Sex Difference in the Number of Adipose Cells from Genetically Obese Rats

It is well known that there is no increase in the number of adipose cells in many kinds of experimental obesity. In *genetically obese mice*, obob obese *hyperglycaemic*[1,2] or yellow obese[3] syndromes as well as in hypothalamic obesities produced by aurothioglucose in mice[4] or stereotaxic lesions in rats[5], there is only an enlargement of the adipose cells. The same results are obtained in rats made obese by a high fat diet[6]. There is only one *known* exception to this rule: obesity induced by a high fat diet in female mice[7], in which the number of cells is increased by a factor of 2.5. Because male animals were used in all experiments except the last, the question arises whether there may be a sex difference in the adipose tissue development in obese animals.

Genetically obese rats (fa/fa)[8] 2.5 months old and their litter mates, fed with my control diet, were used. Whole perigenital fat pads were weighed and treated as previously described[9]: portions of histological sections on slides, projected on the ground glass of a Projectina microscope, were counted for their adipose cells at a magnification of ×130. The mean volume was calculated and the number of adipose cells estimated by the ratio of fat pad weight to mean adipose cell volume ×0.91 (0.91 is the measured density of the pads).

Table 1 shows that the body weights of obese rats doubled in the females but increased less in the males. The weight of perigenital fat pads increased 3.2 and 9.7-fold in males and females respectively. Adipose cells enlarged 4.6 and 4.0-fold in obese males and females respectively. In male obese rats there were significantly fewer epididymal adipose cells than in their litter mates, but a 2.3-fold increase in their number was observed in the parametrial cells of the obese females.

Table 1 Weights of the Body and of the Two Perigenital Fat Pads of 2.5 Month Old Genetically Obese Male and Female Rats and Their Litter Mates

	Lean litter mates	Obese
Males		
Body weight (g)	276 ± 16.0	366 ± 17.3
Epididymal fat pad weight (g)	2.82 ± 0.260	9.00 ± 0.327
Adipose cell volume (× 10^3 μm^3)	276 ± 25.5	1,275 ± 85.2
No. of adipose cells (millions)	9.34 ± 0.458	6.50 ± 0.256
Females		
Body weight (g)	165 ± 2.4	320 ± 13.6
Parametrial fat pad weight (g)	1.64 ± 0.189	15.9 ± 0.71
Adipose cell volume (× 10^3 μm^3)	379 ± 46.6	1,536 ± 43.5
No. of adipose cells (millions)	4.08 ± 0.292	9.48 ± 0.460

The cell volume of the obese groups increased, but cell number increased only in the female group (2.3 times). All differences are highly significant ($P < 0.01$). Values are mean ± s.e. There were six animals in each of the four groups.

Hypertrophy of the adipose cells in the obese females could only account for a parametrial fat pad weight of 1.64 × 4.0 = 6.64 g—9.26 g less than the actual weight. And so about 70% of the weight of the pads was due to formation of new fat cells which were themselves hypertrophied.

These results clearly established a sex difference in the development of perigenital adipose tissue. They are in good agreement with the literature which records no increase in fat cell number in male obese rats or mice[1-6]. But there was a marked hyperplasia in the parametrial adipose cells in the females which was very similar to those observed in female mice made obese by a high fat diet[7].

Exceptions to the rule that only hypertrophy is observed in male adult animals had been claimed: there is hyperplasia of adipose cells in rats after repeated injections of insulin[9,10] or meal feeds[11]. In these animals there was no increase in the weight of the epididymal fat pad, and adipose cell volume seemed to be reduced; the difference, however, were very small. The principal effect, in the two cases, was a great increase in the total DNA content of the pads (+50% for meal fed rats[11] and +100% for rats treated with insulin[10]). Such differences cannot be the effect of a marked hyperplasia: the weight of fat pads was unchanged and cell volume very slightly smaller; so it can be concluded that there was an increased DNA content in stromal structures. Such an effect has been established in hypophysectomized rats after injections of somatotrophic hormone: the rate of DNA synthesis of supporting and vascular structures was enhanced, but there was no effect on division in adipose cells[12]. In my rats, the histological appearance of perigenital adipose tissue was the same as that described for rats made obese by a high fat diet[6]. There was no difference, except for the size of the cells, between fat tissues of control and obese rats, although there was a marked and similar hyperinsulinism in males as in females in the obese group (to be published later).

I thank A. Alexis for technical assistance.

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Received December 15, 1970.

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Polychlorinated Biphenyl absorbed from Sediments by Fiddler Crabs and Pink Shrimp

POLYCHLORINATED biphenyls (PCBs) are manufactured in the United States, Europe, and Japan and used as plasticisers, flame retardants, insulating and heat exchange fluids and in many other products[1]. Structurally related to DDT, soluble in lipid but relatively insoluble in water, and extremely persistent in the environment[2], they have been reported in many marine and estuarine organisms[3]. One PCB, 'Aroclor 1254' (Monsanto), was reported in water, sediments, and biota from Escambia Bay, Florida[4].

In August 1969, pink, white and brown shrimps (*Penaeus duorarum*, *P. setiferus*, and *P. aztecus*) from Escambia Bay were found to contain whole body residues of 'Aroclor 1254' at concentrations as high as 14.0 p.p.m. Most was concentrated in the hepatopancreas; residues in seven composite samples of at least five shrimps ranged from 0.6 to 120.0 p.p.m. In April 1970, fiddler crabs (*Uca minax*) collected at three stations along the lower Escambia River and upper Escambia Bay had individual whole body residues of 0.45 to 1.5 p.p.m.

To determine the distribution of 'Aroclor 1254' in the estuary, forty samples of water were collected in glass bottles; twenty sediments were collected with a core or dredge sampler, and many estuarine organisms were captured by trawl and dredge. These were analysed by gas liquid chromatography[4]. The largest accumulations of 'Aroclor' were found in the sediments. In one survey (February 1970), concentrations ranged from 0.6 to 61.0 p.p.m. in a sample taken at the discharge point of the industrial plant that accidentally released the chemical and at eleven stations extending 16 km downstream (Fig. 1). 'Aroclor' was not detected in sediments collected above the plant, but soil samples from the bank near the mouth of the river 6.5 km downstream from the source had 1.4 to 1.7 p.p.m. of this chemical. Subsequent samplings from these stations in the Bay showed little change in the amounts of the chemical even after nine months. We therefore investigated whether shrimp and fiddler crabs could accumulate 'Aroclor 1254' from the sediments.

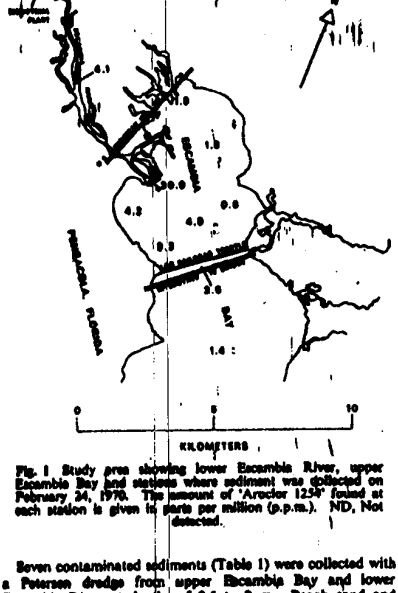

Fig. 1 Study area showing lower Escambia River, upper Escambia Bay and stations where sediment was collected on February 24, 1970. The amount of 'Aroclor 1254' found at each station is given in parts per million (p.p.m.). ND, Not detected.

Seven contaminated sediments (Table 1) were collected with a Petersen dredge from upper Escambia Bay and lower Escambia River at depths of 0.6 to 9 m. Beach sand and additional samples of sediment collected upstream from the contaminated section of the river served as uncontaminated controls. Four kilograms of each of the sediments and ten adult pink shrimp (*P. duorarum*) from a population which had previously shown no contamination with 'Aroclor' were placed in each of nine aquaria. Water at 17° C flowing at a rate of 12 l./h, with an average salinity of 27 parts per thousand (p.p.t.), covered the substratum to a depth of 10 cm.

Fiddler crabs (*U. longisetor*) were exposed to 'Aroclor' by placing them on five sediment substrates collected as described. Four kilograms of each of the sediments were placed in the upper end of five aquaria inclined 10 degrees so as to simulate a natural environment. Ten crabs from a population which had no contamination were placed in each aquarium with one serving as a control. Water with an average salinity of 27 p.p.t. at 13° C flowed at 3 l./h through the lower end. Both shrimp and crabs were fed fish containing < 0.03 p.p.m. 'Aroclor 1254' each day for 30 days. Five crabs were removed from each aquarium, killed and rinsed with a 50% mixture of acetone and water to remove 'Aroclor 1254' from material adhering to the exoskeleton. To ensure enough tissue for analyses, individual crabs and pooled samples of hepatopancreas from shrimp were mixed with anhydrous Na_2SO_4 and extracted with petroleum ether in a Soxhlet apparatus; the extract was purified with 'Florisil'[5]. 'Aroclor 1254' was quantitated by gas liquid chromatography[4]. The ratio of individual 'Aroclor' isomers maintained integrity in the sediments and tissues of test animals throughout the investigation.

Table 1 'Aroclor 1254' in Individual Fiddler Crabs and Hepatopancreas from Pink Shrimp

Sediment	'Aroclor 1254' in sediment (p.p.m. dry weight)	Average and range of 'Aroclor 1254' in five individual fiddler crabs (p.p.m. wet weight)	'Aroclor 1254' in combined hepatopancreas from four or more shrimp (p.p.m. wet weight)
Sandy silt *	61.0	80.0 ± 25.0	240.0
Silt †	30.0	17.0 ± 9.0	6.1
Sandy silt *	5.7	—	9.8
Silt †	4.9	3.6 ± 0.9	1.3
Sandy silt *	4.1	—	6.7
Silt †	2.5	3.2 ± 0.9	1.1
Silt †	1.4	—	0.2
Sand control *	ND	0.3 ± 0.2	< 0.2
Silt control †	ND	—	< 0.2

The samples of crab and shrimp were kept for 30 days on naturally occurring sediments containing 'Aroclor'. Mortalities occurred in some groups of shrimp which could not be attributed to 'Aroclor 1254'.

* Sediment which contained particles of sand > 354 μm in diameter.

† Sediment which contained particles of sand 354 μm or less.

—, No sample.

ND, Not detectable.

Fiddler crabs and shrimp exposed to the contaminated sediments accumulated 'Aroclor 1254' in their tissues by ingesting contaminated sediment particles or by absorbing the leached chemical from water. They sifted the sediments before ingesting the particles. In most cases, the amount of 'Aroclor' in individual crabs or in shrimp hepatopancreas was directly related to the amount in the sediments. We analysed the distribution of the chemical in the sediment in relation to particle size and found it was evenly distributed. Large concentrations of 'Aroclor' residues may also accumulate in the hepatopancreas of shrimp exposed to sandy silts because the chemical leaches from the sediments and is absorbed through the gills. Three and a half parts per billion (p.p.b.) were in the effluent water from the aquarium with sandy silt (61.0 p.p.m. 'Aroclor'); the water from the silt (30.0 p.p.m.) had only 0.5 p.p.b. Even though fiddler crabs were not continuously covered with water, they could have obtained 'Aroclor' from the water as they wetted their gills.

We have demonstrated experimentally that 'Aroclor 1254' can enter the estuarine food chain from sediments. Others[6] have identified this chemical in higher trophic life such as fish, and since PCBs are widespread in the world[7], we should

become more alert to the presence, movement and effects of these chemicals in the environment.

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Received September 17; revised December 10, 1970.

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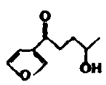
A Lung Oedema Factor from Mouldy Sweet Potatoes (*Ipomoea batatas*)

We have already described the toxic properties of sweet potato tubers infected with *common mould*[1,2]. The toxic properties are not directly attributable to the fungi because poisons were not found when the organisms were grown in autoclaved sweet potato slurry. The experimental infection of viable sweet potato slices or of the intact tubers nevertheless resulted in the production of many furanoterpenoid compounds, some of which are markedly toxic for laboratory and commercial animals.

As well as the previously recognized hepatotoxins ipomeamarone [(+)-ngaione][3] and ipomeamaronol[2], we have also isolated a more potent toxin called lung oedema factor (LO) from ether and chloroform extracts of both artificially and naturally contaminated sweet potatoes[4], which recalls that natural outbreaks of mouldy sweet potato poisoning in cattle all describe respiratory distress related to lung oedema as the principal sign of disease[4,5]. On the other hand, hepatotoxicity which might be attributable to ipomeamarone or ipomeamaronol has not been reported in most enzootics.

Fresh tuber slices inoculated with sweet potato slurry-shake cultures of *Fusarium javanicum* and incubated for approximately 6 days at 22°–24° C contained significant quantities of all three toxins. We partially purified these metabolites by partitioning crude ether extracts of infected sweet potatoes on silica gel columns using elution with a hexane-ethyl acetate gradient. The column eluates were monitored by thin-layer chromatography (*TLC*) (*silica gel*; 9 : 1 benzene-methanol). Ehrlich's reagent produced twelve to fifteen coloured spots representing furanoterpenoids on TLC strips developed from the crude ether extract. Preliminary toxicity assays *in mice* showed that the LO factor was associated with a reddish-purple spot at $R_f = 0.4$ which contained numerous compounds by gas chromatographic analysis.

Column eluate enriched with LO, consisting chiefly of the reddish-purple TLC spot material, was purified further by preparative gas-liquid chromatography (GC) of trimethylsilylated (TMS) product. A single GC peak corresponding to the TMS ether of lung toxic material was obtained. We removed the TMS group by acid hydrolysis to give pure toxin, a colourless oil with a pungent odour. Spectral evidence (Table 1) indicates that its structure is 1-(3′-furyl)-4-hydroxy-1-pentanone.



We propose the trivial name, 4-ipomeanol, for this compound which is a dihydro derivative of ipomeanine, 1-(3′-furyl)-1,4-pentanedione, another known metabolite of sweet potatoes[6]. There are no reports indicating toxicity for ipomeanine.

The empirical formula of 4-ipomeanol, $C_9H_{12}O_3$ (molecular weight 168), was deduced from the mass spectrum by exact mass measurement of the P-H_2O ion. (We measured a value of 150.070 ± 0.002; $C_9H_{10}O_2$ requires a value of 150.068.) The β-furylketo moiety was indicated by the characteristic nuclear magnetic resonance (NMR) signals for the furyl protons[7], by the stretching frequency of the conjugated carbonyl group, by the ultraviolet spectrum[8] and by the intense *m/e* 95 ion (β-C_4H_3O-CO$^+$). The third oxygen function is a hydroxyl group (ν 3,390 cm^{-1}). Double irradiation NMR showed that the hydroxyl group was embodied in a methyl carbinol. The chemical shifts of the remaining two methylene groups were consistent with the unbranched form of the structure.

Table 1 Spectra of 4-Ipomeanol

Infrared, cm^{-1} (neat)	Ultraviolet nm (cyclohexane)	NMR, δ p.p.m. (CCl_4)	MS, *m/e* (%)
3,480 (OH)	243 (ε 3,000)	1.15 (3H, d, J = 6Hz, 3-CH_3)	168 (5) Molecular ion
3,110 (furyl)	211 (ε 5,100)	1.67 (2H, m, 3-CH_2)	153 (2) M-CH_3
2,940		2.50 (1H, broad s, OH)	150 (6) M-H_2O
2,900		2.83 (2H, t, J = 7Hz, 2-CH_2)	124 (5) M-CH_2-HCO
1,675 (conj. C=O)		3.73 (1H, m, 4-CH)	121 (2) M-H_2O-HCO
1,560 (furyl)		6.75 (1H, m, 4′-CH)	110 (47) OH furyl-C-CH_2
1,510 (furyl)		7.42 (1H, m, 5′-CH)	95 (100) furyl-CO
1,160 880 (furyl)		8.07 (1H, m, 2′-CH)	

4-Ipomeanol is acutely toxic to mice, producing the lung oedema and pleural effusion previously observed with crude sweet potato extracts. Doses of toxin (1–6 mg) given intraperitoneally to mice produced immediate signs of general illness, followed quickly by dyspnoea which gradually increased in severity to the time of death, apparently from anoxia, in 5 to 8 h. Comparable quantities given orally were usually followed by a short period of apparent latency (2 to 3 h) followed by increasing respiratory distress leading to death within 24 h.

At post-mortem examinations, clear fluid, sometimes as much as 1 ml. or more, dripped from the nostrils when the animal carcass was held with its head downwards. The lungs were frequently congested and surrounded by 1–2 ml. of clear pleural fluid. Microscopic sections of the lungs stained with haematoxylin and eosin showed intra-alveolar oedema and congested blood vessels. In some animals there was a slight increase in abdominal fluid but we observed no significant pathological changes in abdominal viscera.

We have also isolated an isomer of 4-ipomeanol, tentatively assigned the structure 5-(3′-furyl)-5-hydroxy-2-pentanone, which is under investigation.

Sweet potatoes offered for sale in food stores often contain ipomeamarone, ipomeamaronol and 4-ipomeanol[9]. The exact