# Synergistic Action of Russell Viper Venom and Tissue Thromboplastin Extracts.* (23842)

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In 1945 it was noted that when an extract of acetone-dehydrated rabbit brain was mixed with Russell viper venom, a marked synergistic effect was produced as measured by the one-stage prothrombin time(1). Gollub, Kaplan and Meranze(2,3) have reported a similar synergistic action when rabbit lung and brain extracts were combined. A further study on the effect of mixing various tissue extracts and Russell viper venom on the prothrombin time seemed warranted.

*Materials and methods.* The rabbit brain was extracted with acetone and the extract prepared as previously described(4). The other tissues studied were thoroughly perfused with tap water and homogenized in a Waring blender with 3 parts physiological saline to 1 part tissue. Russell viper venom (Stypven) was supplied by the Burroughs-Wellcome Co. Labile factor (factor V) deficient human plasma was obtained by storing normal oxalated plasma at 4°C until the prothrombin time became 30 seconds or longer. Stable factor (factor VII) deficient bovine plasma was prepared by passing it through a Seitz filter according to the directions of Koller *et al.*(5). The prothrombin time was carried out by the technic of Quick.

*Results.* Prothrombin time of fresh human plasma using acetone-dehydrated rabbit brain as the thromboplastin reagent is 12 seconds. It has not been possible to find a tissue extract which yields a significantly shorter value, but curiously it is possible by mixing various tissues such as brain and placenta, and lung and placenta (Table I) to obtain a prothrombin time shorter than with either constituent alone. The synergistic action between Russell viper venom and various tissue extracts is more pronounced than between various tissues. Particularly striking is the short prothrombin time of 6 seconds obtained on human plasma with a mixture of rabbit brain and Russell viper venom.

In the preceding experiments normal fresh plasma was used. On diminishing the labile factor by storage, the prothrombin time with rabbit brain is prolonged. Thus, the value recorded in Table II is 32.5 seconds. With Russell viper venom alone, the prothrombin time is exceedingly prolonged and with the combination of brain extract and venom, no synergistic action is observed. When stable factor, or factor VII, is removed by filtration through a Seitz filter, a synergistic action with brain and venom is still obtained although the prothrombin time is only reduced to 19.5. It is likely that some prothrombin is removed by filtering plasma through a Seitz filter. A plasma from a patient with congenital stable factor deficiency with a prothrombin time of 27.5 seconds with rabbit brain extract, and 15.5 seconds with venom gave 6 seconds with the 2 reagents combined, the same as is obtained on normal plasma. In marked contrast, plasma from a case of congenital true hypoprothrombinemia showed little synergistic response to the combined rabbit brain-venom thromboplastin reagent. On the other hand, plasma from patients receiving Dicumarol showed a significant response to the combination of brain and venom reagent. An average value of 8.5

TABLE I. Synergistic Action between Tissue Thromboplastins and Russell Viper Venom as Measured by One-Stage Prothrombin Time on Fresh Human Plasma.

| | Prothrombin time (sec.) | | | | |
|---|---|---|---|---|---|
| | With reagent alone | With mixtures* | | | |
| | | Venom | Placenta | Brain | Lung |
| Venom† | 23 | | | | |
| Placenta‡ | 11½ | 11 | | | |
| Brain | 12 | 6 | 8½ | | |
| Lung‡ | 13 | 8 | 10 | 12 | |
| Liver‡ | 46 | 8½ | 15 | 16½ | 15 |

* Equal volumes were mixed.
† 0.01% solution.
‡ Human placenta was used; lung and liver were obtained from freshly killed rabbits.

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TABLE II. Synergistic Action between Rabbit Brain and Russell Viper Venom Tested on Plasma in Which Labile Factor and Stable Factor Were Experimentally Removed.

| Plasma | Rabbit brain | Russell viper venom | Rabbit brain + venom* |
|---|---|---|---|
| | Sec. | | |
| Normal | 12 | 17 | 6 |
| Labile factor exp. depleted | 32.5 | ∞ | 31.5 |
| Stable " " " | 230 | 75 | 19.5 |
| Congenital stable factor deficiency | 27.5 | 15.5 | 6.0 |
| Congenital hypoprothrombinemia vera | 20.5 | 65.0 | 16.5 |
| After Dicumarol: | | | |
| Subject 1 | 35 | 65.0 | 8.5 |
| 2 | 33 | 58 | 8.5 |
| 3 | 37 | 50.5 | 9.5 |
| 4 | 46 | 35 | 8.5 |
| 5 | 36 | 36.5 | 8.5 |

* A 0.1% solution of Russell viper venom was mixed with an equal vol of rabbit brain extract.

seconds was obtained.

*Discussion.* Tissue extracts probably contain several agents which have thromboplastin activity and the potentiation resulting from mixing various tissue extracts may, perhaps, be due to a "reshuffling of compounds in the thromboplastin complex or a rebalancing of functionally different thromboplastins," as Gollub and his associates(2) have proposed. There is also the possibility that since the tissues such as lung and placenta contain at least traces of blood, plasma thromboplastin may be generated, thus giving an additive effect to the preformed thromboplastin present in the tissues. The synergistic effect of Russell viper venom on rabbit brain extract is difficult to explain.

From the results recorded in Table II, it is evident that the activity of Russell viper venom is greatly influenced by labile factor, and when the latter is markedly depleted, as in stored plasma, the synergistic action of rabbit brain and venom is absent. Likewise, in the depletion of prothrombin, as in congenital hypoprothrombinemia vera, the synergistic action of brain and venom is very slight. The activity of Russell viper venom as measured by the one-stage prothrombin time is markedly influenced by both labile factor and prothrombin as is also rabbit brain extract, and when the two are mixed, very little synergistic action is observed if either of these two basic clotting factors is diminished.

Stable factor (factor VII) is not needed for the activity of Russell viper venom. Thus, the prothrombin time using venom as the thromboplastin reagent is the same for plasma from a patient with marked congenital reduction of stable factor as for normal plasma; and when the mixture of venom and brain extract is used, a prothrombin time of 6 seconds is obtained for both plasmas. The results obtained on the plasma of patients receiving Dicumarol are particularly significant. With rabbit brain, prothrombin times varying from 33 to 46 seconds were obtained while with venom, the range was 35 to 58, but there was no close correlation of the two sets of results. When a mixture of brain and venom was used as the thromboplastin reagent, an average value of less than 9 seconds was obtained. This is in harmony with the conclusion of various investigators that Dicumarol reduces the stable factor to a much greater degree than it does prothrombin. Rabbit brain furnishes a thromboplastin reagent for the prothrombin time test which is much more sensitive than Russell viper venom for stable factor deficiency. The substitution of the latter for rabbit brain can be fraught with danger in the control of Dicumarol therapy, as Wilson(6) has pointed out.

*Summary.* As measured by one-stage prothrombin time, mixtures of various body tissues may have thromboplastin activity greater than that of either of the tissues in the mixture. A marked synergistic action as to thromboplastin activity occurs between body tissue extracts and Russell viper venom. This effect is absent if the plasma lacks either prothrombin or labile factor (factor V). The thromboplastin activity of Russell viper venom is not influenced by the lack of stable factor (factor VII) in contrast to the high sensitivity observed with rabbit brain thromboplastin.

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## Further Studies on Urinary Aldosterone in Human Arterial Hypertension.* (23843)

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Two years ago, using a bioassay determination of a purified urinary aldosterone fraction, we reported a significant 2-fold increase in mean urinary aldosterone excretion of groups of severe essential and malignant hypertensive patients as compared to a group of normal subjects(1). Subsequent chromatographic study of this fraction revealed the possible occurrence of 6 other ultraviolet absorbing substances(2). This finding permitted elaboration of a new specific physico-chemical method for determination of aldosterone isolated from urinary extracts(3).

Because of extensive clinical and experimental evidence showing that adreno-cortical hormones with a predominantly mineralocorticoid activity may be directly concerned with the mechanism of arterial hypertension (4), we have applied this new chemical method to the study of 39 patients with essential, renal and malignant hypertension and another group of 11 patients with pheochromocytoma, Cushing's syndrome, primary aldosteronism, and coarctation of the aorta.

*Method and subjects.* The method used was previously reported(3). It is based essentially on isolation of aldosterone in a very high degree of purity and on its determination and identification by ultra-violet absorption at 239 m$\mu$, reduction of blue tetrazolium and absorption spectra in 100% phosphoric acid and concentrated sulfuric acid. In the last 2 years we have had repeated difficulties with eluates from Whatman paper 1 and 2, whether it was specially selected for chromatography or not. Residues of blank eluates from these papers contain a number of interfering substances which react readily with a yellowish brown color after addition of tetramethylammonium hydroxide and blue tetrazolium(5). It has therefore been necessary to add a final purification procedure for separation of aldosterone from these interfering substances eluted from the Whatman paper.† A chromatographic glass column (internal diameter: 7 mm) is filled with Kieselgur (Hyflo-Super Cell, Johns Manville) 500 mg/500 mg water. The eluate residue of the aldosterone zone obtained in the last system (Bush B 5) is dissolved in 5 ml of benzene saturated with water. 15 ml of petroleum ether (D 0.67-0.69) are added and the mixture transferred to the column. The first eluate is discarded. Aldosterone is eluted by the second solvent fraction containing benzene (30 ml) and petroleum ether (10 ml). The Kieselgur and the solvents used are purified according to Hegedüs *et al.*(6) and Schindler *et al.*(7). Each patient was studied as follows: complete history with special emphasis on psychosomatic and emotional aspect of the patient's personality; detailed physical examination with special attention to the state of arteries, fundi and heart, electrocardiogram and teleoroentgenogram of heart, urine analysis with special attention to sediment, blood urea, phenolsulfonphtalein ex-

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† We found recently that these contaminants interfere little with the isonicotinic acid hydrazide reaction described recently by Weichselbaum and Margraf and which depends on the presence of a $\Delta^4$-3 ketone group (8).