

gest that this antibiotic may be effective in extra-intestinal amebic infections such as hepatic abscess as well as in amebic colitis and amebic dysentery.

Eleven cases have since been successfully treated, with no recurrence of symptoms.

References

1. ANDERSON, H. H., et al. *Amer. J. trop. Med.*, 1948, **28**, 373.
2. HEWITT, R. I. Personal communication.
3. NELSON, E. *Amer. J. trop. Med.*, 1947, **27**, 545.

Studies on Arthropod Cuticle. III. The Chitin of *Limulus*^{1, 2}

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Through the years there has been sporadic discussion as to whether or not the chemical chitin is a single compound, identical in all animals and fungi. The consensus of opinion, based on the chitosan color test, nitrogen values, and X-ray diffraction diagrams, is that it is a single compound in the sense that it is always a polymer of identical acetyl glucosamine units (1, 3). However, some exceptional cases have been recorded that require further investigation. The outstanding exception within the phylum Arthropoda is the cuticle of the horseshoe crab, *Limulus polyphemus*. Fränkel and Jellinek (4) isolated and identified glucosamine crystals from *Limulus* that were identical with similar preparations from ordinary crab shells, but their chitin preparation had a low nitrogen value (5.51% in contrast to 6.89% theoretical for acetylated glucosamine). On the basis of analyses giving a carbon-nitrogen value of 10 to 1 (in contrast to 8 to 1 for ordinary crabs) they postulated that the chitin of *Limulus* is made up of units similar to those in chitin from other arthropods, but that there is an additional ethyl or two methyl groups substituted somewhere in the units which, like the acetyl group, are hydrolyzed off during the preparation of glucosamine. To make this suggestion one has to assume that the alkali purification is reasonably complete (since chitin, having no known solvent, cannot be purified by recrystallization).

Reinvestigation of this question has led us to the conclusion that the purification procedure is at fault. Our first nitrogen analyses of alkali-purified chitin from *Limulus* were comparable to those of Fränkel and Jellinek; however, using a more complete purification process involving prolonged treatment with an oxidizing agent (KMnO₄ followed by NaHSO₃) after the treatment with hot alkali (2), we have obtained a pure white residue which gives a Kjeldahl nitrogen value of 6.06%. This,

although significantly higher than the value recorded by Fränkel and Jellinek, is still considerably lower than the nitrogen values of chitin purified from the cuticles of other arthropods. It is suggestive of the removal of additional impurities, but is inconclusive, since chitin is not completely resistant to hot alkali. In an attempt to settle the point, we had X-ray diffraction pictures made of chitin purified from the cuticle of *Limulus* and from a local species of crayfish (*Cambarus* sp.).³ The X-ray pictures are identical, and agree with those previously published for chitin (2, 3). We are forced to conclude that the chitin units of *Limulus* are not substituted in the manner suggested by Fränkel and Jellinek and that they are almost certainly identical with those found in the cuticles of other arthropods. Probably differences do occur on the polymer chemical level (5, 6), but on the constituent unit level the substance appears to be the same for all arthropods.

This finding necessitates a reconsideration of the chemical components of cuticle. As we have pointed out previously (6), there is no assurance that anyone has ever prepared pure chitin. Since, unlike cellulose, chitin is not known to occur in any approximation to the pure state (usually less, commonly much less, than half the dry weight of the cuticle) and since no solvent for it is known, all assumptions of purity have been based on inconclusive data. For another component to be present in the extracts studied would require only that it agree with chitin in being insoluble in and not destroyed by treatment with water, weak acid at room temperatures, hot or cold alkali at any concentration for short periods, strong oxidizing agents at room temperatures, and ordinary organic solvents, and that it be present in low enough percentage not to greatly disturb the nitrogen values. It must also be either amorphous or of nearly identical lattice spacings or else the amount present in *Limulus* chitin would be detectable in the X-ray diffraction pictures.

Accepting the idea that chitin is made of the same units in all arthropods, the discrepancies between theoretical and actual nitrogen values presumably represent unremoved components of the cuticle. If we assume that the unknown component contains no nitrogen⁴ we can make preliminary estimates of the amount present based on the nitrogen determinations. Considering only some of the recent values, we have 6.6% N for lobster chitin (2), 6.45% N as an average figure for insect chitin with 6.28% N as the low value obtained from hardened puparia (3), and 6.06% N as the highest value obtained by us for *Limulus* chitin. Calculating on the basis of 6.9% N representing pure chitin, we obtain the following degrees of purity for materials studied by these authors: lobster 96%, average insect 94-95%, hard puparia 91% and *Limulus* 87% (the material used by Fränkel and Jellinek being only 80% pure).

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² The work described in this paper was done under contract between the Medical Division, Chemical Corps, U. S. Army and the University of Minnesota. Under the terms of this contract the Chemical Corps neither restricts nor is responsible for the opinions or conclusions of the author.

³ X-ray patterns made by Dr. W. N. Lipscomb, of the Chemistry Department.

⁴ The unknown component cannot contain more nitrogen than chitin does, and if it contains any nitrogen then the contaminant percentage will be even higher than these calculations.

We conclude that Fränkel and Jellinek's suggestion of a substituted chitin is incorrect and that *Limulus* almost certainly contains the same chitin units as other arthropods. Accepting this, it follows that all so-called pure chitin used for analytical work contains at least several percent of an unknown contaminant, that in hardened cuticles this may be over 5%, and that in *Limulus* it is considerably more than 10%. The identity of the contaminant is at present only a matter of conjecture. It would be of considerable interest to know whether it is as yet unrecognized compound⁵ or a percentage of one of the known components that is so strongly bound to the chitin that it is not removed by the purification procedure.

References

1. CAMPBELL, F. L. *Ann. entomol. Soc. Amer.*, 1929, **22**, 401.
2. CLARK, G. L. and SMITH, A. F. *J. phys. Chem.*, 1936, **40**, 863.
3. FRAENKEL, G. and RUDALL, K. M. *Proc. roy. Soc. Lond., Ser. B*, 1947, **134**, 111.
4. FRÄNKEL, S. and JELLINEK, C. *Biochem. Z.*, 1927, **185**, 384.
5. RICHARDS, A. G. *Ann. entomol. Soc. Amer.*, 1947, **40**, 227.
6. RICHARDS, A. G. and KORDA, F. H. *Biol. Bull.*, 1948, **94**, 212.

Temperature Measurement inside the Body Using a Thermistor¹

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In recent years thermistors have been increasingly applied to the measurement of temperature in animal and man (1, 3). A thermistor has three important advantages over a thermocouple for measurement of temperature in the body. Due to its high temperature coefficient ($-3\%/^{\circ}\text{C}$) it is approximately ten times as sensitive as a thermocouple, assuming the same order of sensitivity of detecting device. Because of its high impedance (several thousand ohms as compared to a few ohms) lead resistances and switch contact resistances are relatively unimportant; so it is not necessary to take special pains in the wiring of associated circuits. Finally, there is no necessity for maintaining a constant reference temperature.

For these reasons it was decided to incorporate a thermistor as the sensitive element in fine catheters for measuring temperatures inside the animal body. One type of catheter for measuring the temperature inside joint spaces and muscles (2) consisted of a radio-opaque plastic tube 0.063 cm OD, 0.036 cm ID, varying in length from 15 cm to 1.5 meters. Catheters of larger diameter have also been made for intracardiac and intravascular

observations. The thermistor was placed flush with one end, the lead wires coming out the other end.

The thermistor used for this application was Western Electric Type V597 having the following characteristics:

Diameter	0.4 mm
Temperature coefficient at 38° C	-3.1 %/° C
Dissipation constant (in still air)	0.1 mm/° C
Thermal time constant (in still air)	1 sec.

Since it was supplied with two 0.025-mm bare platinum leads 8 mm long, it was necessary both to splice these wires to a pair of copper leads, and to insulate the platinum wires and spliced junctions from each other. No. 40 enamel covered copper wire was used for the lead.

TABLE 1

TEMPERATURE MEASUREMENTS IN THE DOG*

	°F
Superior vena cava	98.9
Coronary sinus	99.0
Right ventricle	99.0
Pulmonary artery	98.8
Inferior vena cava	98.8
Hepatic vein	98.9
Renal vein	98.4
Azygos vein	98.6
Femoral vein	96.8
Femoral artery	98.0
Thigh muscle (2 in.)	96.5
Thigh surface	92.8
Rectal	99.1

* Room temperature 73.0° F.

A connection was effected by wrapping two turns of the platinum wire tightly about the copper lead. A conducting silver paste was then applied to assure satisfactory electrical contact. In order to insulate the two platinum wires from each other, a very fine rayon thread was wrapped tightly along each wire beginning at a point on the copper wire a little below the junction. The whole assembly was then dipped into a bakelite solution, care being taken not to immerse the thermistor bead itself. After the bakelite had been baked, the whole assembly was threaded through the plastic tubing. The end containing the thermistor was then sealed with a small amount of polystyrene, care again being taken not to coat the thermistor bead which protruded a few tenths of a millimeter beyond the end of the tube.

The unit was connected into a conventional Wheatstone bridge using a Rubicon galvanometer of sensitivity 0.007 $\mu\text{a}/\text{mm}$ as an indicating instrument. For recording purposes, the output of the bridge was connected to a pen-writing oscillograph. The overall accuracy of the device was $\pm 0.05^{\circ}\text{C}$.

Vascular and other temperatures obtained with the thermistor units are shown in Table 1. The dog was under heavy Nembutal anesthesia. The catheter was passed through the right external jugular vein, and its position was recognized roentgenoscopically.

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⁵ The possibility of another type of substituted glucose chain with a side group that would give the same lattice unit dimensions as an acetylated amine group is conceivable.