausible explanation of the change of absorption *in vivo*. It may be noted that the ratio of absorptions at 230 and 270 $m\mu$ was much higher in the egg acids than in the artificial mixture prepared by catalysis, suggesting a more selective mechanism in the treatment of elaeostearic acid by the hen.

Comparison of the treatment of polyethylenic acids by the cow and the hen. It may be noted that the two animals differ in their treatment of polyethylenic acids in three directions. (1) In the cow the polyethylenic acids of the butter fat are present almost entirely in "absorptive" form. In the egg and abdominal fat of the hen, unless tung oil has been given, they are present in "pro-absorptive" form. (2) When marine acids are given the components responsible for absorption at 270 $m\mu$ cannot be traced in either "absorptive" or "pro-absorptive" form in the butter fat. In the egg fat of the hen they are present in "pro-absorptive"

form. (3) When tung oil is given to cows only a small percentage of apparently unchanged elaeostearic acid appears in the butter. With the hen the elaeostearic acid is apparently converted into an acid absorbing at $230\text{ m}\mu$.

SUMMARY -

1. Fatty acids from the egg and abdominal fats of hens given diets with no added oil, or with the inclusion of sperm oil or linseed oil contained polyethylenic acids in the "pro-absorptive" state.

2. The dietary fat had a marked effect on the amount of polyethylenic acids present in the egg fat as revealed by spectroscopic examination after refluxing with KOH. Similar effects were observed for the abdominal fat.

3. With sperm oil added to the diet the increases of absorption after refluxing with KOH were about equally great at 230 and $270\text{ m}\mu$. With the diet free from fat, and with linseed oil included, the increase in absorption at $230\text{ m}\mu$ was greater than at $270\text{ m}\mu$.

4. The inclusion in the diet of tung oil which absorbs intensely at $270\text{ m}\mu$ caused a marked increase in the preformed spectroscopic absorption at $230\text{ m}\mu$ in the egg and the body fatty acids. This change in the position of the absorption maximum is probably to be ascribed to the loss of one of the conjugated unsaturated linkages of elaeostearic acid.

5. The above results suggest that there are marked differences in the treatment of polyethylenic acids by the cow and the hen.

REFERENCES

- Miller & Burr (1937). *Proc. Soc. exp. Biol., N.Y.*, **36**, 726.
Moore (1937). *Biochem. J.* **31**, 138.
— (1939). *Biochem. J.* **33**, 1635.