# THE COMPOSITION OF THE BODY FAT OF SMALL GREEN CHIRONOMIDS

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During the winter months, the banks of the Nile near Khartoum abound with small non-biting midges, *Tanytarsus lewisi* Freeman (Diptera, Chironomidae), which emerge from the surface of the river shortly after sunset and fly around in the immediate neighbourhood for about 1½ hr. In an endeavour to relate variations in the incidence of these insects ('nimitti') to seasonal and climatic factors, collections were made every evening for some considerable time; the insects trapped for this purpose provided the source of material used for the experimental work described below.

The trap used, which was designed by Dr A. J. Henry, Government Analyst, consisted of a wooden box, 56 × 56 × 45 cm., which was placed on a table about 4 ft. high, situated on a first-floor verandah of a building on the south bank of the Blue Nile at Khartoum. One side panel (56 × 56 cm.) was removed, and the whole box was painted dull black, inside and out. A metal can, 23 cm. deep and 18 cm. in diameter, lined with black paper inside, was placed centrally on the bottom of the box, the open end uppermost, and a high-powered electric light bulb was suspended just above the top of the can. This was illuminated each night from 6.30 to 8 p.m. when the insects would swarm around the lamp and fall down into the can. Once inside the can it seems impossible for them to escape, and it was furthermore noticed that practically no insects fell outside it. The next morning the dead insects were dried for 5 hr. in a hot-air oven at 50° C. and weighed. Approximately 18,000 insects weighed 1 g. and as many as a million have been caught by this procedure in one evening.

The air-dried insects were analysed with the following results:

| | % |
|---|---|
| Loss at 110° C. | 4·39 |
| Ash | 5·64 |
| Protein | 60·68 |
| Chloroform-soluble fraction | 11·71 |
| Alcohol-after-chloroform extract | 17·78 |
| | 100·20 |

EXAMINATION OF THE CHLOROFORM-SOLUBLE FRACTION

This was a very dark brown evil-smelling oil, having the following characteristics:

| | |
|---|---|
| Free fatty acids (as oleic acid) | 22·28 % |
| Saponification value | 205·5 mg. KOH/g. |
| Unsaponifiable matter | 7·45 % |

The figure for the saponification value must be regarded as approximate only, as the dark colour of the mixture obscured the end-point very considerably. The unsaponifiable matter, removed in the usual way by extracting the saponified oil with ether, was golden yellow in colour, had a pleasant fragrant smell and tended to crystallize radially on standing. The alkaline solution after extraction was acidified and the fatty acids were recovered by re-extraction with ether. This fraction represented 88·38 % of the whole and had the following characteristics:

| | |
|---|---|
| Iodine value (Wijs) (1½ hr.) | 137·2 |
| Thiocyanogen value (20 hr.) | 93·02 |
| Mean molecular weight | 280 |

BROMINATION OF TOTAL NON-VOLATILE FATTY ACIDS

0·90 g. of this fraction was dissolved in 20 c.c. of ether and cooled to 0° C. Bromine was added drop by drop, and a white precipitate appeared. This was allowed to stand in a refrigerator for 24 hr., when it was centrifuged and the mother-liquors carefully decanted. The deposit was stirred up with a further 20 c.c. of ice-cold ether, and again centrifuged and decanted. The residual crystals weighed 0·1594 g. (17·7%). On attempting a melting-point determination, no sharp melting-point was obtained below 210° C., when decomposition with blackening occurred. This compound is not, therefore, the bromination derivative of linoleic acid, but is derived from a tetra-ene or penta-ene acid similar to those present in fish oils.

The mother liquors which had been decanted from these crystals were evaporated to dryness on a water-bath, and treated with hot petroleum ether. An insoluble heavy oily fraction was obtained, and on cooling the petroleum ether, a crop of colourless crystals was also obtained. After twice recrystallizing from petrol ether, this compound had m.p. 114° C., and was evidently the bromination product of linoleic acid present in the original oil.

IDENTIFICATION OF THE LESS UNSATURATED FATTY ACIDS

5 g. of the original oil were subjected to the usual Reichert-Meissl-Polenske process, after saponification with glycerolic sodium hydroxide in the usual way. The Reichert-Meissl value was 1·91 and the Polenske value 0·52.

The distillates from the Reichert-Meissl and Polenske determinations were evaporated to dryness, and the following residues were obtained:

*Reichert-Meissl:*

| | |
|---|---|
| Total residue | 0·0256 g. |
| Phenolphthalein added | 0·0050 g. |
| Weight of sodium salt of water-soluble volatile acids | 0·0206 g. |

This was equivalent to 1·74 c.c. N/10-NaOH (titration figure for Reichert Meissl value).

The molecular weight of the water-soluble steam-volatile acids is therefore 96 (butyric acid 88, valeric acid 102).

Percentage water-soluble volatile acids present: 0·37 %.

*Polenske:*

| | |
|---|---|
| Total residue | 0·0168 g. |
| Blank on indicator and alcohol | 0·0028 g. |
| Weight of sodium salts of Polenske acids | 0·0140 g. |

This was equivalent to 0·52 c.c. N/10-NaOH, corresponding to a molecular weight of 247 for the Polenske acids, which was present to the extent of 0·26 %. It is considered that this finding has no real significance, being due in all probability to a small portion of the non-volatile acids having been carried over by mechanical splashing.

The residue from the Reichert-Meissl-Polenske distillation flask was recovered by extraction with ether, and 4·7802 g. were obtained (95·60 %). This material was saponified by refluxing with excess N/2 alcoholic caustic potash for 1 hr., and the unsaponifiable matter extracted; 0·3748 g. (7·496 %) was obtained. The extracted soap solution was acidified and the liberated fatty acids were recovered by ether extraction (88·10 %). This had a mean molecular weight of 280·0.

## MILD OXIDATION OF TOTAL NON-VOLATILE FATTY ACIDS

These non-volatile fatty acids were oxidized under the conditions described by Lapworth & Mottram (1925). 3·50 g. fatty acids were taken and dissolved in 1400 c.c. water containing 3·5 g. of caustic soda. 490 g. of ice were added to the mixture and then a solution of 5·32 g. potassium permanganate dissolved in 250 c.c. of water, cooled in ice to 0° C. The addition of the solution took 3 min., and the reaction mixture allowed to stand for a further 12 min., the whole being kept thoroughly cooled with ice. In this way it was hoped to produce only hydroxylation of the unsaturated fatty acids and avoid any oxidative split to dibasic acids and short-chain monobasic acids. The mixture assumed the brilliant green colour of manganate, which was finally decolorized by the passage of sulphur dioxide. The liberated acids were allowed to stand overnight in the refrigerator to coagulate. The mixture was then filtered at the pump, and the filtrate extracted with petrol ether. The residue was transferred to a beaker, and boiled up thoroughly with the same petrol ether, the solution being filtered into a separating funnel, washed with water until neutral, and evaporated. The residue was a hard brittle fat (1·1904 g.) equivalent to 34·0 % of the total non-volatile fatty acids. This had a molecular weight of 271·9, and m.p. 54° C., and was evidently mainly stearic acid in admixture with small amounts of palmitic acid.

The residue insoluble in petrol ether was boiled up with about 1200 c.c. of water and filtered hot at the pump, a further 800 c.c. boiling water being used for

washing. The filtrate and washings were united and evaporated to about 1 l. and then cooled in the refrigerator overnight. The deposited crystals were filtered off at the pump, and washed with ice-cold water. 0·6631 g. was obtained, and a further 0·0998 g. was recovered by evaporating the filtrate to dryness. By repeated recrystallization from ethyl acetate, two compounds were isolated, melting at 148° C. (more-soluble fraction) and 172° C. (less-soluble fraction), and had molecular weights 345 and 351 respectively. These compounds are therefore the well-known pair of isomeric tetrahydroxystearic acids corresponding to *cic-cis*-9, 12, octadecadienoic acid—the linoleic acid commonly found in seed fats, and whose presence in this insect fat is thus established.

The water-insoluble residue was digested with boiling ethyl acetate, filtered, concentrated and cooled. A white crystalline deposit was obtained which was further recrystallized twice from ethyl acetate, in which it was readily soluble at the boil, but had a low solubility at 0° C. This compound had m.p. 116° C. and molecular weight 313, and was evidently dihydroxystearic acid probably contaminated with a small amount of dihydroxypalmitic acid, arising from the mild oxidation of oleic acid (and minor amounts of palmitoleic acid) in the original mixed fatty acids.

The fatty material contained in these insects is therefore probably a mixture of glycerides of palmitic, stearic, oleic, palmitoleic, linoleic acids and a polyethenoid acid containing four or five double bonds, and although no quantitative estimate of the composition is given here, the fat is clearly closely similar to those usually occurring in fresh-water fish (Lovern, 1932 et seq.).

It is interesting to note that in all probability the eggs of these insects are laid on the surface of the water, and sink to the bottom where they hatch out in the mud. The larvae spend most of their time in the mud, in which they feed on algae and other similar materials. The pupae, which do not feed, remain on the bottom until they rise to the surface preparatory to emerging as adult insects, which also do not feed, and have only a very short life, probably only a few hours. Thus virtually all the feeding takes place in the aquatic phase. As the insects of course do not return to the water, their emergence represents a considerable loss of protein and fat from river circulation every year. The insects form an important source of food material for the fish, the composition of the fat of which is so closely similar to that of the insects, which in turn resembles that present in algae (Lovern, 1936), all three types containing notable proportions of polyethenoid acids.

## SUMMARY

The body fat of the midge, *Tanytarsus lewisi* (Chironomidae), has been analysed, and has been found to contain major amounts of polyethenoid fatty acids normally associated with the fat of fish. It is interesting to note that this insect does all its feeding while in the larval stage on the bed of the river, under water, and that the body fat resembles that of algae, and plankton, which probably are the main sources of food, and differs considerably from the composition of most terrestrial insects hitherto recorded.

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