Action of Human Plasma on the Isolated Frog Heart
Observations on Subjects with and without Essential Hypertension

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Plasma samples from certain patients affect the staircase phenomenon of the frog heart. Comparison of 3 groups of subjects, normal controls, patients without essential hypertension, and patients with severe essential hypertension shows that plasmas from the last group generally abolish the staircase, whereas plasmas of the other 2 groups exhibit little or no such activity.

The subject of this report is a study of the effect of human plasma on two bioassay systems, the isolated frog heart and the hamster cheek pouch. The experiments were designed to compare the action of plasma samples from patients with essential hypertension with those from 2 control groups.

Method
Selection of Material
The subjects for this study were separated into 3 groups.

Group I. Eighteen hospitalized patients with severe essential hypertension. The information provided by history and clinical studies in this group was sufficient so that known causes of hypertension could be excluded with reasonable certainty. All patients had high blood pressures which, except for certain periods during which they received antihypertensive agents and during the terminal phase of one patient, remained elevated at bed rest. A range of pressures was determined for each patient; the mean for the group was 185/110-225/140. Individuals with mild or transient elevations of blood pressure were not studied. The known duration of the illness averaged 8 years. All patients showed some evidence of secondary changes commonly associated with hypertensive disease. Seven patients exhibited vascular abnormalities in the fundus oculi of grade 3 or 4 (Keith-Wagener-Barker classification), 6 had suffered cerebrovascular accidents and 1 had hypertensive encephalopathy. Three patients were in severe cardiac failure, some clinical symptoms of myocardial insufficiency were present in an additional 5 and all but 1 showed at least electrocardiographic or radiographic evidence of cardiac abnormalities. Since an attempt was made to include in this group only patients with essential hypertension and to exclude cases of elevated blood pressure secondary to chronic renal disease, the incidence of renal abnormalities is rather low. Seven patients had no evidence of renal disease, there were borderline changes in 3, moderate functional changes or urinary sediment abnormalities in 5, and uremia in 2 cases. Blood samples were drawn without regard for therapeutic regimen.

Group II. Twenty-four patients without essential hypertension. In addition to a number of normotensive individuals with various diseases, a group of patients with secondary hypertension is included. They comprise 5 young women with pre-eclampsia or eclampsia, and also 5 patients with hypertension thought to be secondary to chronic renal disease. The latter patients include 1 man whose hypertension disappeared after removal of an infarcted kidney, 2 women with long-standing pyelonephritis, and 2 men with long histories of recurrent nephrolithiasis and associated pyelonephritis. Clinical classification was made before the assays were performed. The hypertension of the renal disease patients, with the associated changes in heart and blood vessels, was comparable in severity to that of the cases of essential hypertension in group I.*

Group III. Fourteen normal controls, employees of the National Institutes of Health. The criteria for selection were no significant illness and normal blood pressure (one determination, made just before the blood sample was drawn). The age range of these subjects corresponded approximately to that of the essential hypertension group.

Bioassay Procedures
Ten milliliters of venous blood were drawn from nonfasting subjects into an oiled, heparinized syringe. The blood was centrifuged at 2000 r.p.m. for 20 min., the plasma separated and used for the bioassays described below about 1 hour after the blood had been drawn.

* Detailed tabulation of the patients in groups I and II can be obtained from the authors on request.
**Frog Heart Assay.** This assay is based on the fact that when a frog ventricle is stimulated electrically the isometric tension developed varies with the interval between successive stimuli over a certain range, increasing as the stimulation frequency interval is decreased. To achieve any chosen tension level a certain frequency interval is needed, which will be designated as the critical frequency interval (CFI) for that tension level. Certain substances, among them cardiac glycosides, are capable of increasing the CFI needed to achieve any given tension level. For compounds that do not bind to the heart muscle, the effect on the CFI is concentration-dependent and a plot of concentration against the logarithm of the CFI gives an approximately straight line relationship. Once this relationship is obtained using a known compound, the effects on the CFI of unknown substances can be determined and can be compared to the action of the standard compound. The details of this method and its application in the isolation of a cardiac-active material of biologic origin have been described.

For the assays performed in this study, 1 ml. plasma was added to 1.5 ml. Boyle-Conway solution and 0.5 ml. water. (The water was added in order to make the final solution isotonic with frog plasma.) The 3.0 ml. of Boyle-Conway solution bathing the frog ventricle was replaced by this mixture. Activity was tested over the next 10–15 min. by determining the CFI that would maintain maximum tension response. Finally, the plasma mixture was removed and the preparation washed several times with Boyle-Conway solution, so that the heart muscle response could be compared with the preplasma control state. Comparing the CFI in the presence of plasma with the CFI found in the presence of a known concentration of a standard compound (strophantidin/ml. plasma), it was possible to determine a concentration of the activity in each plasma in terms of strophantidin/ml. plasma. Each column represents a range of 0.25 pg. Ordinate: number of plasmas in each activity range.

**Hamster Cheek Pouch Assay.** Human plasma when applied topically to the cheek pouch of the golden hamster according to the technic described by Akers and Zweifach generally causes vasoconstriction of the arterioles. Each plasma sample tested in this study was diluted serially until a threshold constrictor response was obtained. Constrictor activity was expressed in terms of the concentration of 1-epinephrine required to elicit an equivalent threshold constrictor reaction in the same test animal.

**Results**

**Frog Heart Assay.** A summary of the activity of the plasmas in the three experimental groups is presented in figure 1. Means for the different groups have not been calculated, since for some of the hypertensive plasmas only a lower limit was determined. However, it can be seen in figure 1 that the values for most of the normal controls and patients without essential hypertension fell into the concentration range of 0.5 μg. or less. On the other hand, 16 of the 18 patients with essential hypertension had plasma activities greater than 1.25 μg. Thus, group I is clearly separated from groups II and III.

Several other points should be noted. 1. Plasma activity in the essential hypertension group was found to be high, whether or not the patient was receiving antihypertensive medication. 2. No obvious correlations could be made between plasma activity and the severity of complications in any organ system or the duration of the illness. 3. Patients receiving cardiac glycosides were indistinguishable from those who were not.

**Fig. 1.** Bar graph showing activity of plasmas assayed in the 3 experimental groups. Abscissa: activity expressed as microgram equivalents strophantidin/ml. plasma. Each column represents a range of 0.25 μg. Ordinate: number of plasmas in each activity range.
not be detected by this assay, judging by the absence of activity in the plasmas of the 3 digitalized patients in group II. 4. No activity was detected in the plasmas of the five patients in group II with hypertension thought to be secondary to renal disease. The action of 3 of the plasmas in this group appeared to be atypical, in that contracture occurred about after about 10 minutes despite the fact that up to this time little effect on the CFI had been noted. 5. One of the 5 patients in group II with pre-eclampsia or eclampsia had an elevated plasma activity before delivery, which fell to normal postpartum. The plasmas of the other 4 patients were normal.

Hypertensin II* in a concentration of 0.03 U./ml. had no effect on the frog heart CFI. This concentration causes immediate and maximal contraction of the isolated rabbit carotid artery strip and is much more than is required to raise the blood pressure of the intact rat.

Hamster Cheek Pouch Assay. This assay was performed on 11 plasmas from group I and 7 plasmas from group III. No differentiation between normal controls and patients with essential hypertension could be made on the basis of the constrictor activity of plasma applied topically to the hamster cheek pouch.

DISCUSSION
It has been shown that the plasmas of a group of 18 patients with severe essential hypertension differ significantly from the plasmas of 2 control groups in their ability to alter the critical frequency interval (CFI) of the isolated frog heart. This action is similar to the action of cardiac glycosides and an estimate of the activity of each plasma has been made by comparing it to the action of a known concentration of the aglycone, strophanthidin. It should not be concluded that the activity of hypertensive plasma is caused by a substance chemically related to the cardiac glycosides. There is no evidence to support such an assumption and it is already known that many compounds chemically unrelated to the glycosides may alter the CFI. These include adrenalin, certain fatty acids and a lysolecithin recently isolated from biologic tissues. It is unlikely that the activity of the plasmas of the hypertensive group (group I) is caused by adrenalin since the action of adrenalin on the CFI is rapid and transient in contrast to the slow development and persistent action of the plasmas. Also, there was no difference between normal and hypertensive plasmas with respect to vasoconstrictor action on the hamster cheek pouch vessels. The presence of adrenalin causes an increased constrictor action on the hamster cheek pouch preparation.

Recent literature contains reports of two substances said to be characteristic of certain hypertensive plasmas. One is hypertensin which has been found to be elevated in cases of hypertension secondary to renal disease as well as late in the course of essential hypertension when renal damage has appeared. The frog heart activity was present in high concentrations in severe hypertensives without clinical evidence of renal damage. Furthermore, hypertensin does not alter the frog CFI. Another substance is said by Schroeder and Olsen to occur in hypertensive plasma and has been called pherentasin. A sample of pherentasin has not been available for testing. However, on clinical grounds it is unlikely that the frog heart activity is due to pherentasin.

The latter has been found in only about 50 percent of the hypertensive venous bloods tested, whereas the frog heart activity was increased in all of our patients in the severe essential hypertension group.

The significance of the plasma activity is unknown at the present time. Attempts to isolate the material responsible for the activity are in progress.

SUMMARY
A study has been made of the action of human plasma on two bioassay systems, the isolated frog heart and the hamster cheek pouch.

Plasma samples from a group of patients with severe essential hypertension have been compared with those of two control groups. Plasma samples from the patients with essential hypertension were shown to have an action
on the contractile properties of the isolated frog heart which is significantly greater than
that of plasma samples of normal controls or patients without essential hypertension.

No differentiation between normal controls and patients with essential hypertension could
be made on the basis of the hamster cheek pouch assay.

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SUMMARIO IN INTERLINGUA

Esseva studiate le action de plasma human super duo systemas de bio-essayage: le isolate
corde del rana e le sacco genal del hamster. Specimens de plasma ab un gruppo de patientes
con sever hypertension essential esseva comparate con specimens de plasma ab duo gruppos
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essential exercite un effecto super le proprietates contractile del isolate corde del rana que es
significativemente plus grande que le effecto correspondente de specimens de plasma ab
normal subjectos de controlo o ab patientes sin hypertension essential.

Nulle differentia inter normal subjectos de controlo e patientes con hypertension essential
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