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## Observations on Calcareous Corpuscles of Larval *Echinococcus granulosus* of Various Geographic Origins. (30543)

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It has been reported(1) that calcareous corpuscles isolated from different species of tapeworms differ considerably in Ca:Mg:P:$CO_2$ ratios. As a probable consequence the crystallization patterns which were induced in the amorphous corpuscles by heat showed rather remarkable differences especially with respect to the nature of the P-containing minerals. Evidently the question can be raised whether the observed variations really are species-specific, or a fortuitous consequence of the fact that the worms in all likelihood had different food materials available and thus lived in environments differing in ionic composition. In this respect it should be mentioned that the worms studied were derived from various hosts, or, as was the case with the human parasites *Taenia saginata* and *Diphyllobothrium latum* from people living geographically far apart (Lebanon, Finland) and thus presumably differing in their food habits. It was therefore deemed of interest to study the calcareous corpuscles of a tapeworm that is less host-specific than those used before and that could be secured from different geographic localities. Larval *Echinococcus granulosus* was selected as one of the most suitable species.

*Materials and methods.* Lyophilized scolices of *Echinococcus granulosus* isolated from sheep livers in Chile and from cattle livers in Lebanon were obtained respectively through the courtesy of Dr. M. Agosin and Dr. R. Thorson. Mixed *Echinococcus* material isolated from liver and lung cysts of New Zealand sheep, first stored in alcohol and then dried, was contributed by Dr. D. J. Morseth.

The calcareous corpuscles were isolated by the ethylenediamine procedure described previously(2) and the methods of analysis coincided with those detailed earlier(2,3).

*Results and discussion.* Despite their varied origins the parasites contained essentially identical amounts of calcareous corpuscles (Table I) and there was little variation in Ca and $CO_2$ content of the latter. The Chilean material, however, contained somewhat more Mg and less P than the corpuscles isolated from either the New Zealand or the Lebanon samples. All corpuscles contained appreciable amounts of hydrogen which, when calculated as water, corresponded rather closely to the weight losses observed upon heating to 300°C (Table II), indicating probably that during heating tightly bound water was lost. It should be noted that the corpuscles of another larval cestode, *Cysticercus fasciolaris*, showed a much less pronounced weight loss even after heating to 400°C(2). Our material contained in all cases some nitrogen, undoubtedly referable to organic material that has not been destroyed by the ethylenedi-

TABLE I. Yield of Calcareous Corpuscles in Percent of Dried Larval Tissues and Major Constituents of Isolated Calcareous Corpuscles in Percent of Dried Corpuscles.

| | Material from | | |
|---|---|---|---|
| | Chile | New Zealand | Lebanon |
| Yield | 14.5 | 13.5 | 14.8 |
| CaO | 20.5 | 19.0 | 21.6 |
| MgO | 13.8 | 9.4 | 8.9 |
| $P_2O_5$ | 2.7 | 5.9 | 5.7 |
| $CO_2$ | 32.0 | 30.7 | 31.5 |
| H | 2.2 | 3.2 | 2.4 |
| ($H_2O$) | 19.8 | 28.8 | 21.6 |
| N | .80 | 1.25 | .76 |
| Protein (N × 6.25) | 5.0 | 7.8 | 4.8 |
| Total accounted for (inorganic compounds + $H_2O$ + protein) | 93.8 | 101.6 | 94.1 |

TABLE II. Weight Loss of Calcareous Corpuscles During Heating in Percent of Original Weight.

| | Material from | | |
|---|---|---|---|
| Heated to (°C) | Chile | New Zealand | Lebanon |
| 300 | 21 | 32 | 21 |
| 450 | 40 | 40 | 34 |
| 600 | 43 | 52 | 47 |
| 900 | 52 | 56 | 53 |

amine treatment. It presumably represented remnants of the organic matrix of the corpuscles.

The weight loss of the *Echinococcus* samples observed upon heating to 900°C (Table II) is largely due to loss of $CO_2$. The difference in weight between the samples heated to 300 and 900°C amounted to 31% of the original weight in both the Chilean and Lebanon samples, a figure surprisingly close to the $CO_2$ percentage determined by analysis (Table I). In the New Zealand sample, however, the weight loss amounted to only 24%, while the experimentally found $CO_2$ content was 30.7%.

In agreement with the experience with other cestode species(2,3) it was found that the corpuscles isolated by means of the ethylenediamine procedure were amorphous, but that heat treatment induced crystallization (Table III). The crystallization patterns of the New Zealand and Lebanon samples were identical at all temperatures employed. The Chilean material, however, showed at higher temperatures a slightly different behavior, by developing at most traces of apatite, or, in some tests, none at all, while apatite could be identified without difficulty in the New Zealand and Lebanon material. This observation is, of course, not unexpected in view of the previously described finding that the Chilean corpuscles contained much less P than those from the other two geographic localities. It should be noted that the mineralogical composition of the New Zealand and Lebanon corpuscles agreed quite closely to that reported for *Taenia taeniaeformis* and *Spirometra mansonoides*(2,3), while the essential lack of apatite in the Chilean material allied it to *Cysticercus cellulosae* which contained still less P and did not form any apatite.

The corpuscles isolated from the New Zealand and Lebanon worms were identical also with respect to minor components (Table IV), while in the Chilean material one of the latter, manganese, was not detected. It should be noted that the *Echinococcus* corpuscles did not contain boron, copper, lead, or tin, elements which have been found in the corpuscles of other tapeworm species(1). In contrast, the former contained cadmium,

TABLE III. Crystallization Patterns of Isolated Calcareous Corpuscles According to X-ray Diffraction Analysis.

| Treatment (18 hr) | Material from Chile | New Zealand | Lebanon |
|---|---|---|---|
| None | amorphous | amorphous | amorphous |
| 300°C | dolomite | dolomite | dolomite |
| 450°C | calcite, magnesium oxide | calcite, magnesium oxide | calcite, magnesium oxide |
| 600°C | calcite, calcium oxide, magnesium oxide, (apatite) | calcite, calcium oxide, magnesium oxide, apatite | calcite, calcium oxide, magnesium oxide, apatite |
| 900°C | calcium oxide, magnesium oxide (apatite) | calcium oxide, magnesium oxide, apatite | calcium oxide, magnesium oxide, apatite |

TABLE IV. Minor Components of Calcareous Corpuscles According to Emission Spectroscopic Analysis (Melpar).

| | Material from | | |
|---|---|---|---|
| | Chile | New Zealand | Lebanon |
| Aluminum | + | + | + |
| Boron | — | — | — |
| Cadmium | + | + | + |
| Copper | — | — | — |
| Iron | + | + | + |
| Lead | — | — | — |
| Manganese | — | + | + |
| Nickel | + | + | + |
| Sodium | + | + | + |
| Strontium | + | + | + |
| Tin | — | — | — |
| Titanium | + | + | + |

+ sign indicates that the compound occurs in the corpuscles; — sign that it has not been demonstrated.

nickel, strontium, and titanium which have not been reported from the previously studied species.

Our present findings indicate that the corpuscles isolated from the same species of tapeworm can show differences when worms from different geographic localities are studied. The differences, however, are minor when compared with the very major ones observed between various species. It is thus probable that the latter are essentially species-specific.

*Summary.* Calcareous corpuscles isolated from larval *Echinococcus granulosus* collected from sheep in Chile and New Zealand and from cattle in Lebanon showed only minor differences in their chemical composition and their heat-induced crystallization patterns. The only definite differences found were that the Chilean material lacked manganese, and that it had a somewhat higher magnesium and a lower phosphorus content than the other samples. As a consequence of the relatively low phosphorus content the Chilean material yielded upon heating at most traces of apatite. Previously described much more pronounced differences in chemical composition and crystallization patterns between corpuscles from different tapeworm species are hence probably essentially species-specific.

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## A Human Placental Fluid Inhibitor to Hemagglutination by H-1 and H-B Viruses: I. Purification.* (30544)

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The H-viruses are a group of small agents that produce a specific deformity in newborn hamsters. Two main types are known which are serologically distinct: H-1 virus which was first isolated from a transplantable human tumor(1,2) and subsequently from human placentas, embryos, and neoplasms(3), and RV, isolated from a rat tumor(4). H-3 is related to RV. A third and rare type of H-virus is HB which has only been isolated from a few fresh human placentas and from one fresh human neoplasm(3). It is serologically unlike either H-1 or RV. All these agents agglutinate red cells from a variety of animal species in distinctive patterns which allow diagnosis of their type(3,5). The hemagglutinating ability of H-1 which has been studied(6) has been found to be remarkably stable. Since it had been reported by Toolan that H-1 and HB could be isolated from human placentas, it was of interest when the same author(3) noted that all human placental fluids, even when extracted with Kaolin to remove non-specific inhibitors, still prevented the hemagglutination of H-1 virus with

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