Table II. Titers of anti-guinea-pig RBC antibodies in culture fluids of (i) normal rat thymocites, (ii) thymocites treated with RNA-IC from anti guinea-pig serum and (iii) thymocites treated with RNA-IC preincubated with ribonuclease

Titers	Slide hemagglutination test			Immunofluorescence test		
	Thymocites alone	Thymocites + RNA-IC	Thymocites + ribonuclease-treated RNA-IC	Thymocites alone	Thymocites + RNA-IC	Thymocites + ribonuclease-treated RNA-IC
1:2	—	++++	—	—	++++	—
1:4	—	++++	—	—	++++	—
1:8	—	+++	—	—	++++	—
1:16	—	±	—	—	+++	—
1:32	—	—	—	—	—	—

plaques of hemolysis in this case were very large and evident.

Results. Thymus and spleen cells of rats injected with RNA-IC from anti guinea-pig RBC serum, showed several hemolytic plaques in the antibody plaque formation test in comparison with the same cells from normal rats or from rats injected with ribonuclease-treated RNA-IC. The number of hemolytic plaques was greater in the thymus than in the spleen of the animals sacrificed 24 h after RNA-IC injection, about the same in the animals sacrificed 48 h after injection, and greater in the spleen than in the thymus of the animals sacrificed 72 h afterwards (Table I). Similar results were obtained with small thymus and spleen fragments (Figure 1). The culture fluid of rat thymocites, which had been in contact for 48 h with RNA-IC from anti guinea-pig RBC serum, had agglutinating power towards the same RBC at a dilution of 1:12, and the immunofluorescent test gave positive results till a dilution of 1:25. Culture fluids of normal thymocites or of thymocites which had been for 48 h in contact with ribonuclease-treated RNA-IC gave always negative results (Table II).

Conclusions. The presence in the serum of immunized animals of a RNA carrier of the antibody template capable of eliciting a rapid antibody response in vivo and in vitro is confirmed. The point of action of this RNA-IC is found in the lymphoid cells both in vivo and in vitro. In the animals injected in vivo with RNA-IC, specific antibodies appear rapidly in the thymus and rapidly disappear: the decrease of antibody production in the thymus corresponds to an increase in the spleen.

Riassunto. Nel siero di animali immunizzati è presente un RNA depositario del modello anticorpale che è capace di indurre la formazione di anticorpi in cellule linfoidi normali in vivo ed in vitro, sia di origine timica che splenica.

L. Michelazzi, A. Novelli, I. Baldini, U. M. Marinari, and G. Nanni

Istituto di Patologia generale, Università di Genova (Italy), June 18, 1965.

Studies on the Action of some Enzymes on the Cyst Wall of Isolated Metacerkariae[1] from the Liver Fluke, *Fasciola hepatica* L.[2]

The wall of the cyst of *Fasciola hepatica* consists of an outer strongly eosinophilic and an inner weakly eosinophilic layer[3]. Recent studies[4–6] have revealed that both layers are subdivided into two strata. Thus the outer layer of the wall consists of a spongy proteinaceous and an inner more fibrous predominantly mucopolysaccharidic layer. The inner layer of the cyst wall again contains an outer mainly mucopolysaccharidic layer and an inner fibrous proteinaceous layer possibly covered by lipids. The occurrence of phenolic substances in the cyst wall has also been pointed out[7]. At excystment the young fluke emerges through an opening in the inner layer. This excystment is induced in vitro by some added proteolytic enzymes but also by external systems devoid of enzymes[3].

As some enzymes act fairly specifically on their substrates, a series of enzymes of different origin and with different substrate specificity were chosen in the following analytical study of the cyst wall. The effect of the enzymic action was studied with light and electron microscopy.

Metacerkariae were obtained according to a method by Boray[8]. Ten of them were incubated at 38°C in small chambers containing 0.2 ml of 0.1 *M* phosphate buffer, pH 7.0 or 0.01 *M* HCl, pH 2.0, together with 0.2 mg of the active enzymes listed in the Table. Others were incubated without addition of enzymes and represented the controls. After 3 h of incubation the metacerkariae were

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washed 3 times in distilled water and studied with a light microscope at × 80 magnification. The metacerkariae were then fixed in buffered OsO_4 for 3–5 days and embedded in Epon[9]. Some specimens were stained with the periodic acid-Schiff (PAS) reagent or toluidine blue[10] and examined with a light microscope, while others were studied with a Siemens Elmiskop I at 60 kv.

The results obtained from observation in the light microscope at × 80 magnification are summarized in the Table.

Controls at pH 7.0 are shown in Figures 1 and 2. Other controls, especially those treated both at pH 2.0 and 7.0, showed a tendency of disintegration of the outer cyst layer. Therefore the effect of the different enzymes was difficult to evaluate.

The enzymes found to disintegrate the outer part of the capsule belong to the protein splitting group, viz. pepsin, pepsin + trypsin, papain, *Asp. oryzae* protease, pronase. The other enzymes tested did not visibly affect the layers of the capsule, with the exception of hyaluronidase which appeared to diminish the stainability with PAS and toluidine blue.

If the metacerkarial wall might be regarded as a substrate for the enzymes tested and the action is visible in the microscope, one might suppose from the results obtained that its *protein* is composed of amino acids joined to peptides poor in proline and hydroxyproline (collagenase), whereas the occurrence of L-forms of aromatic amino acids (pepsin), basic amino acids (trypsin, papain), leucine and glycine (papain) is possible. As the splitting with *Aspergillus oryzae* protease and pronase, known for a strong hydrolytic activity on a variety of proteins, is incomplete, it seems as if the cyst wall, the outer as well as the inner basophilic layer, is fairly resistant.

Effect of enzymes on metacerkariae of *Fasciola hepatica*

	pH optimum	Activator added	Effect[a]
Protein splitting enzymes[13]			
Pepsin, Worthington, 2 × cryst. 1961	~ 2.0		— ?
Trypsin, Worthington, 1 × cryst. 1961	~ 7.0		— ?
Pepsin, followed by trypsin as above			+
Collagenase, Worthington, 1961	~ 7.4		—
Papain, Pharm.	4.0–8.1	0.1 mg cystein	— ?
Aspergillus oryzae protease, T. Astrup, cryst.	6.0–7.0		— ?
Pronase, Calbiochem. 1961	~ 7.0		— ?
Carbohydrate splitting enzymes[13]			
α-Amylase, saliva, purified[9]	4.5–7.0	0.6 mg NaCl	—
Muramidase (lysozyme), Worthington, 1961	4.5–7.0		—
Hyaluronidase, Worthington, 1961	4.5–7.0		—
Lipid splitting enzymes[13]			
Lipase, pancreas, Worthington, 1964	6.0–7.6	0.3 mg $CaCl_2$	—
'Phospholipase C', Worthington, 1961	5.1–5.9	0.3 mg $CaCl_2$	—

[a] Compared with controls the meaning of the signs is as follows: + splitting of the capsule and beginning excystation; — no observable effect; —? changes of the capsule doubtful.

The weaker staining properties of the inner part of the capsule (PAS, toluidine blue) after treatment with hyaluronidase could point to the occurrence of acid *carbohydrate(s)* of hyaluronic acid-, chondroitin sulphuric acid A- or C-type. From the negative results with α-amylase

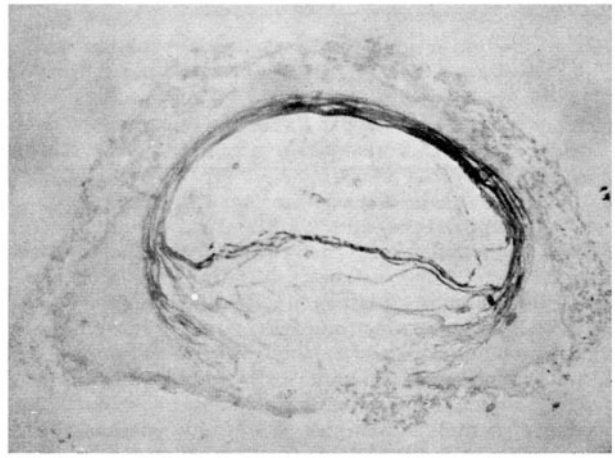

Fig. 1. Cross section of metacerkaria. Outer layer of capsule irregular and partly spongy, partly amorphous. Weakly stained. Inner layer fibrous and strongly stained. Note the morphological difference between the sealing material in the pore for excystment (bottom) and the rest of the cyst wall. Stain: Toluidine blue. Photomicrograph. × 375.

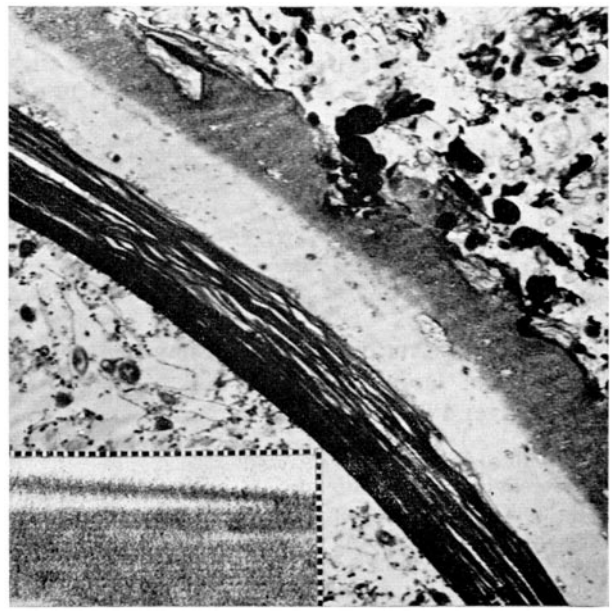

Fig. 2. Cross section of cyst wall showing an outer spongy layer, an intermediate amorphous layer with differences in electron density and an inner fibrous, strongly electron dense layer. Stain: Uranyl acetate. Electron micrograph × 2500. Inset: Enlargement of the fibrous layer showing lamellae with a distance of 7 nm between consecutive elements. The lamellae have serrated profiles with a spacing of 9 nm between the processes. × 140,000.

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and muramidase the occurrence of higher amounts of common α- and β-1.4-glucosidic linkages in neutral or acid polysaccharides might be excluded.

The supposed *lipids* (some unsaturated lipids are osmiophilic and PAS-positive) seem not to belong to common neutral fat or lecithins since no pronounced splitting was observed with lipase or 'phospholipase C'.

The excystment is induced externally by protein splitting enzymes (pepsin + trypsin) which has been confirmed in this study. Excystment has also been observed after treatment with bile, which normally contains protein and carbohydrate splitting enzymes, but also in enzyme free systems[3]. Thus the question whether excystment is an active internal process or not has been discussed[11]. Active internal processes have already been described in other connections with the life cycle of *Fasciola hepatica* when the miracidium emerges through the opening of the eggshell with the aid of a 'hatching enzyme' supposed to have protein splitting properties[12]. Whether the process of excystment depends upon external or internal factors, protein appears to be an important component in the material of the pore.

Conclusion. The effect of protein, carbohydrate and lipid splitting enzymes on the cyst wall of isolated metacerkariae from *Fasciola hepatica* seem to show that at least parts of the outer layer contain fairly resistant protein in which aromatic, basic amino acid, leucine and glycine could be components. The occurrence of acid carbohydrate(s) belonging to the group of hyaluronic acid or chondroitin sulphuric acid A or C in the inner layers is possible.

Zusammenfassung. Die Wirkung von protein-, kohlenhydrat- und lipidspaltenden Enzymen auf die Wand von isolierten Metazerkarien aus *Fasciola hepatica* scheint zu zeigen, dass wenigstens Teile der äusseren Schicht aus ziemlich resistentem Protein, das möglicherweise aromatische, basische Aminosäuren, Leucin und Glycin enthält, bestehen. Das Vorkommen von sauren Kohlenhydraten, insbesondere von Hyaluronsäure oder Chondroitinschwefelsäure A oder C in den inneren Schichten ist wahrscheinlich.

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The Concentration of Lactic Acid in the Human Aqueous Humour is not Determined by the Metabolism of the Lens

In the human subject, there is indisputably a high concentration of lactic acid in the aqueous humour[1], which is very marked in comparison to the plasma level (A/P ratio above 2). This higher concentration does not appear attributable to the glycolytic metabolism of the crystalline lens, as demonstrated by the fact that similar values are observed in eyes with a transparent lens or with cataract, even hypermature cataract. It might be argued that, in cataract, there still persists enough metabolic activity to produce the high concentration of lactic acid.

We were therefore interested in measuring lactic acid in aphakic eyes. This could settle the dispute about the importance of the lens for the high lactic acid content in the aqueous humour.

Our investigations were carried out in 5 patients in whom extraction of the lens of one eye had been performed earlier, and who presented cataract in the other eye. In 4 cases, the intracapsular operation was carried out, in one case the extracapsular variant. In these cases, samples of aqueous humour could be taken practically simultaneously from both eyes, and this avoided the disadvantages of having to compare data relating to different subjects.

As regards the technique of taking samples of aqueous humour and the technique of determination of the lactic acid concentration, the reader is referred to our earlier publications on this subject[1–3].

As can be seen in the Table, there is no significant difference between the concentration of lactic acid in the

Lactic acid concentration in the aqueous humour of the anterior chamber of human eyes with monolateral aphakia. Under I are given the values from the aphakic eye, under II the values from the non-aphakic eye of the same subject. The values are given in m*M* per kg H_2O

Name	Age	Date and kind of operation	Lactic acid concentration	
			I	II
A. Nicola	71	June 1964. Intracapsular extraction	5.03	4.45
F. Gennaro	41	February 1964. Intracapsular extraction	5.23	5.18
D. Francesco	60	October 1962. Intracapsular extraction	4.35	4.78
L. Giacomo	66	March 1961. Intracapsular extraction	4.54	4.28
S. Margherita	70	July 1961. Extracapsular extraction	4.92	4.56
Mean			4.81	4.65
Standard deviation			± 0.31	± 0.34
t			0.94	
P			0.40	

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