Table 2. Summated oxygen consumption in vitro of carp tissues

Tissue[a]	Q_{O2}	Body weight 114 g		136 g		138 g	
	($\mu l \cdot min^{-1} \cdot g^{-1}$)	Weight[a] (g)	O_2 cons.[b] ($\mu l \cdot min^{-1}$)	Weight[a] (g)	O_2 cons.[b] ($\mu l \cdot min^{-1}$)	Weight[a] (g)	O_2 cons.[b] ($\mu l \cdot min^{-1}$)
Trunk[c]	1.10[d]	59.173	65.09	69.680	76.65	72.029	79.23
Head[e]	1.83[f]	27.765	50.81	38.385	70.24	36.961	67.64
Intestine	5.35	2.654	14.20	2.824	15.11	2.501	13.38
Hepatopancreas	4.22	2.358	9.95	3.030	12.79	2.615	11.04
Gonads	2.17[g]	1.750	3.80	2.000	4.34	0.843	1.83
Gill filaments	3.10	1.624	5.03	1.880	5.83	2.096	6.50
Kidney	6.91	0.735	5.08	0.701	4.84	0.723	5.00
Brain	7.60	0.396	3.01	0.405	3.08	0.443	3.37
Ventricle	3.33	0.088	0.29	0.150	0.50	0.133	0.44
Summation		96.543	157.26	119.055	193.38	118.344	188.43
Estimated total O_2 consumption[h] ($\mu l \cdot min^{-1} \cdot g^{-1}$)		1.63		1.62		1.59	

[a] Organ of region. [b] Q_{O_2} of a tissue multiplied by the tissue weight. [c] Trunk region except fins and viscera. [d] Total Q_{O_2} of trunk calculated from Q_{O_2} ($\mu l \cdot min^{-1} \cdot g^{-1}$) and weight proportion (%) of 5 main components, ordinary muscle (1.04, 81.09), skin (1.3, 7.03), scales (0.70, 6.86), bones (1.08, 3.20), and dark muscle (4.26, 1.82). [e] Head region except brain, gill filaments and heart. [f] Q_{O2} of head in small carp of about 2 g. [g] Mean value of Q_{O_2} of testis and ovary. [h] Summated O_2 consumption divided by summated weight.

The estimated oxygen consumption in vitro, 1.63, 1.62 and 1.59, gave the mean value of 1.61 $\mu l \cdot min^{-1} \cdot g$ (wet wt)$^{-1}$ which approximately coincided with oxygen consumption in vivo of the intact animal, 1.67, although 14% of the body were counted out and functional activity of organs was neglected in the former (table 2).

The decline in weight-specific rate of basal metabolism of an intact animal with increasing body size could be explained, partly at least, if tissues with low metabolic rates get larger with growth in weight in proportion to the whole body. This hypothesis is now being examined, based on the present results and figures for the relative growth of tissues and organs.

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0014-4754/83/020160-02$1.50 + 0.20/0

Effect of starvation on enzymes related to lipid metabolism in guinea-pig lungs

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Summary. The changes in activities of acetyl CoA carboxylase, microsomal fatty acid elongation enzyme, choline phosphotransferase, triglyceride lipase, phospholipase A_1 and phospholipase A_2 were followed in guinea-pig lungs at 24, 48 and 72 h after food deprivation. Triglyceride lipase was elevated and phospholipase A_1 and phospholipase A_2 were unaffected, while the other activities decreased. The significance of these findings in relation to food deprivation is discussed.

There is evidence suggesting that an increase or decrease in lipid intake affects the lung lipid content and the activity of lung lining material[2,3]. Faridy[4] has shown that food deprivation resulted in an increased tendency of the lungs to collapse, associated with a lowered content of phosphatidylcholine, the major surface active lipid. Scholtz and Rhodes[5] found a 40% decrease in lung lipid synthesis from glucose in fasted rats. Gross and co-workers[6] have reported changes in various phospholipids in developing lung due to nutritional deprivation. Gross et al.[7] also reported changes in a few enzymes of lipid metabolism after 4 days of fasting in albino rats. However, a systematic and detailed study of the effect of starvation on enzymes related to lung lipid metabolism has not been reported[8]. Therefore, changes in acetyl CoA carboxylase, microsomal fatty acid elongation enzyme, choline phosphotransferase, triglyceride lipase, phospholipase A_1 and phospholipase A_2 have been studied in guinea-pig lungs after 24, 48 and 72 h of fasting. The findings are reported below. We chose to use guinea-pigs, because they do not synthesize ascorbic acid as rats do.

Materials and methods. 24 male guinea-pigs weighing 300–350 g (14 weeks old) from the Small Laboratory Animal Division, Indian Veterinary Research Institute, Izatnager were selected for study. 12 guinea-pigs were kept fasting. Remaining 12 guinea-pigs were provided with food ad libitum. After 24, 48 and 72 h of fasting, 4 animals from each group were sacrificed by exsanguination, and their lungs taken out, washed well in normal saline and immediately chilled in 0.01 M Tris Hcl pH 7.4 containing 0.001 M EDTA and 0.25 M sucrose. The lungs were then homogenized, filtered through muslin and fractionated by differential centrifugation as described by Gross and War-

Activities of enzymes related to lipid metabolism of control and fasted guinea-pig lung

	Group	Hours after fasting 24	48	72
Acetyl CoA carboxylase[a]	Control	1.40 ± 0.20	1.30 ± 0.20	1.48 ± 0.18
	Experimental	0.93 ± 0.12	0.74 ± 0.03	0.61 ± 0.07
Microsomal elongation enzyme[b]	Control	73.90 ± 0.23	70.73 ± 2.35	75.39 ± 3.22
	Experimental	60.27 ± 3.23	50.21 ± 9.27	30.23 ± 1.39
Choline phosphotransferase[c]	Control	120.93 ± 2.74	110.27 ± 8.73	109.33 ± 2.95
	Experimental	100.37 ± 9.45	92.75 ± 3.35	67.35 ± 3.97
Triglyceride lipase[d]	Control	231.79 ± 7.23	223.00 ± 13.95	226.23 ± 9.54
	Experimental	250.30 ± 3.69	301.00 ± 15.23	390.76 ± 7.23
Phospholipase A_1[e]	Control	3.39 ± 0.19	3.53 ± 0.29	3.47 ± 0.11
	Experimental	3.43 ± 0.32	3.69 ± 0.19	3.70 ± 0.21
Phospholipase A_2[f]	Control	2.67 ± 0.31	2.53 ± 0.32	2.59 ± 9.20
	Experimental	2.70 ± 0.15	2.66 ± 0.31	2.76 ± 0.83

All values are mean ± SE (4 animals).
Units: [a]nmoles of [^{14}C]-sodium bicarbonate incorporation into malonyl CoA/min/mg protein; [b]pmoles of [2-^{14}C]-malonyl CoA incorporation/min/mg protein; [c]pmoles of CDP [methyl-^{14}C]-choline incorporation into lipids/min/mg protein; [d]nmoles of fatty acids produced/min/mg protein; [e]nmoles of [^{14}C]-fatty acid produced/min/mg protein; [f]nmoles of lecithin utilized/min/mg/protein.

shaw[9]. The filtered homogenate, 100,000 × g supernatant and microsomal fractions were retained for enzyme assays.

Acetyl CoA carboxylase was estimated in the supernatant fraction obtained after centrifugation at 100,000 × g and partial purification by passing through Sephadex G-25 gel, by measuring the rate of [^{14}C]-sodium bicarbonate incorporation into malonyl CoA by the method of Greenspan and Lowenstein[10]. For partial purification of acetyl CoA carboxylase, 5 ml of 100,000 × g supernatant was passed through a column of Sephadex G-25 (gel bed 2.5 cm in diameter and 30 cm in height) which had been equilibrated with 20 mM Tris-Hcl buffer, pH 7.5, and 1 mM dithiothreitol. Elution was carried out with the same buffer mixture. The recovery of acetyl CoA carboxylase activity was 75–80%. Microsomal fatty acid elongation enzymic activity was assayed in microsomal fraction (recovery 85%) by measuring the rate of incorporation of [2-^{14}C] malonyl CoA into pentane extractable fatty acid as described by Gross and Warshaw[9]. Choline phosphotransferase was assayed in the filtered cell free homogenate by measuring the rate of incorporation of CDP [methyl-^{14}C]-choline into lipids essentially by the method of Mudd et al[11].

In another similar set of experiment, the lungs were homogenized in chilled 0.25 M sucrose, filtered through muslin and the filtered homogenate analyzed for triglyceride lipase, phospholipase A_1 and phospholipase A_2 activities by the methods of Schmidt et al.[12], Shimon and yechezkel[13] and Magee and Uthe[14] respectively. Protein content was estimated by the method of Lowry et al.[15].

Results. A continuous decrease was found in acetyl CoA carboxylase activity of lung tissue as a result of starvation. The values were 34, 43 and 59% lower (all $p < 0.01$) at 24, 48 and 72 h after fasting respectively. Similarly, microsomal fatty acid elongation enzyme activity was found to be decreased at all the stages of the study, the decrease was more prominent at 48 and 72 h of fasting when the activity decreased by 29 and 60% ($p < 0.01$) respectively. Choline phosphotransferase, the enzyme involved in phospholipid biosynthesis was found to be significantly reduced at 72 h after food deprivation. The value was 38% lower ($p < 0.01$) than that of the control. Phospholipase A_1 and phospholipase A_2 activities underwent no change due to food deprivation. The activity of triglyceride lipase showed a continuous increase of 8, 35 and 73% ($p < 0.1$, < 0.01, < 0.01) at 24, 48 and 72 h after fasting respectively. Protein content in whole homogenate, 100,000 × g supernatant and microsomal fraction did not show any significant changes.

Discussion. De novo fatty acid synthesis, which is the more active pathway for fatty acid synthesis in the lung, occurs in a 2-step reaction. The 1st step is catalyzed by acetyl CoA carboxylase[16]. The end product of this reaction is palmitic acid[9], the fatty acid component of dipalmitoyl phosphatidyl choline, which is a major surface active phospholipid. As is shown in the table, food deprivation resulted in a significant decrease in acetyl CoA carboxylase activity, indicating a decrease in palmitic acid biosynthesis.

Another pathway of fatty acid synthesis is by chain elongation of pre-existing fatty acids. By this mechanism long-chain fatty acids may be synthesized from shorter-chain precursors. As shown in the table, there are significant decreases at 48 and 72 h after fasting in microsomal fatty acid elongation enzymic activity; the fatty acid elongation is inhibited drastically due to starvation.

Significant decrease observed in choline phosphotransferase activity in fasted guinea-pig lungs indicates decreased biosynthesis of phosphatidyl choline, the level of which goes down as a result of food deprivation, as reported by Faridy[4] and Jaiswal[17]. An increase in triglyceride lipase activity in the lungs of guinea-pigs deprived of food shows the release of fatty acids from triglyceride to meet with the energy and metabolic requirements. The fact that there was no change in phospholipase A_1 and A_2 activities indicates that the fatty acids are not released from the phospholipids, and the decrease in phospholipids observed in the fasted guinea-pig lungs[17] is not due to breakdown of phospholipids but is due to decreased biosynthesis of phospholipids as shown earlier.

Our data support the earlier findings of Gross et al.[7], and establishes the enzymatic basis for the decreased pulmonary phosphatidyl choline and fatty acid synthesis in addition to triglyceride breakdown in guinea-pig lungs associated with food deprivation.

1 Acknowledgments. Thanks are due to Drs L.N. Singh and C.M. Singh for their valuable suggestion and to Sri Ramesh Pal Singh for his assistance.
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0014-4754/83/020161-03$1.50+0.20/0

About the photostimulation of ATP production in the integument of insects

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Summary. The photostimulation of ATP production in the integument of *Pieris brassicae* has been reexamined, using 2 different methods for ATP estimation. As could be demonstrated with the luciferin-luciferase assay, the so-called photostimulation is an artefact which is due to inappropriate utilization of the Boehringer kit assay.

Vuillaume et al.[2,3] described a photostimulated synthesis of ATP in integument homogenates and in cell-free extracts of the same tegument in the caterpillar of *Pieris brassicae*.

Because of the great potential importance of the newly described system, which would give to the insects a very unusual and surprising position, near to all the chlorophyll-containing plants or bacteria and certain microorganisms like *Halobacterium halobium*[4] which solar energy for phosphorylation, we started a program aimed at the identification of the reactive molecule (or molecules) involved. To increase the sensitivity and specificity of ATP estimation, we adopted the luciferin-luciferase assay[5].

Only very small amounts of ATP were found. None of the qualitative and quantitative results described in the published papers could be reproduced. To understand the origin of such a discrepancy, we felt is necessary to consider again the method used by Vuillaume et al.[2,3] for measuring ATP. In their work, they use an assay kit based on the decrease of NADH, which might be nonspecific in a complex medium.

In fact, depending of the method used, 2 opposite results are obtained. As already described, using the Boehringer kit for ATP assay, an important decrease in NADH is observed with extracts of integuments from *Pieris brassicae*, which can be enhanced under illumination. It is this decrease in NADH which is translated into ATP. But, with the specific luciferin-luciferase assay, only a very low amount of ATP is found and it is rapidly degraded, whether under illumination or in darkness.

Materials and methods. The 5th instar larvae of *Pieris brassicae* were taken under the same conditions as described by previous authors[2,3]: at the end of the instar (mean fresh weight 500 mg), bred from hatching in white light, photoperiod 16/8 h, temperature 20 °C. The homogenate of the integument was prepared as described in darkness with a mechanical Potter homogenizer and the same cold buffer pH 7.2 (0.05 M Tris-HCl, 0.25 M sucrose, 2.5 mM $CaCl_2$). Examination of the extracted integuments under a binocular microscope showed total epidermis extraction.

The Boehringer kit for ATP assay (B.M. assay) from Boehringer-Mannheim based on the following sequence of reactions:

$$\text{glycerate-3-P} + \text{ATP} \xrightarrow{\text{PGK}} \text{glycerate-1,3-P}_2 + \text{ADP}$$

$$\text{glycerate-1,3-P}_2 + \text{NADH} + \text{H}^+ \xrightarrow{\text{GAPDH}} \text{GAP} + \text{P}_i + \text{NAD}^+$$

$$\text{GAP} \xrightarrow{\text{TIM}} \text{DAP}$$

$$\text{DAP} + \text{NADH} + \text{H}^+ \xrightarrow{\text{GDH}} \text{glycerol-1-P} + \text{NAD}^+$$

Abbreviations used: PGK, phosphoglycerate kinase; GAPDH, glyceraldehyde phosphate dehydrogenase; TIM, triose phosphate isomerase; GDH, glycerol phosphate dehydrogenase.

Luciferin-luciferase, Firefly lantern extract (L.L. assay) and ATP were from Sigma. All other chemicals were of the purest quality available from Prolabo or Merck.

Incubation of the extracts under light was as described by previous authors[2,3].

Results. One example of quantitative determinations by both types of assay and different treatments is presented in the table. With the luciferin-luciferase assay, ATP concentration is about 100–1000 times smaller than with the Boehringer assay, and there is no increase after incubation. If a known amount of ATP is added, it is recovered by either of the methods. After 1-h incubation most of this added ATP is hydrolyzed: about 70% with the Boehringer

Amount of ATP detected by 2 different assays

Boehringer assay	ATP mg/g	Luciferin-luciferase assay	ATP µg/g
T=0	0.25	T=0	17.8
T=0+ATP	0.71	T=0+ATP	64.7
T=1 L	1.02	T=1 L	1.1
T=1 L+ATP	1.15	T=1 D	9.7
T=2 L	1.41		

The results are the average of duplicate assays and are expressed in mg or µg of ATP in the total extract obtained from 1 g of original wet integument. T=0, ATP measured without incubation, T=1 L and T=2 L, after incubation, respectively for 1 h or 2 h under illumination; T=1 D after incubation in darkness. All incubations were performed at 20 °C. For controls, ATP was added: 0.5 mg/g for the B.M. assay und 50 µg for the L.L. assay. The caterpillars were bred under white light, with photoperiod 16 h illumination, 8 h darkness during their larval life.