The Lipid Composition of Pigeon Milk

The secretion of 'milk' by pigeons and doves is an unique and spectacular adaptation of the avian crop[1]. Though the phenomenal rate of growth of pigeon squabs has been attributed to their food, the pigeon milk[2–5], the nutritive properties of this feed-stuff have not been properly understood. Many earlier studies[3,6] have been limited to the gross chemical composition of pigeon milk. However, recently it was reported that the pigeon milk has the requisite essential amino acids in adequate proportion[7], which show a close parallelism with those of cow's and human milk[8]. In the present paper the lipid composition of pigeon milk is reported.

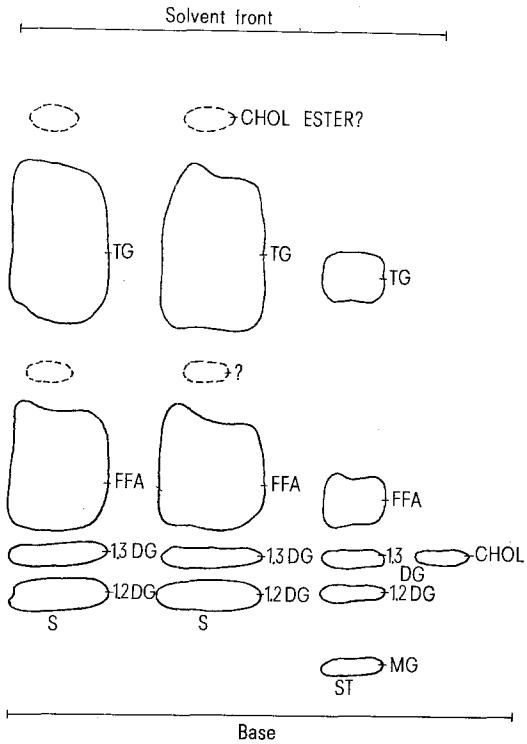
The pigeons were maintened in the laboratory on mixed-grain ration. The pigeon milk was collected[4] from 0–4-day-old squabs as described earlier[9]. The lipids were extracted thrice with chloroform: methanol (2:1 v/v) following the method of Folch et al.[10]. The chloroform layer was evaporated to dryness under reduced pressure and the lipids redissolved in redistilled chloroform. Total lipid was estimated by evaporating the aliquots in duplicate to constant weight.

Suitable aliquots containing about 50 mg lipid were taken in silicic acid columns and the neutral and phospholipids were eluted with chloroform and methanol, respectively. The solvents were evaporated in vacuo and the lipids taken in a known volume of pure chloroform. Total phospholipids and neutral lipids was determined gravimetrically in duplicate aliquots. 0.1 ml aliquots each of neutral and phospholipid extract were analyzed by thin layer chromatography (TLC) on silica gel (NCL-Poona) plates along with known standards. The plates were developed in a chloroform: methanol: water (65:25:4 v/v) and hexane: ether: glacial acetic acid (30:6.0:0.5 v/v) solvent systems for phospholipids and neutral lipids, respectively. The spots were developed using crystalline iodine and identified with reference to known standards.

Phospholipids were quantitatively estimated by determining the lipid phosphorus content in each TLC component[11,12], cholesterol was estimated colorimetrically[13] The methanolic esters of fatty acids[14] were analyzed in a gas liquid chromatogram (GLC) using nitrogen as a carrier gas at 175 °C and relative pressure of 15 lbs/sq.

It can be seen from Table I that the lipid content of pigeon milk is very high (7.75% and 30.64% by wet wt. and dry wt., respectively). This appears to be in proportion to its high amount of proteins (54.06% by dry wt.)[7]. Phospholipids and neutral lipids amount to 43.49% and 56.54%, respectively. Amoung the phospholipids lecithin is the major component followed by spingomyelin and cephalin. The proportion of lysolecithin is about 1/3 of lecithin. Cholesterol forms about 35% of the neutral lipids. Other major neutral lipid components (Figure) are triglycerides and free fatty acids. The diglycerides have been resolved into 1,2 and 1,3 diglycerides. Monoglycerides are absent.

Table II shows the fatty acid composition of pigeon milk. Among the unsaturated fatty acids oleic acid (39,4%) and linoleic acid (12.6%) are in good proportion as palmitic acid (17.6%) and stearic acid (14.0%) are among the saturated ones. Compared to cow's and human



Thin layer chromatography of the neutral lipids of pigeon milk. Solvent system: Hexane, ether, glacial acetic acid (v/v 3.0:6.0:0.5). S, sample; ST, standard; MG, monoglyceride; 1,2-DG, 1,2-diglyceride; 1,3-DG, 1,3-diglyceride; CHOL, cholesterol; FFA, free fatty acids; TG, triglyceride; CHOL-ESTER, cholesterol ester.

Table I. The lipid composition of pigeon milk

1. Total lipids (g/100 g wet wt.)	7.75
2. Total lipids (g/100 g dry wt.)	30.64
3. Phospholipids (mg/100 mg lipids)	43.49
4. Neutral lipids (mg/100 mg lipids)	56.54
5. Cholesterol (mg/100 mg lipids)	20.92
6. Lysolecithin lipids (μmoles/100 mg)	12.61
7. Spingomyelin (μmoles/100 mg)	32.19
8. Lecithin (μmoles/100 mg)	37.09
9. Cephalin (μmoles/100 mg)	20.99

[1] D. S. Farner, in *Biology and Comparative Physiology of Birds* (Ed. A. J. Marshall; Academic Press, New York 1960), vol. 1, p. 411.
[2] L. L. Reed, B. L. Mendel and B. H. Vickary, Am. J. Physiol. *98*, 273 (1932).
[3] W. Dabrowska, Mém. Inst. natn. Polonais Econ. rurals Pulawij *13*, 276 (1932).
[4] D. M. Pase, A. M. Paul and E. M. Frank, Growth *16*, 273 (1952).
[5] S. N. Hegde, Indian Zool. *1*, 1 (1970).
[6] W. L. Davies, Biochem. J. *33*, 898 (1939).
[7] S. N. Hedge, Curr. Sci. *41*, 23 (1972).
[8] S. N. Hedge, Proc. Soc. biol. Chem. India *39*, 22 (1970).
[9] S. N. Hedge and B. Neelakantan, Indian Zool. *1*, 75 (1970).
[10] J. Folch, M. Lees and G. H. S. Stanby, J. biol. Chem. *226*, 497 (1957).
[11] G. R. Bartlett, J. biol. Chem. *234*, 466 (1959).
[12] G. V. Marinetti, J. Lipid Res. *3*, 1 (1962).
[13] T. C. Stadtman, *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan; Academic Press, New York 1957), vol. 3, p. 392.
[14] A. T. James, Meth. biochem. Analysis. *8*, 1 (1960).

milk[15], pigeon milk has a higher percent of unsaturated fatty acids, particularly the linoleic and linolenic acids which are known to be the essential fatty acids for tissue metabolism[16].

Though the biological[2] and chemical assays[17] of pigeon milk for vitamins A, B1, B2 and C have revealed a low concentration, it was reported, based on the growth attained by the squabs, that their amount is adequate[2]. Moreover, it may be mentioned in this context that chicks when orally fed with small amounts of pigeon milk (0.25 g/day/chick in addition to standard ration) showed a significant increase in growth compared to controls, the increased growth being continued for many weeks even after the feeding of pigeon milk was stopped[17]. In view of these observations, it is suggested that an unidentified growth-promoting factor(s) coupled with the dietary balance of essential fatty acids and amino acids[7] might contribute to the phenomenal rate of growth in pigeon squabs[18].

Zusammenfassung. Taubenmilch ist lipidhaltig und enthält zwei wesentliche Fettsäuren: Linol- und Linolensäure. Ein nicht identifizierter Faktor zusammen mit einer fett- und aminosäurehaltigen Diät ist imstande, auf Jungvögel wachstumsfördernd zu wirken.

S. N. HEGDE

Department of Zoology, Karnatak University, Dharwar-3 (Mysore State, India), 25 February 1972.

Table II. The fatty acid composition of pigeon milk

Fatty acid	Percentage
1. Unsaturated	
Palmitoleic acid	8.2
Oleic acid	39.4
Linoleic acid	12.6
Linolenic acid	5.0
2. Saturated	
Palmitic acid	17.6
Myristic acid	0.7
Stearic acid	14.8
Arachidic acid	1.7

[15] P. L. ALTMAN and D. S. DITTMER, *Metaboilsm* (Federation of American Societies for Experimental Biology, Maryland 1968), p. 5.
[16] B. L. OSER, *Hawk's Physiological Chemistry* (McGraw Hill, London 1965), p. 373.
[17] S. N. HEGDE, unpublished results.
[18] I am grateful to Professor C. J. GEORGE for initiating me to this study and guidance. Thanks are also due to Prof. J. GANGULY and Dr. P. S. SHASTRY of the Indian Institute of Science, Bangalore, for their help in the analysis of lipids. The work was financed by a U. G. C. Research Scholarship.

Induction of Brain Tumours in High Yield by Administration of N-Ethyl-N-Nitrosourea to Newborn Rats

Though it has long been possible to induce experimental brain tumours in animals using topical application[1] or direct implantation[2,3] of carcinogenic polycyclic hydrocarbons, tumours of the central and peripheral nervous systems can now be induced much more easily by systemic administration of carcinogens such as N-methyl-N-nitrosourea[4].

Transplacental induction of such tumours has been studied with the very effective and less toxic N-ethyl-N-nitrosourea (ENU) in single i.v. doses of 5–80 mg/kg administered to rats in late pregnancy[5,6]. Tumours of the central and peripheral nervous systems occurred in 193 of 222 offspring of the treated mothers, and it was estimated that the nervous system of the foetal rat is about 50 times more sensitive to ENU carcinogenesis than that of the adult animal.

ENU is also an effective carcinogen for the nervous system of the rat when administered as a single dose to the newborn animal[7,8] and we used this more convenient method in our pathological studies which will be reported in detail elsewhere[9]. We were particularly interested to observe that our experiments yielded an unexpectedly high proportion of brain tumours, indicating that this procedure will be of interest to workers requiring a source of such tumours for therapeutic trials or tumour transplantation experiments. Results with our first series of 34 rats are therefore described briefly here and compared with those of other workers.

Three litters of random-bred Wistar-derived albino rats and 2 litters of random-bred Lister hooded rats were injected s.c. 24 h after birth with ENU freshly dissolved in a little ethanol and made to volume with sterile physiological saline to give a dose of 10 mg/kg body weight. Numbers of animals surviving to weaning were 14/17 albino and 20/21 hooded. They were housed on sawdust in wire cages and maintained on Thompson cube diet 42 and water ad libitum.

When functional neurological disturbances were seen the animals were anaesthetized with ether and killed by per-aortic perfusion of formalin-acetic acid-methanol mixture (FAM) (1:1:8). The brain, spinal cord and main nerve trunks were dissected in toto and immersion fixed in FAM. The blocks were embedded in paraffin wax, sectioned, and stained for routine neurohistological examination[9].

The first animal was killed 197 days after treatment. The last, killed at 680 days, was the only one in which no tumour was found. A total of 71 neural tumours was found, of which 53 (74%) were situated in the brain (Table). The preferred site was in the cerebral hemisphe-

[1] A. M. SELIGMAN and M. J. SHEAR, Am. J. Cancer *37*, 364 (1939).
[2] A. WEIL, Arch. Path. *28*, 777 (1938).
[3] P. PAOLETTI and E. GROSSI-PAOLETTI, Archo ital. Patol. Clin. Tum. *7*, 166 (1964).
[4] H. DRUCKREY, S. IVANKOVIC and R. PREUSSMANN, Z. Krebsforsch. *66*, 389 (1965).
[5] H. DRUCKREY, S. IVANKOVIC and R. PREUSSMANN, Nature, Lond. *210*, 1378 (1966).
[6] S. IVANKOVIC and H. DRUCKREY, Z. Krebsforsch. *71*, 320 (1968).
[7] H. DRUCKREY, personal communication (1969).
[8] H. DRUCKREY, B. SCHAGEN and S. IVANKOVIC, Z. Krebsforsch. *74*, 141 (1970).
[9] E. L. JONES, C. E. SEARLE and W. T. SMITH, J. Path., in press.