assay; with the luciferin-luciferase assay more than 90% of the added ATP is hydrolyzed within 1 h. If the samples are incubated after perchloric acid deproteinization, this ATP is stable and is measured in both types of assay (results not shown).

Then, instead of the awaited increase, there is an absolute decrease in ATP concentration even with the Boehringer assay, since only 1.15 mg/ATP was found where 1.52 (1.02 + 0.50) was expected.

Very similar results were obtained with larvae reared under red light or in darkness in the same described conditions[2,3]. Between 3 independent sets of experiments, ATP concentration in fresh integument extracts varied considerably (up to 100%), but within each series of incubations, the results are comparable to those of the table.

Discussion. Comparing the data obtained with the specific luciferin-luciferase assay with those based on an extensive sequence of reactions, it is obvious that the 'light-dependent ATP production system (LAPS)' in *Pieris brassicae*[2,3] is an artefact due to the inappropriate utilization of the Boehringer kit, prepared for ATP estimation in human blood. With the luciferin-luciferase assay, there is no measurable LAPS. Not only is ATP not synthesized under illumination, but instead, upon incubation it is used up, probably hydrolyzed. To explain the high ATP concentration found by Vuillaume et al.[2,3], one must suppose that, in the integument extracts, there must be some molecule which is estimated as ATP (like other nucleosides triphosphate) or more likely is involved in some reaction, induced by the kit enzymes, which utilizes NADH as a cosubstrate. The amount of this molecule is changed under illumination. The published values for ATP concentration in integument extracts were rather high, one of the highest concentration known for ATP reaching 4–5 mg/g integument, when rat or rabbit muscle[6] has only about 2 mg/g and *E. coli*[7] about 2 mg/g dry wt.

We must also remark that the so-called ATP synthesis was, for these authors, related to the presence of the pigment Pterobiline in the epidermis where it would act as a photoreceptor, absorbing in the red part of the spectrum and therefore preventing diapause[8-11]. Consequently, all explanations in which diapause is related to pterobiline and LAPS must be viewed with caution.

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Effects of cysteine and N-acetyl cysteine on GSH content of brain of adult rats

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Summary. Intraperitoneal injections of cysteine or N-acetyl cysteine induce a depletion of reduced glutathione (GSH) in rat brain. The doses required to promote GSH depletion are lower than those reported to cause a disseminate neurodegenerative syndrome. Since physiological GSH concentrations are required to maintain cell membranes, we suggest that consideration of the cysteine-induced GSH depletion is important in attempts to understand the mechanism of cysteine-induced cytotoxicity in brain.

We reported earlier[1,2] that cysteine induces a depletion of reduced glutathione (GSH) in isolated hepatocytes and that i.p. injections of cysteine, or its derivative N-acetyl cysteine, also promote a depletion of hepatic GSH[3]. Olney and Ho[4] have reported that very high doses of cysteine result in the death of infant rats. However, sublethal doses of cysteine induce a widely disseminated neurodegenerative syndrome[5]. Here we report that cysteine induces a depletion of GSH in the brain of adult rats.

Materials and methods. L-Cysteine or N-acetyl cysteine were injected i.p. to adult Wistar rats fed with a standard laboratory chow (from Prasa, Vara de Quart, Valencia, Spain).

Solutions of the amino acids were made in water immediately before use and care was taken that the pH was 7.0 ± 0.1. The animals were killed 2 h after the injection, by decapitation. The brain was immediately removed, weighed and homogenized in 10 vol. of a solution of 2% perchloric acid in ice-cold physiological saline. The time that elapsed between killing the rat and obtaining the acid homogenate was always less than 2 min.

GSH was determined under the conditions described by Viña et al.[1]. All values are means ± SD for the number of observations in parentheses.

The concentration of GSH in the brain of untreated fed rats was 3.25 ± 0.38 μmoles/g fresh weight.

Results and discussion. The effect of various doses of cysteine on GSH concentration in rat brain is shown in table 1. It is important to notice that injections of 0.25 g/kg b.wt, of cysteine are sufficient to induce a depletion of GSH to values of 60% of the controls. Morphological alterations were observed when the doses of cysteine used were 0.8 g/kg[5]. However, when N-acetyl cysteine was used, a higher dose (1 g/kg) was needed to induce a depletion of GSH to values of 60% of controls (table 2). N-acetyl cysteine is rapidly deacetylated in liver to yield free cysteine[6] which may, in turn, induce a depletion of GSH in brain.

Table 1. Effect of injection of L-cysteine on concentration of reduced glutathione (GSH) in rat brain

Dose of cysteine (g/kg b.wt)	GSH concentration (μmole/g fresh wt)	% of the controls
0.00	3.25 ± 0.38 (5)	100
0.25	2.04 ± 0.16 (3)*	63
0.50	1.95 ± 0.20 (3)*	60
1.00	1.90 ± 0.05 (3)*	58

Rats were injected (i.p.) with a 1 M solution of cysteine, and killed 2 h after injection. Results are means ± SD for the number of observations in parenthesis. * $p < 0.005$.

Table 2. Effect of injection of N-acetyl cysteine on concentration of reduced glutathione (GSH) in rat brain

Dose of N-acetyl cysteine (g/kg b.wt)	GSH concentration (μmole/g fresh wt)	% of the controls
0.00	3.25 ± 0.38 (5)	100
0.25	2.69 ± 0.18 (3)*	83
0.50	2.49 ± 0.29 (3)*	77
1.00	1.95 ± 0.15 (3)**	60

Rats were injected (i.p.) with a 1 M solution of N-acetyl cysteine, and killed 2 h after injection. Results are means ± SD for the number of observations in parentheses. * $p < 0.05$; ** $p < 0.005$.

It is well established that normal GSH concentrations are important to maintain plasma membrane integrity[7].

Mitchell et al.[8] showed that the liver necrosis induced by paracetamol (acetaminophen) overdose is due to GSH depletion. It is known that cysteine may be spontaneously oxidized to give cysteine disulphide and H_2O_2[9]. We have postulated[2] that the cysteine-induced GSH depletion in hepatocytes is due to formation of H_2O_2 with subsequent damage to cell membranes.

Olney et al.[5] suggested that a possible mechanism of cysteine induced cytotoxicity could be related to the conversion of L-cysteine to more acidic derivatives. However, the oxidation of cysteine to yield cystine and H_2O_2 is a much more rapid reaction, and hydrogen peroxide is known to be a very toxic substance, which could account for the widespread lesions observed after injection of cysteine, in contrast with the more localized lesions observed when rats are treated with acidic amino acids, i.e. glutamate. The cysteine derivative N-acetyl cysteine is commonly used as a mucolytic agent[10]. Thus, the fact that high doses of N-acetyl cysteine can also promote GSH depletion in brain should be considered.

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Fluorescence of free bilirubin at room temperature

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Summary. Very weak fluorescence from free bilirubin in liquid solvents at room temperature was clearly recorded. The emission band is peaked at about 540–550 nm and its width is about 75 nm.

Bilirubin (BR) is known to fluorescence at low temperature when in glassy solvents[1-3], and at room temperature when bound to albumins[4,5] and to micellar systems[3]. However, when free bilirubin is in liquid solvents at room temperature, fluorescence is uncertain and controversial[3-5]. Under these conditions emission quantum yields are, in fact, very low ($\leqslant 10^{-4}$) and conventional spectrofluorometers can generally give only ambiguous results. In order to obtain more precise results we used a laser-excited fluorometer with double monochromator and photon-counting electronics. Freshly prepared solutions of purified BR in N-N′-dimethylformamide, chloroform, carbon tetrachloride and water (0.1 N NaOH added) were irradiated with an unfocused Argon laser beam (1–5 mW) and emission spectra were recorded for only a few minutes in order to minimize photo-oxidation processes. In all cases an asymmetrical emission band was observed, with a half-height width of about 2500 cm^{-1} (75 nm) and the intensity peak at about 18,300 cm^{-1} (545 nm) corresponding to a large Stokes shift of about 3600 cm^{-1} (100 nm). Emission intensity was proportional to BR concentration up to saturation and relative quantum yield depended upon solvents: dimethylformamide > chloroform > water (pH 10) > carbon tetrachloride. In basic aqueous solutions sharp peaks were observed on the emission background and they can be attributed to Resonance Raman bands of the BR chromophore, occurring at 1280, 1350, 1590 and 1620 cm^{-1} (Raman shifts).

The actual mechanism of fluorescence quenching is not known but some suggestions can be made: a) collisions of free BR with solvent molecules inducing direct nonradiative decay could be an important quenching mechanism, in agreement with the strong fluorescence enhancement for BR bound to proteins or in rigid solutions; b) another possible quenching process involves specific collisions with oxygen, promoting an efficient intersystem crossing, fol-