

Original
Article

HEMATOLOGIC AND PLASMA BIOCHEMICAL CHANGES FOLLOWING EXPERIMENTAL ADRENAL INSUFFICIENCY

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ABSTRACT

Aim of the Study: The aim of this work is to study the hematologic effects of bilateral adrenalectomy in rats, especially on platelet function; and to relate the observed changes to the associated alterations in plasma biochemical parameters, also, to test the effects of replacement therapy by hydrocortisone.

Materials and Methods: Rats were subjected to bilateral adrenalectomy (group I), sham-operated rats served as controls (group II). Blood samples were obtained from anaesthetized rats 48 hours after the operation. Hydrocortisone was given as a replacement therapy in another group for 48 hours after adrenalectomy (group III) and bled as the other groups.

Results: Adrenalectomized rats showed increased values of PCV and MCV, and decreased MCHC. Other red cell parameters were not significantly changed. Leucocytes showed increased total count, increased percent of lymphocytes and a decrease in neutrophil percent. Biochemical measurements revealed significant increases in levels of plasma calcium and potassium; while values of plasma albumin and lipid parameters (total cholesterol and HDL-cholesterol) were not significantly changed from those seen in the sham-operated controls. Hydrocortisone treatment of adrenalectomized rats brought these hematological and biochemical changes to levels near to those observed in the sham-operated control group. Platelet aggregation: Adrenalectomized rats displayed inhibition of aggregation in response to both ADP and collagen, while treatment with hydrocortisone produced insignificant increase in platelet aggregation compared to adrenalectomized rats.

Conclusion: Adrenal insufficiency produced inhibition of platelet aggregation- induced by both ADP and collagen. This inhibitory response was associated with raised plasma levels of calcium and potassium. The mechanism(s) of this inhibition of platelet aggregation in adrenalectomized rats is discussed.

Key Words: Adrenalectomy, Platelet aggregation.

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INTRODUCTION

The commonest cause of adrenal insufficiency in clinical practice is no longer Addison's disease. Bilateral adrenalectomy is a line of treatment for Cushing's disease, other disorders of the gland e.g. carcinoma as well as advanced carcinoma of the breast and prostate. Adrenal insufficiency is expected to disturb the function of various body systems, most of these are due to cortisol deficiency. The cellular effects of cortisol deficiency are so widespread that one should not be surprised to find almost any biochemical derangement associated with it (Burke, 1985).

Few studies have addressed the effects of glucocorticoids on platelet function. Glucocorticoids have been reported to be inactive on platelet function, unless used at high concentrations; glucocorticoid receptors were detected in human platelets by Moraes *et al.* (2005).

In the present study, we were interested in studying the effect of adrenal insufficiency on blood elements and platelet aggregation, and to study the prevailing biochemical disturbance that would affect the function of blood

platelets. Moreover, the effects of replacement therapy by hydrocortisone were investigated.

MATERIALS AND METHODS

The effects of bilateral adrenalectomy for 48 hours on hematological parameters and platelet aggregation were studied. Plasma albumin, electrolytes and cholesterol parameters were also estimated.

Chemicals:

- Sodium citrate (Adwic chemical laboratories).
- Hydrocortisone sodium succinate (EIPICO).
- Ether (RFCL Limited).
- Pentobarbitone sodium (Abbott laboratories).
- Adenosine diphosphate (ADP) for aggregation (Park CO.).
- Collagen for aggregation (Chronolog CO.).

Animals: This study was performed on albino rats of both sexes weighing 150- 270 g. Rats were purchased from The Egyptian Organization for Biological Products and Vaccines (Cairo), maintained in the Physiology Department Animal House under standard conditions of boarding, and received a meal composed of bread, milk and vegetables; with free access to water.

Experimental protocol: Rats used in this study were allocated into the following groups:

Group (I): Sham – operated controls (n=18): Rats were exposed to the same surgical manipulation except for adrenalectomy.

Group (2): Bilateral adrenalectomized rats (n=18): Rats were subjected to bilateral adrenalectomy according to the method of *Madden and Ramsburg, (1951)*.

Group (3): Hydrocortisone-treated bilaterally adrenalectomized rats (n=18): Rats in this group were subjected to bilateral adrenalectomy and injected intraperitoneally (i.p.) with hydrocortisone (20 mg/kg) immediately after the operation and another injection 24 hours after the operation.

The animals were fed ad libitum and the adrenalectomized groups were maintained on drinking saline (0.9% sodium chloride). Animals of all groups were used for study 48 hours from the operation.

Experimental procedures: On the day of the experiment, overnight fasted rats were weighed and anaesthetized with i.p. injection of sodium pentobarbital (40 mg/kg b.w.). The abdominal aorta was exposed and cannulated. Three blood samples were collected. One ml blood was drawn into a tube containing EDTA and kept at room temperature and used for complete blood picture within 1 hour from blood collection. Three ml of blood were collected into chilled plastic tube containing sodium citrate 3.8% (9 volume of blood to 1 volume of citrate) and gently mixed. The citrated blood was used for preparation of platelet rich plasma (PRP), platelet poor plasma (PPP), and standard platelet rich plasma (SPRP) for measurement of platelet aggregation. A third blood sample was collected in a tube containing heparin and the separated plasma was stored at -20°C for later estimation of biochemical parameters.

Complete blood picture was performed by the use of Sysmex Kx-21N electronic counter, Japan, according to the method described by *Coulter (1956)*. Lymphocyte % and neutrophil% were deduced by examination of blood film.

PRP was prepared for platelet aggregation as described by *Abou-Shady (1987)* and *Abou-Shady et al. (1991)* using Chrono-Log automatic aggregometer (model 540, Chrono-Log Corporation, Harvertown, USA), coupled with computer and printer. The aggregating agents used were ADP at a final concentration of 10µM/L and collagen at a final concentration of 10µg/ml.

Biochemical studies: Plasma levels of calcium were determined according to the method of *Kessler and Wolfman, (1964)*; potassium as described by *Hillmann and Beyer (1967)*; albumin by the method of *Rodkey (1965)*; total cholesterol as described by *Richmond (1973)* and High density lipoprotein (HDL) cholesterol using the method of *Benzie (1979)*.

Statistical analysis: All data were expressed as Mean $\pm$ Standard Error of the Mean (SEM). Results were statistically analyzed according to Student's "t" test for unpaired data. The correlations among variables were determined by linear regression analysis. A probability of $P < 0.05$ was considered statistically significant.

RESULTS

Data of the present study are summarized in tables (1-4) and illustrated in figures (1-4).

Erythrocyte parameters:

Adrenalectomized rats showed significant increases in PCV and MCV, while MCHC was significantly decreased. Non-significant changes were detected in RBCs count, hemoglobin content and MCH compared to the sham-operated controls.

In the hydrocortisone-treated group, levels of MCV were significantly decreased while MCHC increased compared to the adrenalectomized group. This treatment brought these hematological values to near normal levels (table 1).

Leucocyte parameters: As shown in table (2), adrenalectomized rats displayed significant increases in total leucocytic count and lymphocyte percent. The neutrophil percent was however, significantly decreased compared to the sham-operated controls. Hydrocortisone treatment of the adrenalectomized rats produced decreases in WBCs count and lymphocyte percent. The neutrophil percent value was however, increased in comparison with values seen in the adrenalectomized group. All these values were not significantly different from those seen in the sham-operated controls.

Platelet parameters: As displayed in table (3), adrenalectomy in rats produced significant inhibition of both ADP and collagen-induced platelet aggregation. These effects were associated with non-significant changes in platelet count compared to the sham-operated control rats. Hydrocortisone treatment of adrenalectomized rats produced slight increase in ADP-induced platelet aggregation compared to the non-treated group.

Biochemical Studies: Plasma albumin levels were not significantly changed by adrenalectomy in rats compared to their sham-operated controls. Hydrocortisone treatment produced also non-significant change in plasma albumin compared to the non treated adrenalectomized rats. It is to be noted that this regimen of treatment decreased plasma level of albumin significantly below those displayed by the sham-controls (table 3).

Plasma calcium: A significant rise of plasma calcium was observed following adrenalectomy in rats compared to the sham-controls; while treatment with hydrocortisone decreased though insignificantly plasma calcium levels compared to the adrenalectomized group (table 4).

Plasma potassium: A highly significant hyperkalemic effect was seen in the adrenalectomized group. This effect was significantly lowered by hydrocortisone treatment of this group (table 4).

Plasma total cholesterol and HDL-cholesterol levels, showed non-significant changes following adrenalectomy compared with values seen in the sham-operated controls. Moreover, hydrocortisone treatment of adrenalectomized group exerted insignificant effects (table 4).

Correlation studies:

A negative correlation was observed between plasma potassium and ADP-induced platelet aggregation in sham-operated control rats which became more negative and significant ($r = -0.55$, $P < 0.03$) in adrenalectomized rats. A similar relationship was also seen between plasma potassium and collagen-induced platelet aggregation in the adrenalectomized group ($r = -0.38$, NS). A significant negative relationship was displayed, following adrenalectomy, between lymphocyte % and ADP-induced platelet aggregation ($r = -0.048$, $P < 0.045$). This correlation was however positive in both sham controls and hydrocortisone-treated adrenalectomized rats though insignificant. Plasma calcium levels were positively related to ADP-induced platelet aggregation in both sham-operated and hydrocortisone-treated adrenalectomized groups, but this relationship was changed to a negative one following adrenalectomy (figure 4).

Table 1: Changes in erythrocyte parameters in the groups of rats studied

	RBCs count X 10 ⁶ /ul	Hb content (gm%)	PCV %	MCH Pg	MCHC %	MCV fL
Sham-operated controls	5.1±0.23 (18)	10.1±0.32 (18)	26.2±1.29 (18)	20.1±0.35 (18)	39.3±1.04 (18)	51.3±0.66 (18)
Adrenalectomized rats	5.6±0.26 (18)	10.9±0.38 (18)	31.7±1.8 (18)	19.4±0.29 (18)	35.9±0.84 (18)	54.2±0.79 (18)
P	NS	NS	<0.02	NS	<0.02	<0.01
Adrenalectomized + hydrocortisone treatment	5.3±0.18 (18)	10.7±0.38 (18)	27.7±0.96 (18)	20.0±0.16 (18)	38.7±0.49 (18)	51.7±0.66 (18)
P	NS	NS	NS	NS	NS	NS
P*	NS	NS	NS	NS	<0.01	<0.05

Values are Means ± SEM

P: compared to sham controls

P*: compared to adrenalectomized rats

NS: Not significant

In parenthesis is the number of observations

Hb: Hemoglobin content.

PCV: Packed cell volume.

MCH: Mean corpuscular hemoglobin.

MCHC: Mean corpuscular hemoglobin concentration.

MCV: Mean corpuscular volume.

Table 2: Changes in leucocyte parameters in the groups of rats studied

	WBCs X10 ³ /ul	Lymphocyte %	Neutrophil %
Sham-operated controls	3.1±0.48 (18)	59.8±2.45 (18)	34.6±2.14 (18)
Adrenalectomized rats	4.6±0.47 (18)	72.7±3.12 (18)	23.7±2.43 (18)
P	<0.05	<0.01	<0.01
Adrenalectomized +hydrocortisone treatment	3.4±0.48 (18)	62.3±3.32 (18)	27.6±3.97 (18)
P	NS	NS	NS
P*	NS	<0.01	NS

Values are Means ± SEM

P: compared to sham controls

P*: compared to adrenalectomized rats

NS: Not significant

In parenthesis is the number of observations

Table 3: Changes in platelet parameters in the groups of rats studied

	Platelet count X 10 ³ /ul	Platelet ADP (%)	aggregation Collagen%	Albumin (gm%)
Sham-operated controls	744.4±30.16 (18)	65.9±3.69 (18)	71±5.9 (18)	5.0±0.33 (18)
Adrenalectomized rats	685.1±32.34 (18)	45.1±4.61 (18)	50.7±6.38 (15)	4.5±0.20 (18)
P	NS	<0.01	<0.05	NS
Adrenalectomized + hydrocortisone treatment	687.8±26.79 (18)	50.8±5.44 (18)	— —	4.1±0.26 (17)
P	NS	<0.05	—	<0.05
P*	NS	NS	—	NS

Values are Means ± SEM

P: compared to sham controls

P*: compared to adrenalectomized rats

NS: Not significant

In parenthesis is the number of observations

Table 4: Changes in plasma calcium, potassium, total cholesterol and high-density lipoprotein cholesterol in the groups of rats studied

	Calcium (mg%)	Potassium (mEq/L%)	Total cholesterol (mg%)	HDL- cholesterol (mg%)
Sham-operated controls	8.8±0.43 (16)	4.0±0.12 (16)	89.3±4.84 (16)	41.9±2.57 (16)
Adrenalectomized rats	11.1±0.67 (18)	5.8±0.17 (15)	92.5±6.76 (17)	38.2±4.26 (17)
P	<0.01	<0.001	NS	NS
Adrenalectomized + hydrocortisone treatment	10.5±0.99 (13)	4.1±0.11 (16)	96.8±5.43 (16)	47.2±4.42 (16)
P	NS	NS	NS	NS
P*	NS	<0.001	NS	NS

Values are Means ± SEM

P: compared to sham controls

P*: compared to adrenalectomized rats

NS: Not significant

In parenthesis is the number of observations

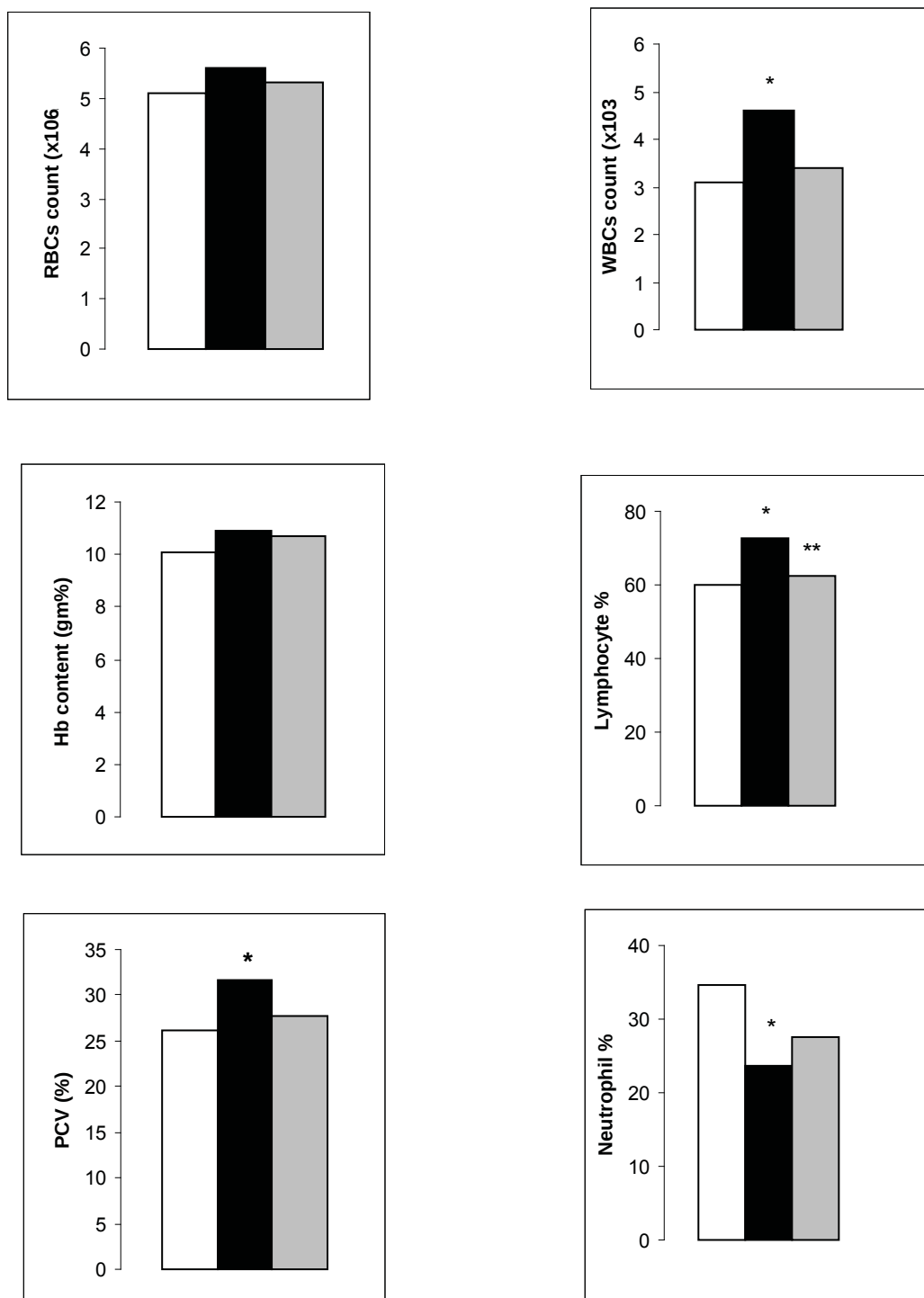


Figure1: Changes in erythrocyte and leucocyte parameters in the groups of rats studied

□ Sham-operated rats ■ Adrenalectomized rats

▒ Adrenalectomized + hydrocortisone-treated rats

*: significance of difference from sham-operated control rats

**: significance of difference from adrenalectomized rats

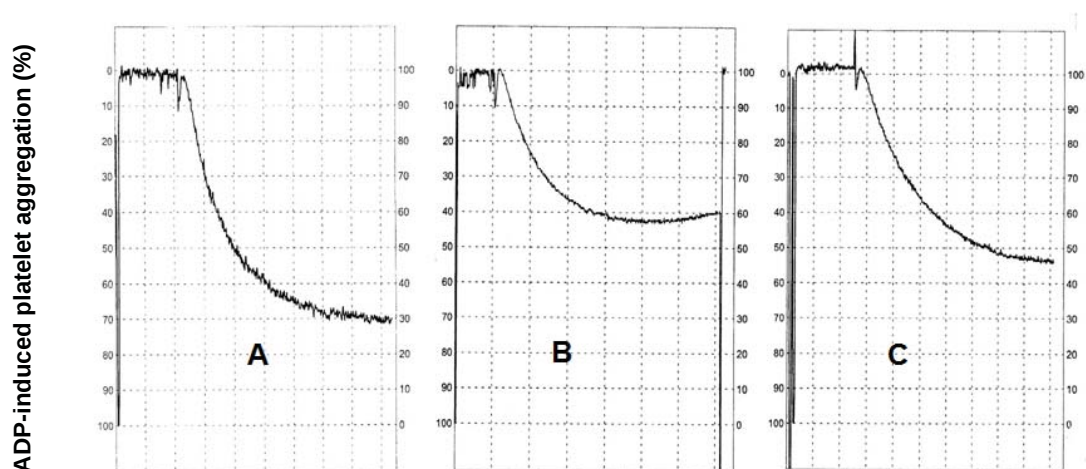


Figure 2: Tracing of ADP-induced platelet aggregation in sham-operated control rats (A), adrenalectomized rats (B) and adrenalectomized – hydrocortisone treated rats (C).

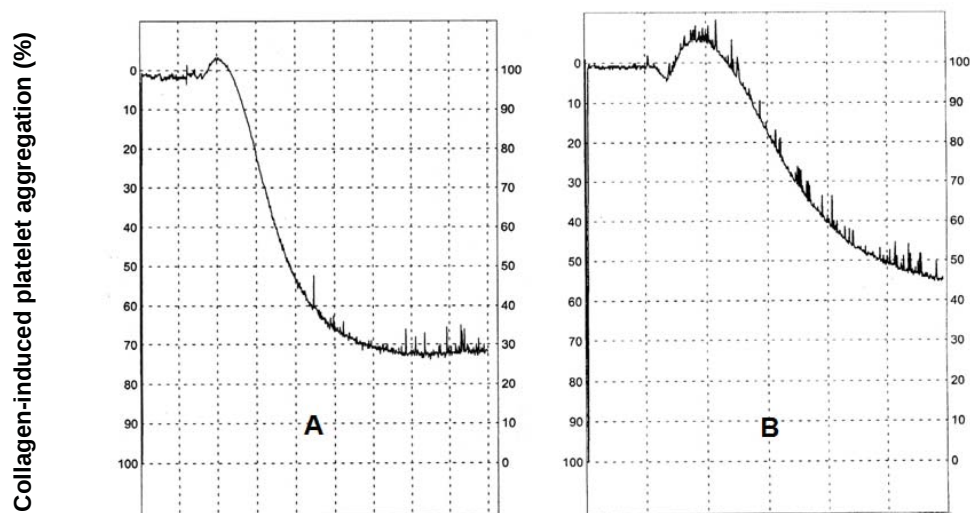


Figure 3: Tracing of collagen-induced platelet aggregation in sham-operated control rats (A) and adrenalectomized rats (B).

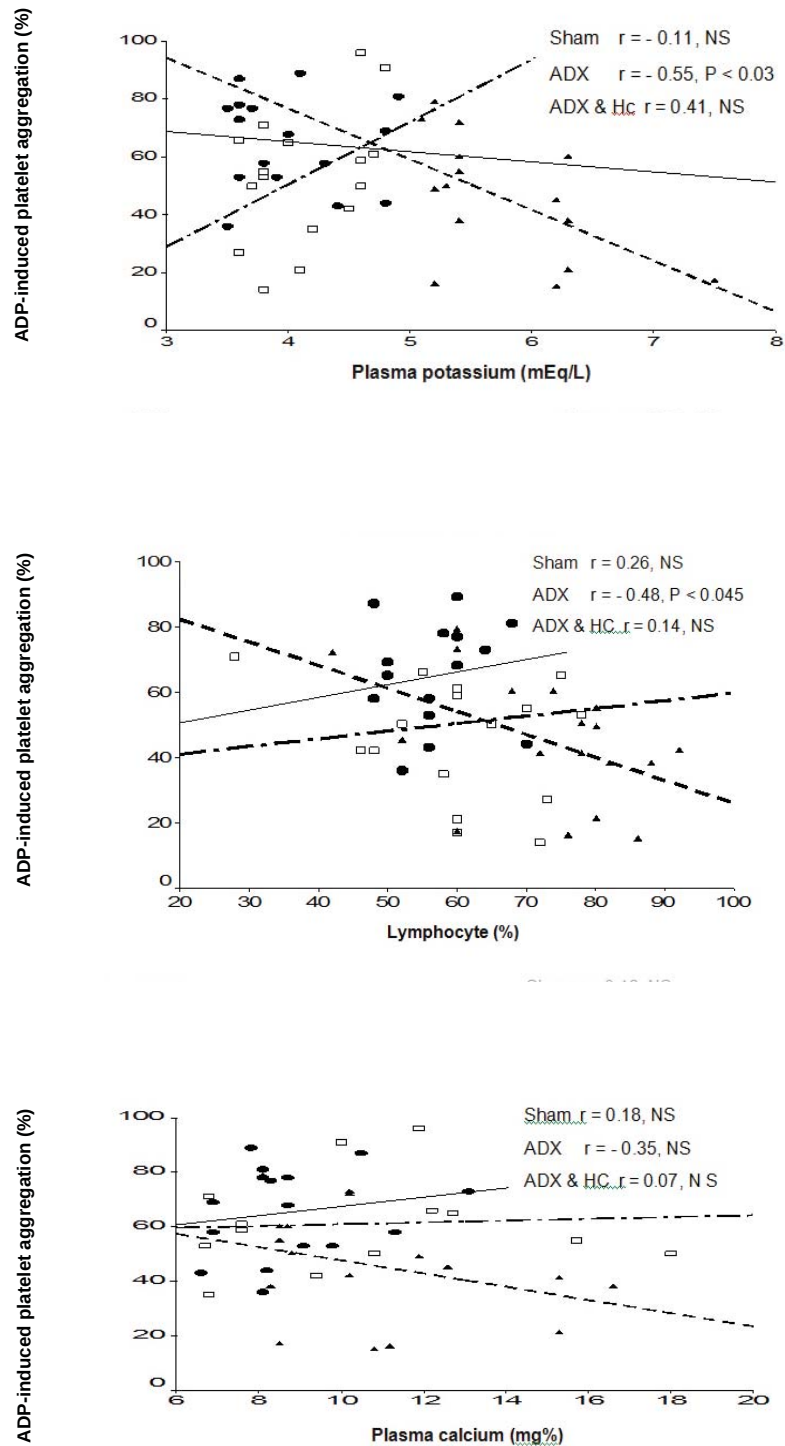


Figure 4: Graphs showing correlations of plasma potassium (mEq/L), lymphocyte % and plasma calcium (mg%) versus ADP-induced platelet aggregation (%) in the groups of rats studied.

● ——— : Sham-operated control rats
 ▲ ——— : Adrenalectomized rats
 □ ——— : ADX & HC : Adrenalectomized + hydrocortisone-treated rats

DISCUSSION

Adrenal insufficiency is usually potentially fatal, unless hormone replacement is instituted (Granner, 2000). Symptoms of adrenal insufficiency may be related first to either glucocorticoid or mineralocorticoid deficiency, but eventually all patients fail to secrete both classes of corticosteroids.

The animal model of glucocorticoid deficiency is for the animal to be adrenalectomized. Most of the symptoms of adrenal insufficiency are found in pure cortisol deficiency, since Swingle *et al.* (1959) showed that steroid deficiency symptoms of adrenalectomized dogs could be reversed by glucocorticoids but not mineralocorticoids.

The aim of this study was twofold; first, to study the hematological effects of bilateral adrenalectomy in rats, with special emphasis on platelet function. Second, to relate the hematologic effects to the prevailing changes in extracellular electrolytes such as potassium and calcium, as well as to the changes in plasma proteins and lipids, in an attempt to throw light onto the relation between the metabolic parameters and hematologic effects. In addition, the possible modulation of glucocorticoid deficiency by replacement therapy given to the adrenalectomized animals was also studied.

Effects on hematologic parameters: The current study, in adrenalectomized rats, showed non-significant changes in red blood cell parameters except for the increased PCV % and MCV and reduced MCHC. These data are in broad agreement with those of Bishayi and Ghosh (2003) who explained the elevated hematocrite value and hemoglobin content by augmented erythrocyte release from the spleen by splenic contraction during corticosteroid insufficiency, since it was accompanied by a decrease in total protein content.

Total leucocytic count and lymphocyte percent were significantly increased; however, neutrophil percent showed a significant decrease in adrenalectomized rats. This neutropenia may be due to sequestration of an increased proportion of neutrophils in marginal tissue pool and is glucocorticoid reversible. This may be explained by the ab-

sence of the regulatory effect of adrenal corticosteroids and the immunopotential in absence of circulating glucocorticoids due to adrenalectomy as proposed by Bishayi and Ghosh (2003). The reversal of most of the changes in the red cell and white cell parameters by cortisol treatment, also indicate that they are due to absence of glucocorticoid effects in adrenalectomized rats.

Effects on plasma calcium: The rise of plasma calcium observed in the present study is in line with previous observations in this department by Abdel Hameed, (1986) and by other investigators Nishino *et al.* (1991). This hypercalcemic response of adrenalectomized rats was expected to enhance platelet aggregation; as elevated plasma calcium causes release of platelet granules, activation of thromboxane A₂ synthesis, and activation of glycoprotein GPIIb/IIIa receptors. As a consequence, this leads to the release of platelet granules containing mediators as ADP and serotonin that activate other platelets. Furthermore, activation of GPIIb/IIIa receptors binds fibrinogen and regulates platelet-platelet interaction (Mills *et al.*, 1968; Fujimura and Phillips, 1983; Fox, *et al.* 1983; Phillips and Baughan, 1983).

Effect on plasma potassium: The data on potassium ion changes confirms the finding of raised plasma potassium levels in adrenalectomized rats; while following treatment with hydrocortisone this hyperkalemic response was significantly reduced. These observations are in line with those of other workers in man and in animals (Fawley, 1967; Cortney, 1969; Abdel Hameed, 1986).

Effects on plasma albumin: The current study showed insignificant changes in plasma albumin following adrenalectomy compared to control rats; while in adrenalectomized rats receiving hydrocortisone replacement therapy, their plasma albumin was slightly and insignificantly reduced compared to their control non-treated rats. Cortisone stimulates the synthesis of hepatic RNA and proteins (Feigelson, *et al.* 1962). On chronic administration of adrenal cortical hormones, the synthesis of plasma albumin is enhanced in animals and in man (Rothschild, *et al.* 1958; 1961; Grossman, *et al.* 1960).

However, in short-term studies, cortisol decreases albumin synthesis in the isolated perfused liver (*Gordon, 1964; Sellers, et al. 1969*), and in vivo as cited by *Jeejeebhoy et al. (1972)*. Data in rats showed an early decrease of albumin synthesis 3 hours after cortisol injection, and increased albumin synthesis after 24 hours (*Jeejeebhoy, et al. 1972*).

Regarding the role of plasma albumin in platelet aggregation, observations by other authors showed that human serum albumin enhances the activity of both inhibitors and stimulators of platelet aggregation. A number of studies also revealed that the overall effect of human serum albumin on platelet aggregation is strongly inhibitory (*Silver, et al. 1973; Remuzzi, et al. 1979*).

Effects on lipid parameters: Previous studies revealed non-significant effects of adrenalectomy on plasma concentrations of free fatty acids or ketones, however, the plasma concentrations of triglycerides were elevated (*Cole, et al. 1982*). The results in this study that there were non-significant changes in the levels of plasma total cholesterol and HDL-cholesterol following adrenalectomy, and in adrenalectomized hydrocortisone-treated rats corroborate and extend similar data reported by other authors (*Berdanier and Burrell, 1980*).

Effects on platelet aggregation: The platelet response obtained in the present study was hypoaggregability instead of the expected increased aggregation produced by hypercalcemia. Therefore, it was thought that some inhibitory factor (s) prevented the aggregating effect of hypercalcemia, prevailed in the adrenalectomized rat model; alternatively a dominant inhibitory condition prevented the proaggregating effect of calcium. A possible candidate of this effect would be potassium ions.

The observation in the current study that by plotting the values of ADP-induced platelet aggregation against the levels of plasma calcium, the positive correlation between calcium in plasma and ADP-induced platelet aggregation among sham-operated controls was changed to a negative relationship in adrenalectomized group. This observation

also suggests that, in adrenalectomized animals a third factor might have interfered with the normal role of calcium in platelet aggregation. This proposal is favored by the striking finding in this study that among all levels of plasma potassium, seen in sham-operated controls and adrenalectomized rats, a negative correlation was observed between plasma potassium levels and ADP-induced platelet aggregation. This relationship was stronger and more negative in adrenalectomized rats, also the same kind of negative relationship was seen between collagen-induced platelet aggregation and plasma potassium.

Our proposal of the inhibitory effect of hyperkalemia on platelet aggregation is supported by the recent finding in humans that high potassium intake attenuated ADP-induced platelet aggregation in healthy men and women (*Kimura, et al. 2004*). They concluded that the prevalence of occlusive stroke is inversely correlated with potassium intake (*Khaw and Connor, 1987; Ascherio, et al. 1998; Iso, et al. 1999; Suter, 1999; Green, et al. 2002*). As a possible mechanism for the effect of potassium supplementation on ADP-evoked platelet aggregation, it was suggested that this phenomenon relates to the link between sodium/potassium gradients across the platelet plasma membrane and platelet cytosolic calcium. The sodium potassium gradients are crucial for maintaining calcium homeostasis through the platelet sodium-calcium exchanger. This exchanger is driven by the transmembrane gradient of not only sodium but also potassium, and therefore rendering platelets highly sensitive to perturbations in cellular sodium/potassium concentrations (*Kimura, et al. 1993; 1999*). Our data on the inhibition of platelet aggregation in adrenalectomized rats in the presence of raised plasma potassium concentration could be explained by potassium inhibition as explained by *Kimura et al. (2004)*. Thus, as a working hypothesis, we suggest that the rise of plasma potassium would diminish the outward potassium gradient across the platelet membrane and retard the forward calcium extrusion of the platelet sodium-calcium exchanger.

Lymphocyte platelet interaction: Our observation in this study of increased percent of lymphocyte in blood following adrena-

lectomy and a significant decrease in lymphocytes by replacement therapy with hydrocortisone, illustrate the role of adrenal cortex in the control of lymphocyte function.

There is a link between platelets and lymphocytes percent that could be seen by the observation in this study of a positive correlation between platelet aggregation induced by ADP and lymphocyte % in sham-operated control rats. This relationship was reversed and became significantly negative following adrenalectomy in rats.

In hydrocortisone-treated adrenalectomized rats, the positive relationship between lymphocyte and ADP-aggregation was resumed as in the control group. These data show that platelets influence lymphocyte function. Previous studies showed that platelets are able to contact with lymphocytes and by this way initiate adhesion of lymphocytes to the extracellular matrix, this migration of T-activated lymphocyte from blood to arterial intima initiates inflammation and progression of atherosclerotic plaque (Solpov, et al. 2007).

The influence of platelets on lymphocyte function is via direct cell contact and/or soluble mediators. Platelets attenuate cytokine secretion and immunosuppressive T helper cells and enhance T cytolytic cells proliferation and cytotoxicity. On the other hand, lymphocytes also regulate platelet aggregation and secretion. This platelet-lymphocyte talk was recently addressed by Li (2008).

From the herein reported data on the antiaggregatory effect of adrenal insufficiency, we may propose one of the following mechanisms for this effect.

First, from the prevailing changes in plasma biochemical parameters, the hyperkalemia demonstrated in this study is probably the dominant antiaggregating factor. This platelet aggregating inhibitory effect of potassium nullified the expected hypercalcemic proaggregatory effect of adrenalectomy.

Second, we demonstrated an interesting link between lymphocyte and platelet aggregation, a positive correlation between platelet aggregation and lymphocyte among

control sham-operated animals and its reversal following adrenalectomy. This may explain the effect of lymphocytosis on platelet aggregation, an effect-effect relationship.

Finally, the above data provided strong evidence for the relationship between the prevailing biochemical changes and the inhibition of platelet aggregation following adrenalectomy. In this regard, additional work should be performed to throw more light on the relation between other biochemical changes in plasma and their effect on platelet aggregation in adrenal insufficiency.

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دراسة التغيرات في خلايا و كيمياء الدم نتيجة لاستئصال الغدة فوق الكلوية

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ملخص البحث

يهدف هذا البحث إلى دراسة تأثير استئصال الغدة فوق الكلوية على خلايا الدم المختلفة وخاصة وظائف الصفائح الدموية وعلاقتها بالتغيرات الكيميائية في بلازما الدم و دراسة تأثير الهيدروكورتيزون على هذه الوظائف. ولقد أجرى هذا البحث على ثلاثة مجموعات من الفئران البيضاء وقد تعرضت المجموعة الأولى لاستئصال الغدتين فوق الكلوية ، والمجموعة الثانية مجموعة ضابطة أجرى لها عملية استئصال كاذبة ، والمجموعة الثالثة تعرضت لاستئصال الغدتين فوق الكلوية ثم تم حقنها مباشرة ثم بعد أربع وعشرون ساعة بالهيدروكورتيزون. وقد تمت دراسة هذه المجموعات بعد ثمانية وأربعين ساعة من إجراء العمليات.

وقد أظهرت النتائج أن استئصال الغدة فوق الكلوية قد أدى إلى زيادة في حجم كرات الدم المكلسة، وفي حجم كرة الدم الحمراء الواحدة بينما كان هناك نقص في تركيز الهيموجلوبين في كرة الدم الحمراء الواحدة. وكذلك وجدت زيادة ذات دلالة إحصائية في عدد كرات الدم البيضاء وفي النسبة المئوية للخلايا الليمفاوية زيادة ذات دلالة إحصائية بينما وجد نقص في النسبة المئوية للخلايا البيضاء المتعادلة. وقد أظهرت القياسات الكيميائية عدم التغير في مستوى الألبومين والكوليستيرول الكلى والكوليستيرول عالى الكثافة، بينما كانت هناك زيادة ذات دلالة إحصائية في تركيز كل من الكالسيوم والبوتاسيوم في البلازما. وأما بخصوص الصفائح الدموية فقد انخفضت نسبة تجمعها باستعمال كل من الأدينوسين ثنائى الفوسفات والكولاجين.

وأما في المجموعة التى عولجت بالهيدروكورتيزون فقد كانت الزيادة في نسبة تجمع الصفائح الدموية بسيطة وليس لها دلالة إحصائية. أما بالنسبة لباقي التغيرات في خلايا الدم والتغيرات الكيميائية فقد أدى العلاج بالهيدروكورتيزون إلى إعادة أعداد ونسب خلايا الدم المختلفة والقياسات الكيميائية إلى نفس القيم الموجودة في المجموعة الضابطة.

ويستنتج من هذه الدراسة أن نقص وظائف الغدة فوق الكلوية يؤدي إلى انخفاض في نسبة تجمع الصفائح الدموية باستعمال الأدينوسين ثنائى الفوسفات والكولاجين، وكان ذلك مصحوبا بارتفاع نسبة الكالسيوم والبوتاسيوم.