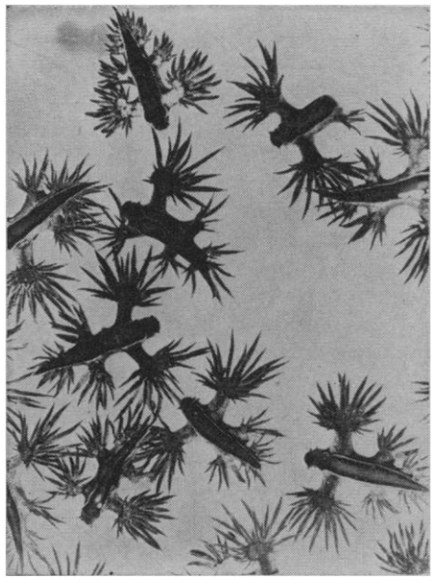

Fig. 1. *Glaucilla* floating in the usual attitude, upside down near the seawater surface, buoyed up by bubbles of air gulped into the stomachs. [Photograph by J. Myers.]

stung by numerous small blue invertebrates brought in with the surf. Although the stings were not severe (mild pain for 1 or 2 hours), specimens were sent to the Australian Museum from where they were passed to us for examination. They proved to be nudibranch mollusks (sea slugs) of the planktonic family Glaucidae (Fig.1), of which two species occur sporadically in eastern Australian waters—*Glaucus atlanticus* Forster, 1777, and *Glaucilla marginata* Bergh, 1868. Hitherto nudibranchs were not thought to be harmful to man. It has been known, however, that

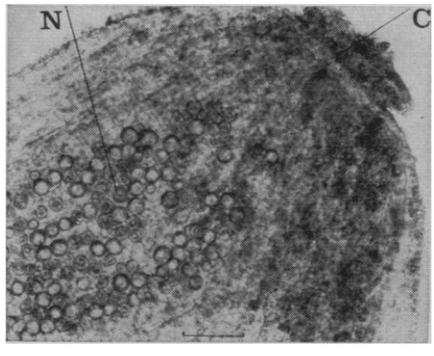

Fig. 2. Photomicrograph of an unstained squash preparation of the tip of a ceras (dorsal papilla) of *Glaucus*, showing the nematocysts causing injuries to bathers at Port Stephens in February 1968; *N*, nematocysts in cnidosac; *C*, cnidopore (scale 100 μm).

many eolidiform species utilize for defense (against fish and perhaps other predators) the nematocysts (stinging cells) of the coelenterates upon which they prey (*1*).

Microscopic preparations of the dorsal papillae or cerata of the Port Stephens specimens of *Glaucus atlanticus* revealed (Fig. 2) that the cnidosacs contained nematocysts which corresponded exactly with nematocysts of the venomous siphonophore *Physalia utriaulus*, the Portuguese man-of-war. It became clear that *Glaucus* and *Glaucilla*, which feed upon the chondrophores *Velella* and *Porpita* and the siphonophore *Physalia*, store the nematocysts of *Physalia* preferentially and employ them for their own defense. Other nematocysts are customarily digested and were found in the lumina of the digestive gland diverticula and in food vacuoles in the digestive cells. With respect to size, the nematocysts of *Physalia* fall into two classes which do not usually overlap (*2*). Australian *Glaucus* and *Glaucilla* utilize in their cnidosacs principally the largest nematocysts (which possess the longest penetrants when discharged). This is the explanation for the painful stings experienced by bathers at Port Stephens. Specimens from the Gulf of Aden, St. Helena, and Japan in the natural history collections of the British Museum were also examined, and the same type of *Physalia* nematocysts was identified.

Both *Glaucus* (*3*) and *Glaucilla* (*4*) are therefore potentially harmful to man over their whole geographical range. Both are warm-water species; the latter is restricted to the Pacific Ocean, but the former (despite the specific name *atlanticus*) occurs in the three major oceans.

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25 April 1969

Purine Metabolism in Heterozygous Carriers of Hypoxanthine-Guanine Phosphoribosyltransferase Deficiency

Abstract. *The urate pool and daily turnover of urate, together with the rate of incorporation of glycine into urate, were measured in three asymptomatic mothers who had sons with various degrees of deficiency of hypoxanthine-guanine phosphoribosyltransferase activity. Two of these mothers had abnormally increased values for the urate pool, urate turnover, and 24-hour urinary excretion of uric acid. These two mothers also had reduced hypoxanthine-guanine phosphoribosyltransferase activity and increased adenine phosphoribosyltransferase activity in erythrocyte lysates. All three mothers showed an abnormal increase in urate production, as judged by the rate of incorporation of glycine into urate.*

The Lesch-Nyhan syndrome, characterized by mental deficiency, choreoathetosis, and self-mutilation, is associated with gross overproduction of urate and excessive glycine incorporation into urate (*1*). Patients with the disorder have abnormal activities of the phosphoribosyltransferase enzymes involved in the reutilization of the purine bases; in erythrocyte hemolyzates the activity of the enzyme for hypoxanthine and guanine is virtually absent, whereas that for adenine is increased (*2*). The disorder occurs only in males and is inherited in an X-linked fashion. In the mothers of patients with the Lesch-Nyhan syndrome (obligate heterozygotes), cultures of skin fibroblasts have two cell populations (*3*); one population has normal, and the other shows no hypoxanthine-guanine phosphoribosyltransferase (E.C. 2.4.2.8) activity. However, enzyme activities in hemolyzates of erythrocytes have not been distinguishable from normal (*4*, *5*). Studies of urate turnover in such heterozygotes have also been normal, although preliminary studies suggested that *de novo* purine synthesis might be increased (*5*, *6*). A few patients with overproduction gout have also been described as having erythrocyte hypoxanthine phosphoribosyltransferase activities of less than 10 percent of normal (*5*), and other patients have manifestations intermediate between such gouty subjects and those with the full Lesch-Nyhan syndrome (*7*).

The three families studied illustrate

Table 1. Phosphoribosyltransferase activity in erythrocyte hemolyzates of the three families studied, expressed as nanomoles per milligram of protein per hour. Incubation mixtures contained (final volume, 200 μl): 55 m*M* tris-Cl buffer, at *p*H 8.5 for hypoxanthine and guanine substrates and at *p*H 7.4 for adenine; 5 m*M* magnesium chloride; 1 m*M* 5-phosphoribose 1-pyrophosphate; 0.52 m*M* hypoxanthine-8-^{14}C [12,200 disintegrations per minute (dpm)/nmole] or 0.36 m*M* guanine-8-^{14}C (64,300 dpm/nmole) or 0.46 m*M* adenine-8-^{14}C (13,400 dpm/nmole) (Schwarz BioResearch), and between 0.1 and 0.5 mg of protein from erythrocyte hemolyzates dialyzed overnight at 4°C in 0.001*M* tris buffer, *p*H 7.4. Samples were incubated at 37°C for 20 minutes, and reactions were terminated by the addition of 4 μmole of sodium ethylenediaminetetraacetate and cooling. Nucleotide was separated from base by adsorption onto small columns of Dowex-1-*X*8, 200 to 400 mesh, in the chloride form, with subsequent elution.

Substrate	Phosphoribosyltransferase activity						
	Normal mean ± S.D. (28 subjects*)	Family W		Family J		Family L	
		Mother	Father	Mother	Father	Mother	Father
Hypoxanthine	70.4 ± 14.5	81	85	31	80	12	77
Guanine	82.1 ± 24.2	54	64	14	50	11	50
Adenine	14.6 ± 5.2	24	24	40	16	29	22

* No sex difference.

this spectrum of clinical manifestations of hypoxanthine-guanine phosphoribosyltransferase deficiency. In family W, two males (17 and 15 years) exhibited hypoxanthine phosphoribosyltransferase activities of less than 0.04 nmole per milligram of protein per hour. Their clinical affection was typical of the Lesch-Nyhan syndrome. In family J, two males (27 and 17 years) exhibited hypoxanthine phosphoribosyltransferase activities of 0.37 and 0.22 nmole per milligram of protein per hour. They had suffered from joint pains and renal colic before the age of 10 years and showed a moderate degree of mental deficiency with some spasticity, but no tendency to self-mutilation. However, the propositus in family L (35 years) had a pleasant personality and had been self-supporting until the age of 25 years when he developed gout which progressively incapacitated him. He had minimum neurological signs in the form of pes cavus and dysarthria and a hypoxanthine phosphoribosyltransferase activity of 0.82 nmole per milligram of protein per hour. Activities of phosphoribosyltransferase for hypoxanthine, guanine, and adenine for the parents of these patients are shown in Table 1.

Table 2. Characterization of basal urate metabolism in the mothers of three patients with hypoxanthine-guanine phosphoribosyltransferase deficiency who were taking diets adequate in calories and protein but essentially free of purines for 5 days before the study began and for 7 days after. Serum urate was measured by the spectrophotometric uricase method (*11*). The urate clearance was estimated from the urate content of a timed specimen of urine (24 hours for mothers W and J and three periods of 30 minutes each for mother L) and the corresponding plasma urate concentration. The miscible urate pool and daily turnover rate (urate production) were assessed by the intravenous administration of between 11 and 21 mg of ^{15}N-lithium urate, followed by the determination of the amount of urinary uric acid excreted and its ^{15}N enrichment, during the following 7 days (*9*). The rate of incorporation of glycine into urate was measured by the simultaneous oral administration of 10 μc of ^{14}C-glycine and the determination of the ^{14}C content of the pure crystalline uric acid isolated from the seven subsequent 24-hour specimens of urine (*9*). The percentage of the glycine incorporated into urinary uric acid was then corrected to the amount incorporated into produced urate by reference to the percentage of excretion of the ^{15}N-labeled urate in the 7-day period.

Test	Mother W	Mother J	Mother L	Upper limit of normal
Age (years)	38	60	55	
Weight (kg)	39.8	69.5	57	
Serum urate (mg/100 ml) (*11*)	4.5	6.8	4.9	6.0
Urate clearance (ml/min)	5.7	6.8	18.7	
Miscible urate pool (mg) (*8*)	526	1804	1596	20 mg/kg
Urate production (mg/24 hours) (*8*)	448	1227	803	10 mg/kg
Urinary urate excretion (mg/24 hours) (*9*)	367	773	761	600
Urinary creatinine excretion (mg/24 hours)	683	1124	841	
Intravenous urate excreted (%) (*9*) (A)	73.8	61.5	99.3	82
Glycine incorporated into urinary urate (%) (*9*) (B)	0.50 (6 days)	0.77 (7 days)	1.27 (7 days)	0.29 (7 days)
Glycine incorporated into produced urate (%) (*9*) $(\frac{B}{A} \times 100)$	0.67	1.25	1.28	0.37

Abnormalities of basal urate metabolism were also present in the asymptomatic mothers of these patients (Table 2). Mother J and mother L showed abnormal increases in the urate pool and the rates of production and renal excretion of urate (*8*, *9*). Mother W showed only a marginal elevation in the rate of urate production. In all three mothers, however, the percentage of glycine incorporated into uric acid was increased, as judged by the measured incorporation into urinary uric acid and the calculated incorporation into produced uric acid (*9*). Only mother L showed the abnormally high ratio of urine urate to urine creatinine characteristic of the Lesch-Nyhan syndrome, gouty patients with partial phosphoribosyltransferase deficiency, and some of the unaffected members of their families (*5*). Despite these abnormalities, both of enzyme activity and of urate metabolism, none of the mothers had ever suffered from gouty arthritis, and only the one who had the greatest degree of urate overproduction had an increase in the serum urate concentration. However, mother W, who had normal hypoxanthine-guanine phosphoribosyltransferase activity and the least abnormality of urate metabolism, had the sons who showed the most severe manifestations of the disease. Mother J, on the other hand, had a moderate reduction of hypoxanthine-guanine phosphoribosyltransferase activity and a moderate increase in adenine phosphoribosyltransferase activity, and had sons who appeared clinically to have a less severe form of the Lesch-Nyhan syndrome. Mother L had the lowest hypoxanthine-guanine phosphoribosyltransferase activity of the three mothers and yet her son had the least severe clinical affection. In an X-linked condition such as this, it would be expected that heterozygous females would have enzyme activities intermediate between the normal and the deficient states. However, in common with the situation in glucose-6-phosphate dehydrogenase deficiency, the X-linked recessive form of Hurler's syndrome and orotic aciduria (*10*), the enzyme activity in the heterozygotes may also be either close to normal or considerably decreased.

The study of abnormalities of urate metabolism in the absence of gouty arthritis may also contribute to our understanding of the pathogenesis of gout. Although mother W overincorporates glycine, her rate of urate production is only marginally abnormal.

Other examples of enhanced ^{14}C-glycine incorporation into urate occurring with nearly normal values for urate production have been observed both in patients with primary gout and also in patients with psoriasis (*9*), and, although this might possibly be related to a reduction in the size or turnover rate of the glycine pools, the exact metabolic explanation for this phenomenon has not been elucidated. Mother J has a considerably elevated production rate, but maintains an only moderate increase in her urate pool and serum urate concentration by a high urinary urate excretion and efficient extrarenal disposal. Mother L, with a high renal clearance of urate and normal renal function, maintains a normal serum urate by excreting almost all of her produced urate by the kidney each day. Her ability to excrete such a high proportion of her produced uric acid in each 24 hours is supported by the finding that she is able to excrete a similarly high proportion of the administered ^{15}N-urate within 7 days. Perhaps appropriate studies may yet enable some abnormality of urate metabolism to be detected in all such heterozygotes.

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Peroxidation of Subcellular Organelles: Formation of Lipofuscinlike Fluorescent Pigments

Abstract. *Lipid peroxidation of subcellular organelles gives fluorescent products which have fluorescence and excitation spectra similar to those of lipofuscin pigments. Fluorescence and excitation spectra and total fluorescence in the 460-nanometer region are useful for qualitative identification and quantitative measurement of the Schiff base product, a molecular damage site of lipid peroxidation which develops during some aging processes.*

Lipofuscin pigments accumulate in animal tissues, especially the brain and heart, as a function of age. The pigments have characteristic fluorescence spectra with a maximum at 470 nm when excited at 365 nm. Formation of the pigments appears to involve peroxidation of polyunsaturated lipids of subcellular membranes (*1*). Malonaldehyde, a major product of peroxidation of polyunsaturated lipids, reacts with primary amino groups of amino acids and proteins in a cross-linking reaction (*2*). The Schiff base product, RN=CH–CH=CH–NH–R, has fluorescent maximums in the 450- to 470-nm region and excitation maximums in the 360- to 390-nm region. Fluorescent products are also produced when malonaldehyde undergoes similar reactions with amino groups of nucleic acids and their bases and with phospholipids. Thus a number of properties identify this cross-link as a chromophoric molecular damage site of lipofuscin pigments.

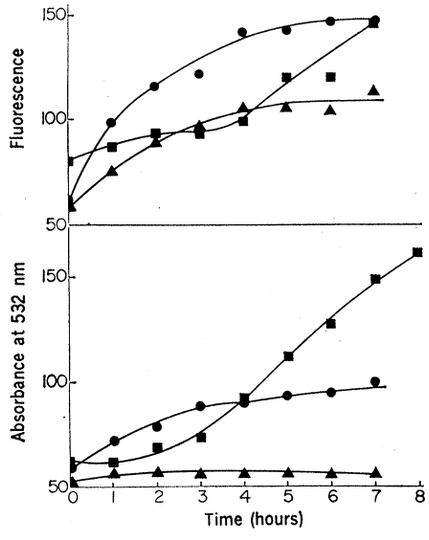

Fig. 1. Fluorescence at 460 nm (top) and thiobarbituric acid reactants (bottom) in the oxidation products of lysosomes (triangles), mitochondria (circles), and microsomes (squares). Fluorescence is given in units per milligram of protein per milliliter, and thiobarbituric acid reactants in absorbance at 532 nm per milligram of protein per milliliter.

The damaging effects of lipid peroxidation are well documented for mitochondria (*3*), microsomes (*4*), and lysosomes (*5*). Lipid peroxidation in vivo is a basic deteriorative reaction in vitamin E deficiencies (*6*), in hepatotoxic mechanisms (*7*), in cellular mechanisms of oxygen toxicity (*8*), and in cellular mechanisms of aging processes (*9*). Fluorescent lipofuscin pigments have been observed in many pathological processes (*10*), especially in vitamin E deficiencies and aging processes, where their deposition has been correlated with lipid peroxidation dam-

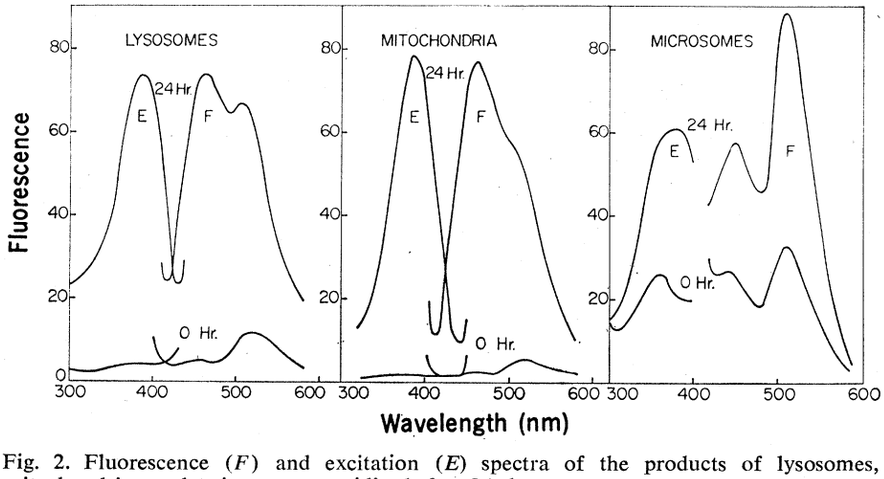

Fig. 2. Fluorescence (*F*) and excitation (*E*) spectra of the products of lysosomes, mitochondria, and microsomes oxidized for 24 hours.