The skeleton of freshly collected coral colonies was cleaned by a jet of sea water from a high pressure hose without fixation or following fixation in 10% neutral formalin in sea water or in Clarke's acetic ethanol. The skeleton of this species yields 0.1% by weight of organic material upon demineralisation. Clean, whole pieces of skeleton were demineralised in 2% hydrochloric acid or in 10% (w/V) ethylene dinitrilo tetraacetate made up and adjusted with both di- and tetrasodium salts to pH 7.0–7.3. Light, polarised light and phase contrast microscopy revealed three organic constituents: (1) occasional filaments of limeboring algae (e.g. *Ostreobium* sp.), (2) a dispersed network of fibers approximately 1 μ in diameter and (3) a transparent, milky, regionally birefringent matrix which appears amorphous in light and phase contrast microscopes.

No soluble or fibrous proteins or amino acids were found by histochemical or microanalytical techniques in whole skeleton, demineralised skeleton, or supernatant demineralising fluid. Fibers and matrix showed no change in their optical properties following the elution with methanol of a histochemically detectable lipid component, and they gave positive histochemical tests for 1,2 glycol groups. The fibers disappeared in chitosan preparation[3] and the matrix became readily soluble in 3% acetic acid and gave a strong positive reaction for chitin. The X-ray diffraction powder patterns of chitin purified in the classical manner[4] from soft integument of the shore crab, *Hemigrapsus nudus*, and from wings of the cockroach, *Periplaneta americana*, were compared with those of demineralised skeleton of *P. damicornis*. The d values of these patterns are given in the Table, and they are in close agreement with previously published values for chitin[5]. The patterns were obtained by Dr. K. J. PALMER of the U.S. Dept. of Agriculture Western Regional Research Laboratories, Albany, California, using Cu K-α radiation from an X-ray tube operated at 40 kv and 15 ma. It is concluded that chitin is the major constituent of the organic component of the skeleton of *P. damicornis*. Whether it exists in the α- or β-form has not been determined.

The d values and relative intensities of X-ray diffraction powder pattern rings of chitin from a coral, a crab, and an insect

Pocillopora damicornis	*Hemigrapsus nudus*	*Periplaneta americana*
9.6 s	9.6 s	9.6 s
7.0 w	7.0 w	7.0 w
4.64 s	4.64 s	4.64 s
	3.85 vw	3.85 vw
3.40 m	3.39 m	3.39 m

Relative intensity of rings within patterns is indicated by s-strong, m-medium, w-weak, and vw-very weak and diffuse. The d values are given in Ångström units.

Small bits of demineralised coral skeleton were dried on Formvar-copper grids and shadowed with Pt–Pd and examined with an RCA EMU 3 electron microscope. The matrix was observed to consist of fibrils of average diameter 200 Å which showed no longitudinal periodicity or character other than a tendency for fasciation.

With one notable exception[6], published accounts of the details of molecular structure of chitin[4,5] are concerned with chitin which has been purified by treatment with diaphanol or boiling normal potassium hydroxide. The skeleton of *P. damicornis* presents a source of chitin which need not be subjected to these treatments in order that its structure be studied.

Chitin is known also from the calcified skeletal elements of Hydrozoa, Mollusca, Crustacea, Diplopoda and Ectoprocta. In the species which have been examined, fibrous protein is intimately associated with chitin and, except in some Crustacea[7], it is present in much greater amount by weight than chitin. The skeleton of *P. damicornis* differs from all calcified structures previously described in its lack of fibrous protein and typical mucopolysaccharides. One cannot disregard the possibility of a gel precursor[8] or early crystal environment which is lost upon further calcification and the possibility of a role played by very small amounts of lipid and undetected constituents in the calcification of this skeleton. It is suggested here that this system is far simpler structurally and perhaps biochemically than the calcifying systems which have been studied to date.

Résumé. L'auteur démontre que la partie organique du squelette du corail, *Pocillopora damicornis*, est constituée par de la chitine. Cette chitine semble d'exister sous une forme très pure, sans être associée à la protéine, comme le sont toutes les autres chitines calcifiées connues.

S. A. WAINWRIGHT

Department of Zoology, University of California, Berkeley (USA), August 2, 1961.

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Absence of Adverse Effects in Spleen Extract. Protected Guinea-Pigs during Second Post-Irradiation Year[1]

In previous studies[2–6], it was demonstrated that homologous as well as heterologous cell-free spleen extracts reduce the mortality of mice and guinea-pigs exposed to the $LD_{75/20}$ of ionizing radiations. These short-term observations are now supplemented by a study of the fate of long-term survivors.

Material and Methods. Guinea-pigs which were alive 365 days after exposure to 650 r measured in air of Co^{60}

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γ-radiation administered under 4π geometry in a 2400 curie radiation source[7] were used in this study. This dose resulted in 75% mortality within 20 days. From various experiments 12 animals which had served as saline controls (Group A) and 16 guinea-pigs which had been treated with cell-free mouse of guinea-pig spleen extracts (Group B) were available. Maintenance and observation of the animal took place as previously described[6].

Results. The following observations were made:

(a) Mortality. During the second post-irradiation year, 5 out of 12 saline-treated animals, or 41.7%, and 6 out of 16 spleen extract treated animals, or 37.5%, died. In agreement with the almost equal mortality in both groups, Table, column 2 indicates no obvious difference in death distribution. The mean survival times were 590 and 565 days, respectively. Statistical analysis by means of Student's 't' test showed that the difference in mean survival time between groups A and B was insignificant ($p > 0.60$).

Death distribution and weight changes of guinea pigs dying during the second post-irradiation year

(A) Saline controls

Animal No.	Post-irradiation day of death	% Weight changes
1346-3	512	− 8.6
1346-5	541	+ 5.3
1346-A4	588	+ 20.0
1346-7	603	− 12.9
1332-10	708	− 2.5
Average	590	+ 1.3

(B) Spleen treated

Animal No.	Post-irradiation day of death	% Weight changes
1347-3	407	+ 99.1
1347-4	542	+ 79.9
1333-4	556	+ 94.8
1347-1	568	+109.9
1333-5	606	+ 4.8
1348-7	709	+ 15.9
Average	565	+ 67.4

(b) Body Weight Changes. At death the body weight of the animals was established. Pertinent data are listed in column 3 of the Table which indicates that the saline-treated animals showed instances of loss of body weight as compared to the starting weight, resulting in an average weight corresponding to that of zero day of the experiment. In contrast to this, the spleen extract treated animals all showed weight gain, averaging 67% above the starting weight. Student's 't' test revealed that the difference between groups A and B was highly significant ($p < 0.02$).

(c) Autopsy Findings. The macroscopic observations made at autopsy did not indicate a specific cause of death and showed in all of the animals dying before the 600th post-irradiation day only a more or less pronounced pneumonia. In addition, a complete absence of fat depots in the saline-treated animals was noted.

Discussion. Death distribution and body weight changes of irradiated saline control and spleen extract treated guinea-pigs clearly indicate that the latter group of animals did not succumb eventually from an anaphylactic reaction causing severe emaciation of the animals, the so-called 'secondary disease'. On the contrary, the notable weight gain of the spleen extract treated animals observed during the acute phase of the radiation syndrome, which paralleled the reduced mortality during the first 20 days after irradiation, remained in evidence until death.

In the absence of adequate data on guinea-pig gerontology, the question whether the animals died during the second post-irradiation year due to natural or radiation-induced premature ageing must be left undecided at this time.

The importance of the present investigations for a possible clinical application of spleen extracts consists in the fact that even crude cell-free spleen extracts can be administered to guinea-pigs, a species well known for its susceptibility to allergic manifestations, without producing deleterious late effects within a period of time comparable to approximately one half of the maximum life span of this species.

Zusammenfassung. Todesalter und -gewicht von mit Milzextrakt behandelten Meerschweinchen und von Kontrolltieren im zweiten Jahr nach γ-Bestrahlung mit einer 75% letalen Dosis werden verglichen und das Fehlen von schädlichen Spätwirkungen der Milzextrakttherapie aufgezeigt.

F. ELLINGER, T. STRIKE, and B. LINDSLEY

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Antagonism of Chlorpromazine by β-Melanocyte Stimulating Hormone (β-MSH)

Previous communications from these laboratories have described the actions of β-melanocyte stimulating hormone β-MSH[1,2] and of chlorpromazine[3], on spinal reflexes of cat spinal cord. It was found that β-MSH potentiates spinal reflexes, whereas chlorpromazine depresses them. In an extension of the studies on chlorpromazine[4], it was found that this drug is capable of depressing the positive intermediary potential of cat spinal cord. The positive intermediary potential was initially described by GASSER and GRAHAM[5] as a negative potential associated with increased central excitatory state. The conclusions of GASSER and GRAHAM were later verified by LLOYD and MCINTYRE[6] who used somewhat modified techniques. Thus, the observation that chlorpromazine depresses the

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