1-, 2-, and 4-lb rates appeared normal. Bluegrass, bentgrass, crabgrass, plantian, and chickweed were inhibited in this experiment. Observations will be continued to determine whether the treated plants will survive the winter. During the course of this study there were several weeks without rain, and the inhibition periods might be altered in a more normal growing season.

In one experiment it took much more chemical to inhibit growth of tomato plants when the chemical was used as a soil drench than when it was used as a spray. Maleic hydrazide used as a pre-emergence treatment on a variety of different plants had no inhibiting effect when used at a rate of 8 lb per acre. When applied as a dust to corn seed before planting it had no effect on the amount or time of germination, but the seedlings suffered a marked retardation of growth. As with foliage, the amount of inhibition was directly proportional to the concentration applied.

Reference

1. CURTIUS, TH. and FOERSTERLING, H. A. *J. pr. Chem.*, 1895, **51**, 371.

A Preliminary Report of the Successful Treatment of Amebiasis with Aureomycin[1]

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Amebiasis, or human infection by the protozoan parasite *Endamoeba histolytica*, is a world-wide and prevalent infection. Although a great deal of work has been done with amebicidal drugs, the treatment of this condition remains unsatisfactory. Under certain conditions, currently used amebicidal drugs, especially emetine, involve danger of toxic reactions.

In several cases of other infections treated with aureomycin hydrochloride, we observed an alteration in the gross character of the stool and a reduction in the fecal bacterial flora. This evidence of local intestinal effect, combined with its known systemic action, led us to investigate the use of aureomycin in amebiasis.

Three cases of infection with *E. histolytica* have been treated with aureomycin. While this is a small series and the patients have not yet been observed for an adequate period following therapy, we feel that the results are sufficiently encouraging to warrant a preliminary report so that investigation of this apparently useful drug in amebiasis might be furthered.

W. W., a 27-year-old white male complaining of flatulence, heartburn, diarrhea, and malaise was found to have trophozoites and cystic forms of *E. histolytica* in his stool. After the administration of 7.0 g of aureomycin by mouth in divided doses over a 3-day period, the stools became negative for *E. histolytica* and the patient became asymptomatic. At this time, the aureomycin blood level was 8 μg %. Therapy was continued until the patient had been given a total of 19 g. Six negative stools have been obtained since treatment.

No stool is reported as being negative until it has been studied by fresh saline and iodine preparations, by iron hematoxylin stains, and by culture.

B. C., a 63-year-old colored male was examined because of paraumbilical pain, abdominal fullness, and constipation. *E. histolytica*, in both the trophozoite and cystic forms, was demonstrated in the stool. After 6.75 g of oral aureomycin in divided doses over a 3-day period, the stools became negative. This patient received a total of 21.75 g of aureomycin. All of the gastrointestinal symptoms disappeared and 14 stool examinations over a period of 3 weeks have been negative for *E. histolytica*.

A. T., a 43-year-old colored female was found to have trophozoite and cystic forms of *E. histolytica* in her stools. She complained of rather severe indigestion, epigastric pain, and flatulence. In this case, the stools became negative after 15 g of aureomycin. The blood level of aureomycin at this time was 8 μg %. The patient received a total of 22.7 g of aureomycin. Seven negative stools have been observed since treatment.

In the first two cases there were no toxic reactions to aureomycin. The last patient complained of nausea for 48 hr after the initiation of treatment. No other reactions were observed.

While it was recognized that *in vitro* and *in vivo* action of antibiotics is frequently quite different, *in vitro* studies were carried out. Hewitt (*2*) demonstrated amebicidal activity of aureomycin *in vitro* on the NRS strain of *E. histolytica*. Since the NRS strain has been carried in culture for many years, we thought it might be of some interest to test *in vitro* activity of aureomycin on the three strains isolated from the above cases. Cultures were isolated and maintained in Nelson's egg yolk alcoholic extract medium (*3*). The first and second subcultures from one case, the third subculture from the second case, and the fifth subculture from the third case were used for the assay study. Inoculation of several tubes was made from each strain. These were examined at the end of 24 hr and only heavily positive cultures were used to test the activity of aureomycin. At the time of examination, aureomycin was introduced into the cultures in amounts varying from 0.2 to 3.2 mg/cc of overlay. The tubes were then examined at 6, 18, and 48 hr after the introduction of aureomycin. All ameba were destroyed at the end of 6 hr exposure in the tubes having 0.8 mg or more aureomycin per cc. Most of the ameba in tubes having 0.2 and 0.4 mg/cc were destroyed at the end of 18 hr. At the end of 48 hr no ameba were found in any of the tubes containing aureomycin, while all of the control cultures were heavily positive.

A much larger series of cases with adequate follow-up examinations after treatment will be necessary for an evaluation of aureomycin in the therapy of amebiasis. The work of Anderson, *et al.* (*1*) has shown that there is a sufficient number of cases in Memphis to allow us to accomplish this. The high blood levels obtained sug-

[1] Aureomycin furnished through the generosity of the Lederle Laboratories, Pearl River, New York.

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 ($KMnO_4$ followed by $NaHSO_3$) 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 nitrogen[4] 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).

[1] Paper No. 2447, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

[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.