INVESTIGATION INTO THE RESERPINE EFFECT ON SOME HEMOSTASIS INDICATORS IN WHITE RATS

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It is a well known fact that preservation the blood in liquid state within the vascular system is the result of complex nerve-humoral effects. Some of them affect blood coagulation, while others — the anti-blood coagulation mechanisms. The evidence available of the role played by the vegetative system in blood clotting suggests that the sympathetic-adrenal system accelerates clotting, whereas the parasympathetic nervous system delays it (9, 11).

The pharmaco-physiological analysis of blood coagulation using reserpine is worthy of special interest. Reserpine administration leads to a gradual liberation of epinephrine (E) and norepinephrine (NE) by the sympathetic neurons and adrenal medulla, with a peak effect within 24 hours. Under the effect of reserpine and background impulsation the labile, mediator significant norepinephrine deposit is also liberated. As a consequence, a severe disturbance of adrenergic transmission in the CNS and in the periphery occurs (12, 15, 18, 19, 21, 25). Along with that, the tone of the parasympathetic nervous system is increased and the adreno-hypophyseal system — stimulated (6, 20). Researches into blood clotting 24 hours after reserpine administration, in the presence of post-reserpine adrenergic axonal block, would enable us to reach certain conclusions concerning sympatho-adrenal influence on coagulation.

Material and Method

The experiments were conducted on 61 healthy white rats, divided up into experimental and control groups. The animals experimented upon were injected with 2 mg/kg weight Rauvasedin — Germed, under sterile conditions, subcutaneously, and the controls — with physiologic saline solution at the same dose. In control group II (untreated), no injections were made. Before the beginning of the experiment, the thrombocytes in all animals were studied after the method of Perlick (24), and at the end of the experiment investigations were performed of the recalcification time according to Houel, prothrombine time — after Quick, and thrombcelastogram (TEG), using for the purpose Hartert's thromboelastograph, type Helige. The prothrombin factor in the experimental and control groups was calculated in relation to the averaged normal value in control group II.

Results and Discussion

The study of thrombocytes after injecting reserpine shows the following values: in the test animals, 536200±31000 mm³ and 358100±24400 mm³ at the beginning and within 24 hours respectively. It is a matter of a sub-

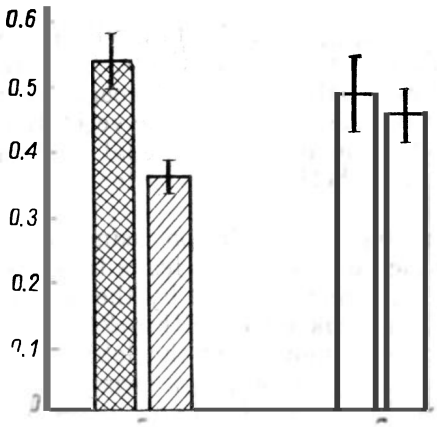

Fig. 1. Thrombocyte count m/mm³
E — experimental; *C* — control
leftside columns — before the experiment
rightside columns — at the end of the experiment

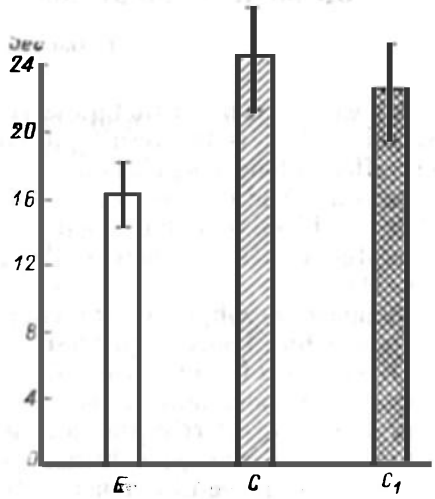

Fig. 2. Recalcification time according to Houel.
E — animals experimented upon (treated with reserpine)
C — control group I (treated with physiologic saline solution)
C_1 — control group II (untreated)

stantial decrease equal to 33.21 per cent, statistically reliable at $p>0.001$ In the controls — at the beginning 494400±62000 mm³, and at the end of the experiment 472800±37700 mm³ — the reduction is insignificant (4.36 per cent) and statistically unreliable, and is interpreted as a fluctuation within normal physiological limits.

At the end of the experiment, the recalcification time after Houel in the test animals is 16.54±1.60 sec, whereas in the controls — 24.30±3.31, i. e. a substantial shortening with 47.72 per cent is present, statistically reliable at $p>0.05$. The recalcification time in control group II is 22.21±3.14 sec, hardly differing from that of the first control group.

The prothrombin time shows values accordingly 11.56±1.16 sec for the animals experimented upon, 12.33±0.17 sec for control group I, and 12.38±±0.47 sec for control group II, i. e. a reduction amounting to 6.66 per cent is present. The prothrombin factor calculation of both experimental and control groups relative to the mean time of control group II shows 104.67±±4.84 for the rats experimented upon, and 100.56±1.72 for the control rats — a difference of 6 per cent which is statistically unreliable.

Studies on the thromboelastogram show the following changes: in the rats experimented upon R is shortened with 14.28 per cent, whilst K displays 36.45 per cent higher values in comparison with the controls. The maximum range mA and mE are accordingly 31.51 per cent and 41.06 per cent lower in the animals experimented upon. From the results presented above it can be seen that only mA proves to be statistically reliable ($p < 0.01$).

Our results indicate that 24 hours after reserpine administration practically all coagulation indicators inder study are shortened, the reduction of thrombocytes inclusive. Emphasis should be laid on the considerably shortened mA and mE recalcification time, and on the strong reduction of thrombocyte count.

The changes in recalcification and prothrombin time are coincident, up to a certain degree, with the epinephrine and norepinephrine effects on blood coagulation. In this respect, our results in terms of post-reserpine liberation of catecholamines (CA) corroborate both directly and indirectly some of the literature data on the issue (1, 3, 9, 10).

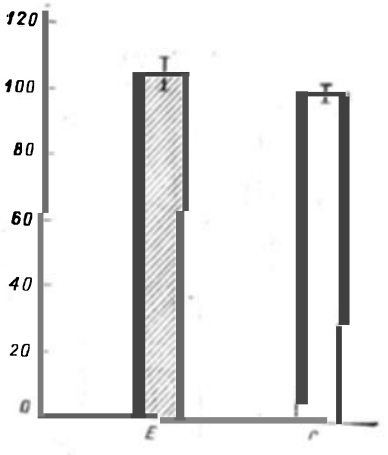

Fig. 3. The prothrombin index of Quick

E — prothrombin index in the rats experimented upon
C — the same for the control animals

In our opinion, the changes in thrombocytes are of no less interest. Their reduction by no means leads to a prolonged coagulation, but contrarily, correlates with the shortening in most of the indicators studied. The data submitted corroborate indirectly the results reported by Markosyan (9) concerning the role of epinephrine, and directly confirm the results of Pletschen and co-authors (22) and Por and co-authors (23) who were successful in demonstrating that reserpine accounts for shortening of the coagulation time. The latter finding is attributed by the cited authors chiefly to serotonin liberation and to the increased agglutination susceptibility of thrombocytes.

Analysis of the data from the thromboelastogram shows a considerable reduction of R among the animals experimented upon, and a strong reduction of mA in comparison with controls. Shortening of the reaction time points to an activation of thromboplastinogen, and correlates with the data concerning prothrombin and recalcification times. The finding of a prolonged coagulation time among the rats experimented upon may be explained by the peculiarities of the graphic appearance of TEG — a slow rise to the normal maximal range which, apart from that, remains reliably low in the test animals — 38.71 ± 6.51 mm against 50.90 ± 2.42 in the controls.

The maximal range reduction in the experimental animals is due to the marked decrease of thrombocytes.

We are prone to attribute the changes described above to the CA liberated by reserpine, which undergo metabolization and reaggregation within certain period of time. In our opinion, the influence of reserpine may be explained in the following way:

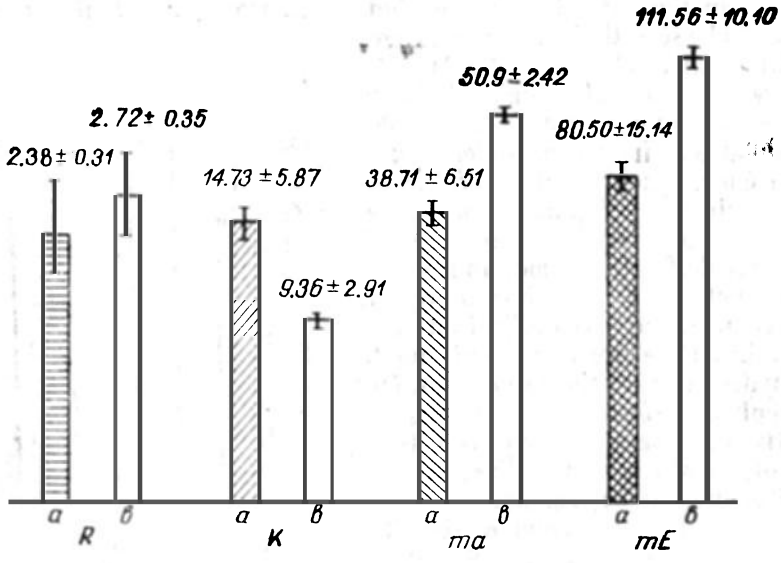

Fig. 4. TEG study

R: a — experimental rats; b — control rats
K: a — experimental rats; b — control rats
mA: a — experimental rats; b — control rats
mE: a — experimental rats; b — control rats.

1. Effect of liberated NE and E which intensify thromboplastin activity and prothrombin activity, shorten the time of recalcification and strongly reduce the thrombocyte count; along with that NE and E enhance the aggregation and adhesion of thrombocytes.
2. Effect of reserpine on the thrombocyte serotonin content which is decreased parallel to the ability of thrombocytes to bind serotonin. As a consequence their agglutination susceptibility is increased.

It is possible that in this particular case some CA metabolites manifest activity in terms of coagulation (5). It is likely that medullary CA, whose liberation is slower and is induced by higher reserpine doses, play a more significant role in this respect. Participation of the serotonin liberated by the tissues, which is an antagonist of antithrombin factors, is by no means ruled out (4, 16, 17). The fact that reserpine stimulates the system hypophysis-adrenal gland (6) should be by all means considered in the interpretation of a similar result. It is well known that the sympathico-adrenal system exerts an essential influence on blood coagulation, and also that its enhanced activity is accompanied by hypercoagulation, and vice versa (2). Furthermore, it exerts influence on the non-fermentative fibrinolytic activity of plasma, increases heparin and lengthens the thrombin time

(7, 8, 14, 19). Some of the above cited studies are indirectly supported by our results.

Notwithstanding the statements made hitherto, our results are somewhat in disagreement with the well known facts about the influence of the

Fig. 5. TEG in experimental rats No No 4 and 5
mA—20 mm; mE—25

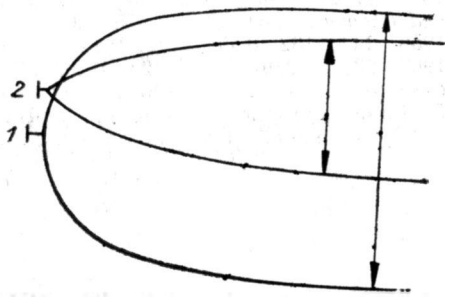

Fig. 6. TEG of control rat No 1
mA=65 mm; mE=186
TEG of experimental rat No 2 mA=32 mm; mE=47

sympathicus, insofar as reserpine, inhibiting the central and peripheral sympathetic tone, should have by all means caused a lengthening of coagulation. In our opinion, it would be possible that the latter takes place subsequent to a rather prolonged reserpine treatment with ensuing more complete liberation of the two intraaxonal CA storages. On the other hand, our data concerning the reserpine induced increase of the parasympathetic tone are likewise in contradiction with literature reports according to which the parasympathetic nervous system lengthens the time of blood coagulation. Obviously, the action of reserpine is quite intricate, and is due equally to direct and indirect effects exerted on a great number of regulating functional linkages and processes. These effects involve equally the coagulation factors and the anticoagulation system whose elucidation requires further researches.

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ИССЛЕДОВАНИЕ ВЛИЯНИЯ РЕЗЕРПИНА НА НЕКОТОРЫЕ ИЗ ПОКАЗАТЕЛЕЙ ГЕМОСТАЗА У БЕЛЫХ КРЫС

Т. Ганчев, Е. Дянков

РЕЗЮМЕ

Авторы исследуют время рекальцификации по Хоуэлю, протромбиновый комплекс, тромбэластограмму и тромбоциты у крыс, третированных однократно резерпином. Устанавливается укорочение времени рекальцификации, протромбинового времени и сильно выраженное снижение числа тромбоцитов. Анализ тромбэластограммы у опытных, третированных резерпином животных показывает укорочение R и уменьшение мА и мЕ, в то время как К было ниже в контрольной группе.

Описанные изменения объясняются освобождением катехоламинов из адренэргических невронов и мозгового слоя надпочечников.