In three of the four chiniquodontid genera, articulation between skull and jaw is still technically of the old quadrate-articular type. The quadrate (with which the quadratojugal is combined) is a small element rather loosely lodged in a pair of notches in the squamosal; the articular is likewise small in size, forming the posterior end of a bar of bone braced against the inner surface of the large dentary; this bar is formed by angular, surangular, and prearticular elements. The posterior end of the dentary lies just above and slightly lateral to the articular, and close to the squamosal near the point where the quadrate is inserted. There is no osseous connection in these three genera between dentary and squamosal, but in consideration of the feebleness of the quadrate and articular, it is probable that the dentary and squamosal functionally took part, through joint ligaments, in jaw support. In a fourth chiniquodontid genus, from the Chañares beds, which I am formally describing elsewhere as *Probainognathus* (*6*), an articular connection between squamosal and dentary is definitely developed. The squamosal extends far down over the cheek, external to the back end of the dentary. On its inner surface here, just above and anterior to the point of quadrate articulation with the articular bone, is a rounded depression with distinct boundaries in which was obviously received the outer surface of the posterior end of the dentary. This is surely the initiation of the articulation which was later to become the more highly developed glenoid-condyle articulation of the mammal.

What is the phylogenetic position of *Probainognathus*? One type of argument is that we should term a mammal any form in which a squamosal-dentary articulation has been initiated. This seems unreasonable here; after all, *Probainognathus* is in other regards very close indeed to the other chiniquodontid genera, and is very far from attaining mammalian conditions in such important features as braincase construction. On the other hand, there are no known features which would bar *Probainognathus* and its close relatives from a position ancestral to mammals of the later Triassic. It is highly probable that we are here dealing with a cynodont close to, if not actually on the line of ascent to the class Mammalia.

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Hepatic Influence on Splenic Synthesis and Release of Coagulation Activities

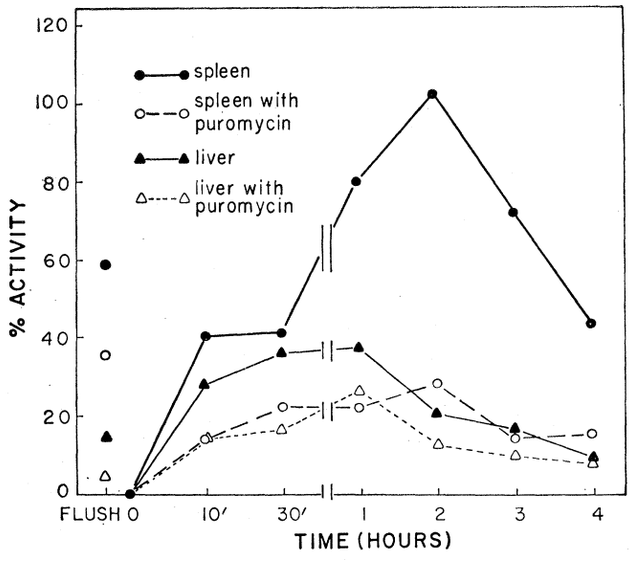
Abstract. *Isolated rabbit liver perfusates contain one or more puromycin-sensitive factors that stimulate factor VIII and IX activities in splenic perfusates. Production of coagulation activity by a complementary process involving organ perfusates suggests existence of coagulation factor precursors. An analogy is made to appearance of high factor VIII activity after plasma transfusions in von Willebrand's disease.*

Activities of factors VII, VIII, and IX that are puromycin-sensitive appear in isolated rabbit organs perfused with oxygenated fluid containing 30 ml of homologous red cells that were washed six times, 60 ml of human albumin (6 percent in Tyrode's solution), and 10 ml of 3.8 percent trisodium citrate (*1, 2*). The washed, packed suspension of red cells also contained platelets and white blood cells. This perfusion fluid contained no coagulation activities, and thorough flushing of the organs with Tyrode's solution followed by preliminary perfusion for 1 hour eliminated most of the stored coagulation factors. The mechanics of perfusion were carried out with a modification of the liver perfusion method of Miller (*2, 3*) adapted for splenic perfusion.

An isolated rabbit liver and spleen were perfused simultaneously as described above, each by an independent circuit. Most of the sequestered coagulation activities were removed from the organs by preliminary 1-hour perfusion with separate volumes of fluid. This initial perfusate, termed the "flush," was then removed, and the respective system was washed thoroughly with Tyrode's solution. Fresh perfusate was then added, and the perfusion was continued for 4 additional hours, the final perfusion period (*1, 2*). However, after 30 minutes of this final perfusion period, the effluents from both perfusion reservoirs were reversed so that the liver effluent now perfused the spleen and the spleen effluent, the liver. In some experiments, puromycin (25 mg) or cycloheximide (4 mg) was added to the perfusates during the final perfusion period. A series of control experiments included identical perfusion of liver-kidney and spleen-kidney combinations. Periodic samples of perfusates were taken, and assays for coagulation factors were performed as described (*1, 2*). Factor VII was assayed in a one-stage system with canine plasma deficient in factor VII (*4*); factors VIII and IX were assayed by the partial thromboplastin method, with canine plasma deficient in factors VIII and IX (*5*). Coagulation activities were expressed as a percentage of that obtained with a pooled standard of normal rabbit plasma which was assigned a value of 100.

Factor VII activity remained unchanged when the effluent of one organ was perfused though the other. When liver effluent was added to the spleen, however, factors VIII and IX activities were markedly different from those of spleen perfusions (*1, 2*). In contrast to these results, addition of spleen effluents to the liver circuit did not alter the coagulation activity.

Figure 1 shows the mean activity of factor VIII from 12 simultaneous liver and spleen perfusions without puromycin and from 6 parallel experiments with puromycin. Figure 2 shows the mean activity of factor IX from these same experiments. The passage of liver effluent through the spleen produced a rapid increase in both factors VIII and IX activities. Each activity reached a peak in 45 to 60 minutes, approached its normal level in plasma, and then declined rapidly. In the presence of puromycin, the liver effluent was without effect on coagulation factor activity in the spleen. The same results were obtained when cycloheximide was used as an inhibitor. Figures 1 and 2 show results of the reverse experiments in which spleen effluents were perfused through the liver. No stimulation of either factor VIII or IX activity was observed. Control experiments with liver-kidney or spleen-kidney perfusions did not show stimulation of factors VII, VIII, or IX activities be-



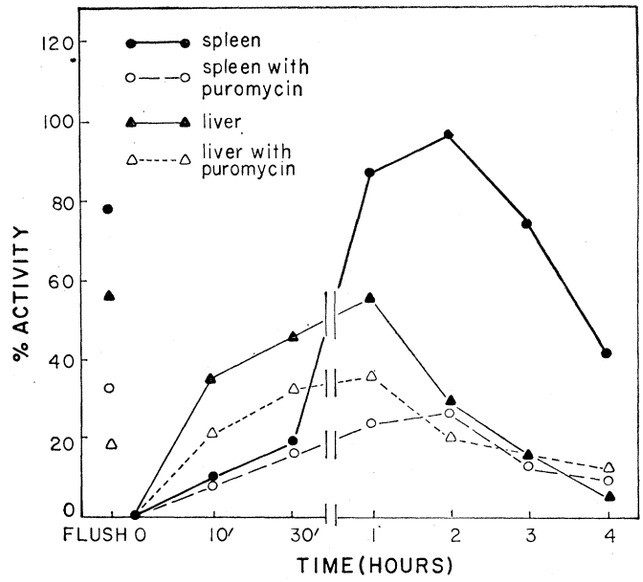


Fig. 1 (left). Mean factor VIII activity of simultaneous liver and spleen perfusions. Open and closed circles denote the activity of splenic perfusate in which liver effluent was added at 30 minutes; open and closed triangles represent the activity of liver perfusates with splenic effluent added at 30 minutes. The open circles and triangles denote experiments with puromycin. Values for the "flush" period represent sequestered factor VIII activity released during the initial 1-hour perfusion period (as described in text). Fig. 2 (right). Mean factor IX activity of simultaneous liver and spleen perfusions. Open and closed circles denote the activity of splenic perfusates in which liver effluent was added at 30 minutes, whereas open and closed triangles represent the activity of liver perfusate with splenic effluent added at 30 minutes. The open circles and triangles denote experiments with puromycin. Values for the "flush" period represent sequestered factor IX activity released during the initial 1-hour perfusion period (see text).

yond that seen with single liver, spleen, or kidney perfusions (*1*, *2*).

Mixing of isolated liver and spleen perfusates in vitro, with or without incubation prior to assay, generated no more factor VIII or IX activity than was present in either sample alone. Thus, the activation process appears to require passage of liver effluent through an intact spleen. In addition, portions of the material with peak activity did not shorten the recalcification time of pooled noncontact activated plasma from normal rabbits. This observation implies the absence of active thromboplastins.

We next investigated the rapid decrease in factor VIII and IX activities in the spleen effluent that occurred after the stimulation induced by liver effluent (Figs. 1 and 2). In six experiments this decline of activity was prevented by the addition of more liver effluent 15 minutes after the peak activity in the spleen effluent had been reached. Effluents from spleens perfused for 3 hours added to liver perfusions did not inhibit factors VIII and IX activities. These results suggested that the spleen was still responsive to liver material during 2 to 4 hours of perfusion, and that the rapid decline in activity probably was not caused by production of an inhibitor substance.

Preliminary characterization of the stimulating factor or factors of liver effluent indicated that it was stable to freezing at −70°C and to heating at 56°C for 30 minutes but was destroyed after 30 minutes at 60°C. Most of the factor IX coagulation activity and 30 percent of the factor VIII activity was destroyed at 56°C for 30 minutes. Dialysis of six liver effluents at 4°C for 2 days against Tyrode's solution did not alter their individual splenic stimulating capacities. Little change in effluent factor IX activity occurred after dialysis, and no factor IX activity could be recovered in any of the dialyzates. The factor VIII activity, however, varied in that there was some reduction after dialysis, and a significant amount of activity (2 to 9 percent) was recovered from the dialyzates. This behavior of factor VIII activity upon heating and dialysis is not understood, but the results suggest that the factor VIII and IX coagulation activities measured have somewhat different properties. The stimulating material of liver effluent appears to be moderately stable to heat and withstands dialysis.

The liver effluent material may contain a nonspecific trophic factor that affects synthesis of more than one intrinsic coagulation factor or some common precursor procoagulant. Alternatively, the liver effluent may contain one or more factors that rapidly convert inactive factors VIII and IX in the spleen to active products. The dramatic response of the spleen to additions of liver effluents provides an analog to the paradoxical increase in factor VIII activity seen when patients with von Willebrand's disease are infused with normal or hemophilia A plasma (*6*, *7*). This unusual response in Von Willebrand's disease has led to the hypothesis that von Willebrand's stimulating factor in plasma is missing in patients with the defect and probably in a precursor of factor VIII (*6*, *8*).

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