on *R. punjabicus*, revealed that the enamel prism pattern is very similar to that of *H. sapiens*.

Interpretation of these structural differences is at present conjectural. The differences in prism packing between living pongids and hominids may be correlated with differences in enamel thickness, pongids having thin enamel while hominids have thick enamel (*9*). The *Ramapithecus* molar exhibits thick enamel and a hominid-like prism pattern. However, before drawing any conclusions about phylogeny (that is, whether *Ramapithecus* is ancestral to later hominids), it will be necessary both to examine a full range of extinct Neogene hominoids and to analyze the functional significance of prism packing and enamel thickness (*10*).

The patterns obtained are reproducible, and the method used is nondestructive. Prism patterns appear to be potentially very interesting for functional analysis and, perhaps, eventually for phylogenetic and taxonomic purposes.

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Fascioliasis: Role of Proline in Bile Duct Hyperplasia

Abstract. *In animals with fascioliasis, extensive hyperplasia of the main bile duct occurs that often results in enlargement of the duct to more than 20 times the normal. We report that proline infused into the abdominal cavity of rats caused hyperplasia of the bile duct resembling that produced in the early stages of the disease. We suggest that* Fasciola hepatica, *which synthesizes and releases large amounts of proline, induces enlargement of the bile duct by a similar mechanism.*

Fasciola and several other fluke genera, among them *Dicrocoelium*, *Clonorchis*, and *Opisthorchis*, cause liver fluke disease. Every year thousands of cases of this disease are reported in humans, and losses from infected livestock total millions of dollars. Regardless of the host or fluke genus causing liver fluke disease, the pathology includes a usually striking enlargement of the main bile duct (*1*). The enlargement is due to cellular proliferation or hyperplasia that results in a thickened duct wall and a much infolded endothelium surrounding the enlarged duct lumen. This process is probably essential to the establishment of the disease, particularly for those flukes (including the genera given above) that inhabit the bile duct, because the normal bile duct is too narrow to accommodate the worms, which are of a relatively large size. Furthermore, the proliferated endothelium in the duct may provide an important source of nourishment to the worms (*2*, *3*).

Because hyperplasia of the main bile duct occurs while immature *Fasciola* are still in the liver parenchyma and long before they actually enter the duct, Dawes (*3*) suggested that the hyperplasia was induced by chemical rather than mechanical stimulation. This hypothesis was confirmed in our laboratory by using *Fasciola* implanted into the peritoneal cavity of rats (*4*). Hyperplasia of the bile duct occurred even though the worms were implanted only in the peritoneal cavity and were encased in a nylon mesh sack that precluded their direct contact with the liver or bile duct. This study also showed that the testing of various substances to determine their potential as inducers of the hyperplasia of fascioliasis could be greatly simplified. Introduction of the inducing agent directly into the bile duct or liver was not necessary because the inducer was effective from the peritoneal cavity. Hence the current investigation was based on this observation. In experiments in which test substances were pumped into the peritoneal cavity of rats we found that the amino acid proline may be the active agent inducing the bile duct hyperplasia of fascioliasis, a finding that could have broad implications not only for understanding a key element in the establishment of liver fluke symbiosis but also in the areas of morphogenetic regulation and tumor growth.

Proline is a major component of the free amino acid pool in bile fluid where its concentration increases by more than 10,000 times during infection by *Fasciola* (*5*). The production of this proline by the parasite is also indicated by the presence within the body of the worm of high concentrations of free proline (*6*) and of proline biosynthetic enzymes which show four to ten times the activity of their mammalian analogs (*7*).

The role of proline in inducing hyperplasia of the bile duct was suggested by several observations. First, proline concentrations in bile from host animals showed a fourfold increase by day 25 of infection, which was more than a month before the flukes entered the bile ducts. Although there was some increase in the other amino acids in the bile fluid, this was minor compared to the increase in proline and was not measurably different from normal until the worms actually entered the duct (*5*). Second, the bile duct tissue is rich in collagen whose biosynthesis is stimulated by increased levels of proline (*8*). Third, it is generally believed that bile duct hyperplasia occurs after stimulation of collagen synthesis (*9*), although a causal relation between the two effects apparently has not been documented.

Table 1. Comparison of lumenal perimeters of bile ducts from rats treated with saline, proline, or a mixture of amino acids. The data are for two experiments (A and B) and are expressed as means ± standard deviation.

Group	Treatment	Perimeter (mm)	
		A	B
1	Saline	2.03 ± 0.712	2.26 ± 0.796
2	Proline	2.94 ± 0.603	3.79 ± 1.421
3	Amino acid mixture	2.01 ± 0.519	2.12 ± 0.353
Significance		$F(2, 18) > .025$	$F(2, 18) >> .01$

To test the role of proline in bile duct hyperplasia, the amino acid was pumped intermittently for several weeks by way of a permanently implanted cannula into the abdominal cavity of rats. We then examined the bile ducts for evidence of hyperplasia. The procedures used will be described in detail elsewhere (*10*). For each experiment we usually used two or three groups of animals that were infused simultaneously for 1 to 2 weeks. Proline was an effective hyperplastic agent in all experiments. We report here only the results of experiments in which we tested both the hyperplastic effect of proline and the specificity of this compound in inducing hyperplasia compared with other free amino acids whose concentrations also increase in the bile fluid of infected animals (*5*).

Rats of approximately equal weight (200 ± 25 g) were used. In each rat, a cannula was permanently implanted in the abdominal cavity. Two to three days after surgery the rats were randomly divided into groups of eight to ten rats each. Once every 12 minutes during the experiments 0.04 ml of sterile infusate was pumped into each rat. The composition of the infusate varied according to the group. Group 1 received physiological saline; group 2, 20.0 m*M* proline in saline; group 3, a mixture of alanine, valine, methionine, leucine, tyrosine, phenylalanine, and histidine, all at 2.0 m*M* concentrations in saline. The concentration of proline was selected because it would result in a daily dose of about 100 μmole. This is approximately equivalent to the amount of proline found in the bile of animals that have been infected for 120 days (*5*, *10*). The concentrations of the amino acids used for group 3 were far in excess of the concentrations of these compounds in the bile of infected hosts.

At the end of the infusion period the animals were killed, and their bile ducts were removed and prepared for histological examination by standard techniques. Previous experiments indicated that the maximum effects of *Fasciola* infections on the main bile duct occurred in the region proximal to the liver, at a point just distal to the bifurcation of the main duct into the liver. Hence, in the present experiments, we sectioned approximately 6 mm of the main duct proximally until just beyond its bifurcation. Five to eight slides, each with 30 to 40 sections were prepared for each bile duct. The slides were labeled in a way that prevented possible bias in the selection and analysis of the bile duct sections.

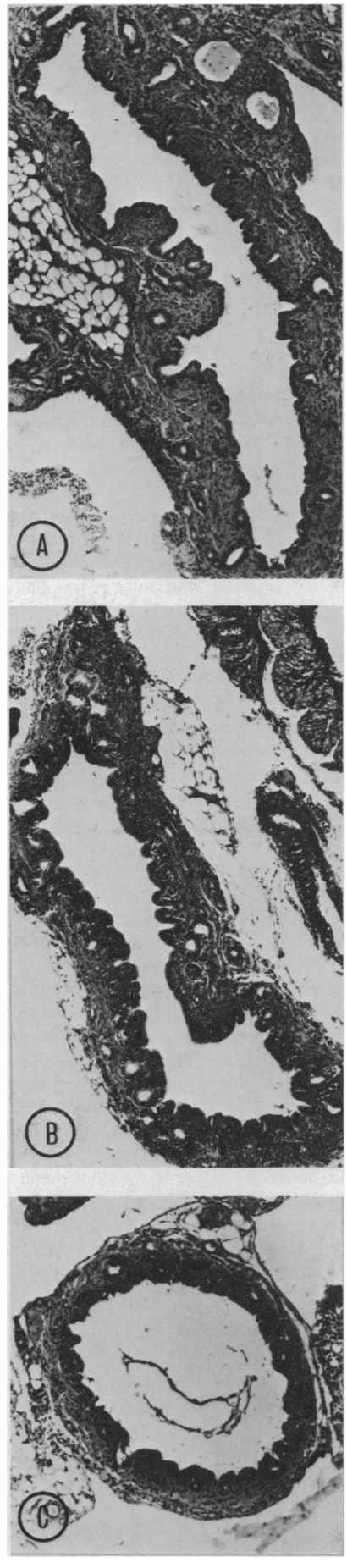

Fig. 1. Photomicrographs of the largest cross section of a rat bile duct enlarged to × 110. Tissues were fixed with formalin and stained with hematoxylin and eosin. In each case sections are typical of the treatment group. (A) Thirty-day infection by *F. hepatica*. (B) Fourteen-day infusion with 20.0 m*M* proline in physiological saline. (C) Fourteen-day infusion with physiological saline.

The most easily observable histological change that occurs very early in fascioliasis is the hyperplasia of the endothelial lining. For this reason we selected the perimeter of the lining as an index for comparing the effects of the different treatments, using the three largest cross sections (those just distal to the bifurcation of each bile duct) for measurement and analysis. A carbon arc microprojector was used to project the selected sections onto large sheets of paper, where tracings of the duct endothelial perimeters were made. Because of the extreme irregularity of the bile duct endothelium, we used the following procedure for perimeter measurements: After the tracing was secured to a cork panel, common pins were set in place closely along the entire inner perimeter of the duct. By threading dental floss between the pins and measuring the length of the floss required to encompass the duct lumen, we determined the perimeter of the duct lumen. An analysis of variance was performed on the data (see Table 1). The means of the duct lumenal perimeters were then compared among treatments by means of the Student-Newman-Keuls test. This test established in both experiments that (i) the mean for the group given proline was significantly different from the means for the groups given saline or other amino acids beyond the .05 level, and (ii) the mean for the group given amino acids did not differ significantly from the mean for the group given saline. When the individual *F* values were combined by the method of Sokal and Rohlf (*11*) we obtained a significance among the three treatments beyond the .005 level. Thus we conclude that the hyperplastic effect of proline was responsible for the highly significant *F* value. Histologically (Fig. 1), the bile duct from the proline-infused animals resembled that found in 20- to 30-day infections with *Fasciola*. In both cases, the ducts are enlarged, the walls are hyperplastically thickened, and blebbing is usually evident in the cells of the proliferated, convoluted endothelium. However, proline-treated ducts show sparse mononuclear cell invasion and sparse giant cell formation. These cellular reactions occur in ducts harboring *Fasciola* but their occurrence in the absence of antigen would not be expected.

Although we have shown that proline can induce a hyperplasia of the bile duct, we have not definitely established that this is the mechanism by which *Fasciola* establishes itself in its mammalian host. Studies with the proline analog, 1-azetidine-2-carboxylic acid (*12*), should answer this question. Should the proline inhibitor fail to interfere with establishment of *Fasciola* in the bile duct or with enlargement of the main bile duct when the flukes are implanted in the abdominal cavity, then some other mechanisms may be operating. However, an inhibitory effect by proline inhibitors would strongly support the hypothesis that proline is the mediator of the hyperplastic response in fascioliasis. In any event, investigations of the effects of 1-azetidine-2-carboxylic acid on *Fasciola* infections should be of interest in determining a possible therapeutic role for this compound. Indeed, Senft (*13*) suggested that proline analogs be tested for their activity against the human blood fluke *Schistosoma* which is also rich in free proline.

If proline is the agent which induces hyperplasia of the bile duct in fascioliasis, it is likely that this mechanism of bile duct enlargement occurs in other liver fluke diseases since high levels of free proline have been found in all trematodes examined thus far.

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Measurement of the Human Magnetic Heart Vector

Abstract. *A unipositional lead system has been developed to record the human magnetic heart vector and to permit comparison with the electric heart vector recorded with a conventional Frank lead system. Recordings made in five normal subjects showed a remarkably consistent relation between the electric and magnetic heart vectors. However, the angle between electric heart vector* R *and* T *waves was markedly different from the magnetic heart vector* R-T *angle. In addition, recordings made in two patients with bundle branch block showed a different relation between the electric and magnetic heart vectors compared to normal subjects. These data support the hypothesis that magnetic measurements have a different sensitivity to some components of cardiac activation compared with body surface potential measurements.*

Since the first recording of the magnetocardiogram (MCG) by Baule and McFee (*1*), considerable effort has been devoted to theoretical analysis of the magnetic field produced by cardiac electrical activity (*2*). While such analysis is complicated by the inhomogeneous nature of the human body, some investigators have concluded that the MCG contains information about intracardiac current sources that is unattainable from surface potential measurements alone (*3*, *4*). For instance, mathematical analysis shows that the electrocardiogram (ECG) is more sensitive than the MCG to cardiac current sources that are radial with respect to the cardiac center (*5*), whereas the MCG is primarily sensitive to tangential sources (*6*, *7*). This finding is applicable to a model of cardiac excitation that is based on a moving depolarization wave front which could be pictured as a double layer of charge, or on a model using distributed current dipoles (*4*).

Several studies have been made to determine whether such sensitivity differences might provide clinically useful MCG data. A-C coupled magnetometers with a typical bandwidth of 0.1 to 40 hertz have been used to record the magnetic field perpendicular to the chest wall (B_n) at multiple precordial locations (*8*, *9*). Such "maps" may aid in the analysis of local cardiac activation events, particularly in the free wall of the right ventricle and intraventricular septum (*9*). Mapping of B_n is analogous to detailed ECG mapping of body surface potentials (*10*) and reflects nondipolar as well as dipolar components of cardiac activation. A systematic analysis of the relation beteen maps of B_n and maps of ECG surface potentials does not appear to have been made.

A different approach to magnetocardiography suggested by Baule and McFee (*11*) is to measure the magnetic heart vector (MHV), which is related to the magnetic dipole moment of the heart. An ideal MHV lead system would detect the magnetic field from the tangential components of cardiac current in an element of myocardium with a sensitivity proportional to the distance of the active element from the cardiac center, and would be equally sensitive to cardiac events occurring in the anterior and posterior portions of the heart. The MHV obtained with such a lead system would have the advantage of being easily compared with the electric heart vector (EHV), such as commonly recorded with the Frank lead system for vector electrocardiography.

Baule and McFee measured the sagittal component of the MHV, but did not construct a lead system capable of measuring all three MHV components. Rosen and Inouye (*12*) recorded the vector magnetic field over the precordium and used the data at the instant of maximum signal to determine the components of a fixed magnetic dipole model. However, signal quality was not optimal, the distortions produced by the torso boundaries and inhomogeneities were not discussed, and apparently no attempt was made to compare these data with the vector electrocardiogram. Matelin (*13*) recorded two orthogonal MHV components. Wikswo (*14*) examined the temporal and spatial dependence of the vector magnetic field around the thorax and interpreted the data in terms of a moving magnetic dipole model.

To overcome the practical limitations of other MCG recording techniques, we have designed a "unipositional" lead system employing a SQUID (superconducting quantum interference device) differential magnetometer system located above the anterior chest wall of a supine subject, with the instrument axis through the estimated center of the ventricular chambers (*4*, *7*). Both pickup coils of the magnetometer are oriented at an angle of 54°44′ to the instrument axis so that three orthogonal components (B_A, B_B, and B_C) of the magnetic field can be recorded with successive 120° rotations of the magnetometer. A linear transformation converts these com-