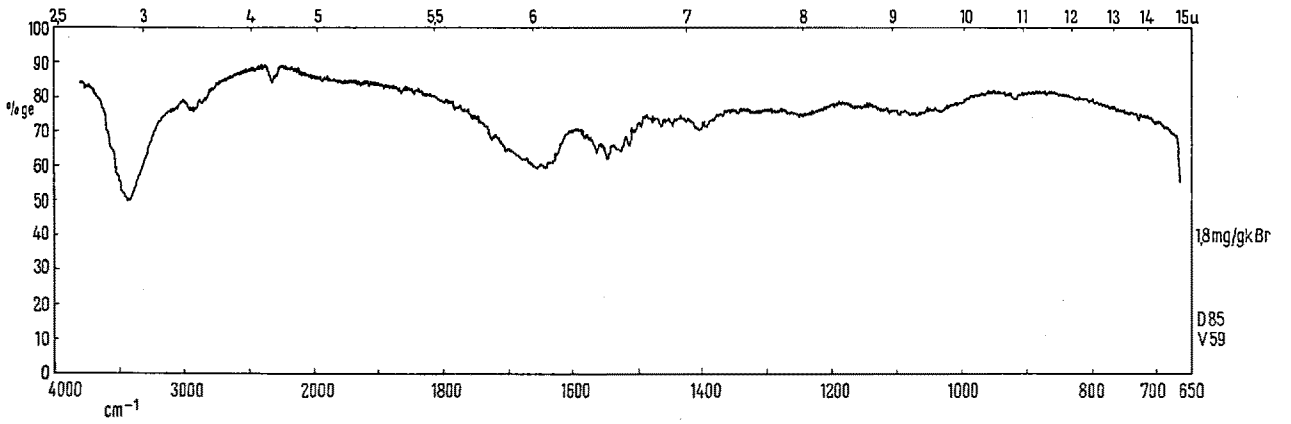

Fig. 2. Penicillinase 1.8 mg/g of KBr. Infrared spectrum of 2.4 min/500 e. 100 cm^{-1}. Vector (Spaltprogramm): 3. Elcctrical Data: D 8.5; V 5.9. Prisma NaCl (Ernst Leitz GmbH, Wetzlar).

several times. The sediment was finally dissolved in $M/1$ Tris buffer of pH 9.0. Very little material remains insoluble with no activity. The solution was therefore mixed with ethanol until the concentration reached 20%. At this point, penicillinase was precipitated by $2n$ phosphoric acid till pH 5.4. After several washings with distilled water, the precipitate was dried in vacuum over phosphorus pentoxyde. The preparation was brownish, hygroscopic, insoluble in water, acetate, phosphate, veronal, and citrate buffers. Only Tris-buffer of high ionic strength was able to dissolve it completely. 1 mg of this substance is equivalent to 500 penicillinase arbitrary units (PAU). Mycobacterial penicillinase is soluble in liquid medium, and remains active at pH between 4.5 and 7.83. Its present insolubility can only be explained by the possibility that the molecule of penicillinase was to some extent denaturated by the organic solvent used for the removal of benzoic acid. This possibility is shown when using other solvents such as acetone, ethanol, benzol, benzyl alcohol, toluol, terc. butanol, sec. butanol etc. All those solvents diminish enzyme activity, and at the same time lower its solubility in Tris-buffer. Most conservative was n-butanol. In view of the fact that the IR spectrum of the enzyme preparations indicated the presence of polysaccharides, further purification was necessary. To a solution of enzyme (in Tris buffer) 5% sodium p-aminosalicylate was dissolved, and PAS precipitated by $3n$ HCl till pH 5.8. The precipitate dissolved in methanol contained only a trace of proteins without activity, and the rest insoluble polysaccharides. Supernatant solution contained protein with an equivalent of 800 PAU/mg. IR spectrum shows no trace of polysaccharides. The preparation is water-insoluble being soluble only in $M/1$ phosphate buffer and $2M$ acetate buffer at pH between 6.8 and 7.8. It is no longer hygroscopic and looks like whitish powder.

Crystallisation of mycobacterial penicillinase was done in the form of mixed crystals. Penicillinase was dissolved in phosphate buffer, in which ammonium phosphate (t) was dissolved to 19% concentration. It is necessary that the amount of buffer is small. The solution was cooled to 4°C, and mixed with 4 Vol of cold (— 20°C) acetone. Very rapidly a crystalline precipitate of phosphates containing all the enzyme in a hybride crystalline form was formed. The precipitate was washed several times with acetone and centrifuged. This crystalline preparation is not water-soluble, but it remains soluble in all the aforementioned buffers. Paper electrophoresis of penicillinase in a $M/15$ phosphate buffer of pH 6.0, and $0.2M$ veronal buffer of pH 8.6 shows only one individual band, which moves very slowly towards anode after 16 h. UV spectar shows two maximal adsorption peaks at 240 and 280 μ, with a linear deflection after 340 μ. IR spectrum of the preparation shows absence of nucleic acids, lipids and polysaccharides, with characteristic waves of carbonyl-groups, suggesting a piperazine structure in the enzyme molecule. After acid hydrolysis this carbonyl-groups wave disappears, fortifying our convinction that the sensibility of mycobacterial penicillinase depends upon the preservation of piperazine linkages[9].

Résumé. La pénicillinase des mycobacteries est précipitée par l'acide benzoïque. L'élution d'acide se fait par n-butanol. L'élimination des polysaccharides étant donné par la précipitation avec PAS la substance devienne mieux soluble. La crystallisation de l'enzyme est obtenue en forme de crysteaux hybrides avec du phosphate d'ammoniaque. L'analyse spectrophotométrique prouve l'absence d'impuretés, l'électrophorèse revèle une bande individuelle d'une motilité ralentie.

M. K. MUFTIC

Tuberculose Research Institute, Borstel/Bad Oldesloe (Germany), July 26, 1961.

[9] *Acknowledgment.* Our thanks are due to Dr. S. SEYDEL for UV and IR spectrophotometric investigations. This work was supported by a grant from the West German Government.

An Anthozoan Chitin[1]

From a study of the organization of the skeleton of the Hawaiian reef coral, *Pocillopora damicornis* L., evidence is presented here that the major constituent by weigth of the organic component of the skeleton is chitin (poly-acetylglucosamine). This is the first record of chitin in the class Anthozoa[2].

[1] Contribution No. 147 from the Hawaii Marine Laboratory.

[2] L. E. R. PICKEN, *The Organization of the Cell and other Organisms* (Clarendon Press, Oxford 1960).

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.

3 F. L. CAMPBELL, Ann. ent. Soc. Amer. *22*, 401 (1929).
4 A. G. RICHARDS, *The Integument of Arthropods* (University of Minnesota, Minneapolis 1951).
5 M. S. FULLER and I. BARSHAD, Amer. J. Bot. *47*, 105 (1960). – E. J. WINKLER, L. A. DOUGLAS, and D. PRAMER, Biochim. biophys. Acta *45*, 393 (1960).
6 N. E. DWELTZ, Biochim. biophys. Acta *44*, 416 (1960).
7 M. LAFON, Bull. Inst. Oceanogr. *45*, No. 939 (1948).
8 W. H. BRYAN, Proc. Roy. Soc. Queensland *52*, 41 (1941).

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}

1 Presented in part during a Meeting of the Informal Society for the Study of Long-Range Effects of Ionizing Radiation in San Francisco, May, 1960.
The present investigations were jointly supported by the Bureau of Medicine and Surgery of the U.S. Navy and the Defense Atomic Support Agency. We should like to express our thanks to the Liaison Officer DASA, LCDR W. H. CHAPMAN, MSC, USN, for his continued support and interest.

2 F. ELLINGER, Radiol. clin. (Basel) *23*, 129 (1954).
3 F. ELLINGER, Proc. Soc. exp. Biol. Med. *92*, 670 (1956).
4 F. ELLINGER, Science *126*, 1179 (1957).
5 F. ELLINGER, Atompraxis *4*, 439 (1958).
6 F. ELLINGER, Atompraxis *6*, 208 (1960).