gate comes in contact with enzymes which break down the aggregate to produce a hormone molecule which is active at the cellular level.

Conclusion. Human growth hormone has been shown to be composed of two components as indicated by tibia assay. One of the components at neutral pH will pass through cellulose dialyzing tubing in the presence of ammonium ion, urea or glycine.

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Myocardial Depressant Factor in Plasma from Cats in Irreversible Post-Oligemic Shock.* (31090)

EUGENE D. BRAND AND ALLAN M. LEFER (Introduced by D. R. H. Gourley)
Departments of Pharmacology and Physiology, University of Virginia School of Medicine, Charlottesville

Post-oligemic shock, irreversible to blood transfusion therapy, has been reversed by therapy with large volumes of water and solutes in the dog(1) and in the cat(2). Because in the studies of Brand *et al*(2), blood or low molecular weight dextran did not reverse post-oligemic shock, it was reasoned that simple cardiovascular support did not wholly account for this effect. The positive correlation of a large urine volume with survival incidence and duration suggested that removal of a toxic factor from the plasma into the urine might be a major mechanism in the observed reversal of shock. Several authors (3,4,5) have found myocardial weakness in irreversible post-oligemic shock. Other workers have postulated the presence of a toxic factor in the blood of rabbits and dogs in post-oligemic shock(4,6,7,8,9) which might act by depressing the heart. In apparent contrast to these findings *in vivo, in vitro* studies of papillary muscles removed from rats and cats early in shock and bathed in a buffer solution show them to function normally (10,11). Taken together, these observations suggest that a myocardial depressant factor is present in the plasma during shock. This substance may diffuse out of the muscle into the bath solution, so that the muscle then functions normally.

In the present investigation we used isolated papillary muscles to test this hypothesis.

Methods. Shock was induced by the method of Brand *et al*(2) in healthy adult cats of either sex. The cats were anesthetized with pentobarbital (35 mg/kg i.p.), heparinized (2250 units/kg), and bled from the femoral artery into a reservoir in which the pressure was maintained between 40 and 45 mm Hg.

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TABLE I. Influence of Different Bath Solutions and Plasmas on Developed Tension in Cat Papillary Muscles.

Solution in bath	% Change in developed tension from indicated control ± SEM	No. of muscles tested	Different from indicated control P
I. Control papillary muscles (from normal anesthetized cats)			
Control plasma (with anesthetic)	—	16	—
Plasma from early shock	−26 ± 6.1*	8	<.001*
Plasma from late shock	−43 ± 9.2*	8	<.001*
Plasma without anesthetic	+ 8 ± 2.3*	6	<.025*N.S.†
Control KH solution	+14 ± 2.3*	12	<.001*
KH at pH 7.05	− 4 ± 2.9†	11	N.S.†
" " " 6.75	− 5 ± 3.2†	8	<.025†
Pentobarbital	− 7 ± 1.7†	6	<.001†
II. Papillary muscles removed from cats early in shock			
Control plasma (with anesthetic)	—	22	—
Plasma from early shock	−27 ± 4.1*	22	<.001*
Plasma from late shock	−62 ± 9.2*	8	<.001*

* Control = control plasma (with anesthetic).
† Control = control Krebs-Henseleit (KH) solution.

When the cats had bled out maximally and then spontaneously taken up from the reservoir 40% of the maximum bleeding volume, the remaining shed blood was reinfused intraarterially.

The term "early" shock refers to that period from 10 to 30 minutes after reinfusion during which mean arterial blood pressure was at normal levels. The term "late" shock refers to that time after reinfusion (avg, 5 hrs, 0.8 to 14 hrs range) when the mean arterial blood pressure had fallen to 60 mm Hg.

Plasma was obtained by centrifuging blood removed either from the femoral artery or from the chest after cardiectomy. All plasma was frozen immediately after collection and thawed to room temperature before testing.

Two papillary muscles were removed from the right ventricle of each cat as described by Lefer(12). Each muscle was placed in a 20 ml chamber containing a modified Krebs-Henseleit (KH) solution(12) maintained at 37° ± 0.1°C. Threshold voltages and length-tension relationships were determined for each muscle. The muscles were then stimulated at a frequency of 1 per second, for a duration of 20 msec at 2 volts above threshold voltage. Isometric contractions were recorded on an Offner Dynograph using Grass force transducers. KH solution at 37°C was used before and after each test solution. Test solutions were added at room temperature (27 ± 2°C).

Results. Table I summarizes the results of our studies.

Muscles from normal and from shocked cats. When the muscles were bathed in KH solution, there was no difference in the function of those removed from normal cats and those from cats early in shock. Both groups of muscles had similar thresholds of excitability, similar developed tensions, and similarly enhanced inotropic responses to potassium and similarly depressed inotropic response to plasma from cats in early and in late shock. These results concur with those reported previously(10,11) indicating that there is no apparent malfunction of myocardial tissue from animals early in shock after it has been washed in a muscle bath.

Reversible myocardial depressant effect of plasma from cats early in shock. Plasma from cats early in shock (Table I) depressed developed tension significantly in both groups of muscles. Complete reversal of this depression was accomplished when the papillary muscles were subsequently bathed in KH solution. These observations confirm the hypothesis that a diffusible and reversible myocardial depressant is present in the plasma early in shock. Threshold of excitability was normal throughout.

Increased and irreversible myocardial depressant effect of plasma from cats late in shock. Plasma from cats late in shock (Table

I) depressed developed tension to a greater degree than did plasma from cats early in shock. Reversal of this effect was only about 50% complete on washing with KH solution.

Pentobarbital, which was added to the KH solution in the bath (at 37°C) to make a concentration equivalent to that in the cat plasma (0.02 mg/ml), depressed developed tension to a small but significant extent (−7% of control). The pentobarbital present in the plasma of control anesthetized cats depressed developed tension significantly also. Developed tension was 8% lower than in plasma from unanesthetized cats.

Plasma potassium in 5 control cats averaged 4.8 ± 0.66 mEq/l. Plasma from 5 cats in early shock contained 6.7 ± 0.13 mEq/l of potassium and plasma from 5 cats in late shock contained 8.1 ± 0.26 mEq/l potassium. Addition of KCl to the KH solution in the bath (at 37°C) to make 9.75 mEq/l potassium resulted in a significant increase in developed tension in papillary muscles from both control and from early shocked hearts (+18 and +13%, respectively). It is highly unlikely, therefore, that the elevation of plasma potassium found in shock accounts for the observed depression of myocardial contractility.

Plasma pH is low in cats in both early and in late shock (avg, 6.95 and 7.10, respectively)(2). Adjustment of the KH buffer from a pH of 7.30 to a pH of 7.05 ± .05, with 1 N HCl, did not significantly depress developed tension but adjustment to a pH of 6.75 ± .05 depressed developed tension significantly (−5%). These data suggest that lowering of the plasma pH in shock may account for a small portion of the depression of myocardial contractility.

Plasma osmolality rises in shocked cats from an average control value of 300 mOs/l to an average of 340 mOs/l(2). The finding of Koch-Weser(13) that raising osmolality 50 mOs/l increased developed tension of cat papillary muscles by 10% suggests that increased osmolality of plasma is not an important mechanism in the depression of developed tension by shock plasma.

The temperature of the solution added to the muscle bath influenced myocardial contractility. Plasma or KH solution, when added at room temperature (27 ± 2°C) to the muscle chamber, resulted in a marked transitory increase in developed tension followed by a fall to below the control level (−11 ± 1.8% and −16 ± 1.8%, respectively; $p < 0.001$ in both instances). The fall was maximal at 5 to 10 minutes and over the succeeding 10 to 20 minutes (as the temperature of the solution approached 37°C) the developed tension returned to the control value. The end point used to measure the response in all of these studies was the minimal developed tension which occurred at 10 to 20 minutes after changing the bath solution. All calculations have been made such as to eliminate this thermal artifact.

Discussion. These data show that plasma from cats in early shock reversibly depresses myocardial contractility. This finding, coupled with our previous observation that "irreversible" post-oligemic shock in the cat can be reversed by infusion of large volumes of a solution of physiologic electrolytes and glucose, gives strong support to the following hypothesis: the cardiovascular failure of "irreversible" shock is due, in large part, to depression of myocardial contractility by one or more diffusible substances in the plasma. Removal of this substance can reverse this pathogenic mechanism of irreversible shock.

Our finding of even greater, and less reversible, myocardial depression with plasma from cats late in shock suggests that late in shock, a higher concentration of the depressant substance(s) accumulates or an additional depressant mechanism has appeared.

Summary. Plasma from cats in the early stage of irreversible post-oligemic shock depressed contractility of isolated cat papillary muscles. This depression was reversed completely by washing. Plasma from cats late in post-oligemic shock depressed developed tension to a greater degree, but this depression was only partly reversed by washing.

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Separation of Trypsin Inhibitors of Human Plasma on DEAE-Cellulose.* (31091)

JOHN W. MEHL, MAI YOUNG PARK,† WILLIAM O'CONNELL‡

Department of Biochemistry, University of Southern California School of Medicine, Los Angeles

It has been recognized for some time that there are at least two trypsin inhibitors in human plasma. One of these is quite stable to temperatures around 60°C and at pH values around 4-5, while the other is quite unstable under these conditions. It has also been possible to demonstrate two components by electrophoresis on paper or starch, the minor component being an α_2-globulin and the major component an α_1-globulin which is unstable to heating and low pH values. The latter inhibitor has been obtained in a highly purified form(1,2,3) and shown to be a very effective inhibitor for chymotrypsin as well as trypsin. The nature of the more stable inhibitor has been in question, since it is present in relatively smaller amounts. The work of Shulman suggested that it may be an α_2-globulin with a molecular weight of about 16,500(4), but that conclusion was based primarily on the properties of material isolated from urine. More recently, a third component has been recognized(5) which combines with trypsin and chymotrypsin to give enzymatically active complexes which can no longer be inhibited by soybean trypsin inhibitor or the inhibitors from plasma. It now seems possible to identify this last component with α_2-macroglobulin(6), and with plasmin inhibitor(7), as well as with at least part of the α_2-trypsin inhibitor(8). In trypsin inhibitor assays it will appear as an inhibitor, since the activity of the trypsin (or chymotrypsin) complex with α_2-macroglobulin is less than that of the free enzyme.

The present work was initiated in an effort to obtain the α_1-trypsin inhibitor by as mild an isolation procedure as possible, and to separate it from other trypsin inhibitors in plasma. Although it has, in some measure, proved successful in this respect, it has also provided further information regarding the multiplicity of trypsin inhibitors in human plasma. It has been found that chromatography on DEAE-cellulose permits the separation of the trypsin-binding activity from the α_1-trypsin inhibitor, as well as the separation of a third inhibitor which is very stable to heat and low pH. The results of the fractionation of approximately one liter batches of ACD plasma will be described, together with some of the properties of the fractions. Since

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† Taken in part from a dissertation submitted by Miss Park to the Graduate School in partial fulfillment of the requirements of the Ph.D. Present address: Hospital of the Children's Society of Los Angeles.

‡ Present address: University College, Faculty of Agriculture, Glasnevin, Dublin, Ireland.