## Thromboplastic and Fibrinolytic Activity in Valves of the Pig Heart

Determinations of concentrations of plasminogen activator and thromboplastin in human and animal tissues have revealed distributions which in many instances could be correlated with patterns of tissue repair. Such observations have led to the formulation of an integrated concept of reparative connective tissue formation regulated by fibrin deposition and resolution[1]. There are only minute amounts of tissue thromboplastin and plasminogen activator in human heart valves with a limited number of fibrinolytically active sites localized to vascular structures in proximal parts of the atrioventricular valves and to parts of the chordae tendineae close to the attachments to the papillary muscles[2]. Occasional sites of fibrinolytic activity in the distal parts of the atrioventricular and semilunar valves were all related to the endocardial lining. Recently, assays of thickened valves from 2 human hearts revealed no marked differences from normal valves. The low plasminogen activator concentrations in the cusps are not surprising since their ground plate consists of a tendinous membrane, a connective tissue structure in which fibrinolytic activity has never been observed. However, since fibrinolytic activity is related in particular to endothelium of the venous system[3] and the endothelium of the large vessels is continuous with the endocardial lining, the low values in all the valves were somewhat unexpected. We wish to report some data on pig heart valves because they are known to be extensively provided with blood vessels in contrast to the mostly avascular human heart valves. The concentrations of tissue thromboplastin and plasminogen activator were determined by extraction methods as described before[2]. These data would also be of interest in view of the recent use of heart valves from pig as substitutes for human aortic valves[4] (Table).

In heart valves of animals vessels extend beyond the margin of insertion into the valve ring Therefore, in this study valves were not divided into a proximal and distal part. The content of tissue thromboplastin was as low as in human valves whereas plasminogen activator concentrations were markedly higher, in particular when compared with the distal parts of the human valves. Interestingly, the myocardium has considerably higher fibrinolytic activity in pig than in other species including man[5]. Usually concentrations of plasminogen activator are lower in animals than in man. Though the usual large individual variations in plasminogen activator concentrations were encountered, even in assays of different samples from the same valve specimen, no marked differences between different valves of the pig heart were observed. As in the human heart the absence of marked differences between the valves of the left and right side of the heart is particularly noteworthy. There are many reports on vessels in heart valves of animal species such as the pig, sheep, dog, cat, horse, cattle and calf[6]. In contrast, valves from rabbit and guinea-pig have been reported as avascular[7]. The results of our fibrinolytic assays are in agreement with the reported presence of vascular structures in pig heart valves. Individual variations and differences observed between different samples from the same specimen could probably be caused by different degrees of vascularization. Since the fibrinolytic assays were performed by an extraction method which incorporates an acid precipitation, the presence of labile activators originating in the blood could be excluded.

The presence of blood vessels in the valves of the pig heart would make them depend less on diffusion of nutrient material and oxygen from blood surrounding their surfaces. Hence, fibrin deposition following valvular injury and disruption of agglutinated platelets, substituting for the missing tissue thromboplastin, would lead to less impairment of valvular nutrition in pig than in man. Fibrin deposits would also be retained for briefer periods because of the higher fibrinolytic activity. The presence of vascular structures accompanied by fibrinolytic activity could possibly explain the infrequency of cardiac disease reported in animal species[8]. The


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Thromboplastic and fibrinolytic activity of pig heart valves

| Animal No. | Thromboplastic activity (U/g) | | | | Fibrinolytic activity (U/g) | | | |
|---|---|---|---|---|---|---|---|---|
| | Mitral valve | Tricuspid valve | Aortic valve | Pulmonic valve | Mitral valve | Tricuspid valve | Aortic valve | Pulmonic valve |
| 3 | 1 | 5 | 1.5 | 14 | 122.5 (66; 179) | 74 | 87 | 95 |
| 5 | 3 | 2.5 | 3 | 3 | 64 | 35 | 23 | 49 |
| 6 | 3 | 4 | 14 | 5.5 | 60 | 45 (43; 47) | 47 | 68 |
| 7 | 4 | 1.5 | 0.5 | 3 | 79.5 (103; 56) | 41 (34; 48) | 44 | 103 |
| Average | 3 | 3 | 5 | 6 | 82 | 49 | 50 | 79 |

Thromboplastic activity is recorded as concentrations of tissue thromboplastin in U/g fresh tissue assayed on human plasma. Fibrinolytic activity is recorded as concentrations of tissue plasminogen activator in U/g fresh tissue. The 2 separate determinations reported in some of the fibrinolytic assays are from 2 different samples from the same specimen. Averages for each specimen were then determined and used in the calculation of the final estimate.

fibrinolytic activity in the valves would not remain after the sterilization procedures used in preparing them as prostheses for human aortic valves[9].

*Zusammenfassung.* Herzklappen vom Schwein wurden auf ihre thromboplastische und fibrinolytische Aktivität untersucht. Die Konzentration von Gewebsthromboplastin war in allen Herzklappen (Segel- und Taschenklappen) gering und stimmt mit der in menschlichen Herzklappen gemessenen überein. Im Gegensatz dazu weisen Schweineherzklappen eine höhere fibrinolytische Aktivität als menschliche Herzklappen auf, wahrscheinlich als Folge der reicheren Vaskularisierung.

PIA GLAS and T. ASTRUP

*The James F. Mitchell Foundation, Institute for Medical Research, Washington (D.C. 20015, USA), 28 October 1968.*

[9] Supported by grant No. HE-05020 from the U.S. Public Health Service, National Institutes of Health, National Heart Institute.

## The Transport of Various Immune Globulins by the New-Born Pig Intestine

The new-born pig obtains a passive immunity to disease by the ingestion and subsequent intestinal absorption of antibodies present in the sow's colostrum. The appearance of the intestinal mucosa shortly after birth suggests that protein is being endocytosed by the mucosal cell[1], but the mechanism which initiates endocytosis or the means by which protein leaves the cell is not known. Any demonstration of selectivity during absorption would help to define conditions critical for the movement of protein molecules. The present work was therefore designed to test whether immune globulins derived from different species would be transported at different rates by the pig intestine used in vitro.

*Materials and methods.* Piglets collected at birth before they could suck the sow were killed by decapitation and everted sacs of small intestine formed and incubated in bicarbonate saline as described previously[2]. Incubation was for 2 h at 37 °C in medium containing glucose, 1 mg/ml, together with a known preparation of IgG (final concentration 5 g/100 ml). Fluid removed from the sacs at the end of incubation was weighed and samples later analysed for sodium using an EEL flame photometer, for glucose using the glucose oxidase method of HANSEN[3] and for IgG using the quantitative immunodiffusion assay of GELL[4]. Intestinal sacs were weighed at the end of incubation and serosal transfers calculated as amount transferred g intestine/h incubation. Values have been represented in each case as per cent change from values found in the presence of porcine IgG to lessen errors due to animal variation. Direct comparisons were made between alternate sacs, one incubated in bicarbonate saline containing porcine IgG and the other incubated in saline containing IgG prepared from a different species. The significance of any difference was assessed using the Wilcoxon test[5]. Human, equine and ovine $\gamma$-globulins (Koch-Light Labs. Ltd.) were stated to contain $\geq 98\%$ $\gamma$-globulins estimated by electrophoresis on cellulose acetate. The purity of bovine and porcine $\gamma$-globulins (Armour Pharmaceutical Co. and Pentex Inc. respectively) was stated to be $> 95\%$. All preparations had been manufactured using the same Cohn fractionation technique.

*Results and discussion.* The purity of these various proteins was first assessed by comparing their sedimentation patterns on ultracentrifugation (Figure 1). Protein sedimenting as the major peak corresponded to 7S globulin (IgG globulin). The small amount of material which sedimented faster was thought to consist of aggregated IgG. The proportion of aggregated to 7S IgG was lowest for ovine and highest for porcine preparations, but the difference between individual samples was small. Equine IgG contained about 5% (w/v) sodium chloride and this was removed by dialysis. The proportion of 7S to aggregated IgG was not changed by this treatment or by subsequent freeze drying (Figure 1 compare E with E(d)). Salt-free equine IgG was used for all further work. The homogeneity of these proteins was next assessed by comparison of their immunoelectrophoretic patterns (Figure 2). Antibodies to porcine, equine and bovine IgG globulins had been raised previously in rabbits. Antisera to human and ovine IgG light chains, prepared in sheep and rabbit respectively, were used to precipitate the whole IgG molecules from these species. Antiserum to bovine IgG produced a precipitin line of partial identity with

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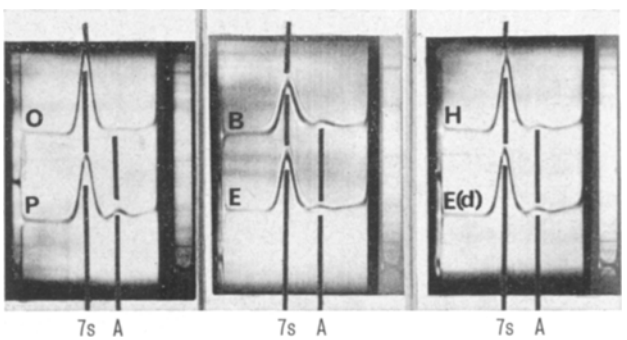


Fig. 1. Ultracentrifugal patterns obtained using commercial preparations of $\gamma$-globulins prepared from 5 different species. The final concentration of each protein, dissolved in phosphate buffer $I = 0.1$, pH = 7.5, was 0.8% w/v. Centrifugation was at room temperature for 55 min at 59,780 rpm O, P, B, H and E, ovine porcine, bovine, human and equine $\gamma$-globulins. E(d), equine $\gamma$-globulin after dialysis. A, aggregated globulin. 7S, globulin having a Svedberg coefficient of approximately 7S.