

# تأثير بعض عوامل الاجهاد على كريات الدم البيضاء ، جلوكوز الدم والهرمونات اللحائية الكظرية في الكتاكيت

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## الملخص

- تناول البحث دراسة تأثير بعض عوامل الاجهاد على الهرمونات اللحائية الكظرية الكريات البيضاء ، وغلوجوز الدم في الكتاكيت وكانت النتائج كالآتي :-
- ١ - تم تقدير الهرمونات اللحائية الكظرية في دم الكتاكيت عمر ٥٢ ، ٥٨ يوما بطريقتين مختلفتين باستخدام تناقس البروتين المصبب *Competitive protein binding assay*
  - ٢ - وبمقارنة الطريقتين باستعمال التريثيم المرقم  $^3\text{H-Corticosterone}$  التقدير الكورتيكوستيرون واستعمال السلينيوم المرة لتقدير الهرمونات اللحائية الكظرية وجد أن الطريقة الأخره تحتاج الى مجهود أقل وو أقصر .
  - ٣ - ثبت أن حقن الحائة الكظرية تسبب زيادة معنوية في مستوى الهرمونات اللحائية الكظرية وكذلك في عدد الخلايا اليفه الايوزين الحقيقية منها والكاذبة كما أن التغيرات في مستوى الغلوكوز والعدد الكلى للكريات البيضاء كان غير معنويا .
  - ٤ - لوحظ أنه تحت تأثير درجة حرارة ٤٠م زاد مستوى الهرمونات اللحائية الكظرية زياده معنويه كما تبين نقص مستوى الغلوكوز في الدم وكذلك عدد الخلايا اليفه الايوزين الحقيقية منها والزائفة هذا وقد نفقت الكتاكيت بعد ساعتين من التعرض لدرجة حرارة ٤٠م .
  - ٥ - لوحظ أن حرمان الكتاكيت من المليقة والماء يسبب زيادة في مستوى الهرمونات اللحائية الكظرية في الدم بعد ١٢ ساعة وكانت الفروق الأخرى في محتويات الدم غير معنويه .
  - ٦ - أكد البحث أن المجهود العضلى العنيف يزيد من مستوى الهرمونات اللحائية الكظرية في الدم بعد ساعة من المجهود كما أزداد محتوى الدم من الغلوكوز بعد ساعتين وكانت الزيادة معنويه في الحالتين . ولم يتغير العدد الكلى للكريات البيضاء .

بسم الله الرحمن الرحيم

الحمد لله رب العالمين

والصلاة والسلام على من لا نبي بعده

وآله

أما بعد فقد حضر هذا الاجتماع المبارك

في يوم الاثنين الموافق ١٠/١٠/١٤٢٥هـ

بمقر الجمعية العامة في مدينة الرياض

على رأس السيد الرئيس الأستاذ الدكتور

عبدالله بن عبدالعزيز آل سعود

والسيد نائب الرئيس الأستاذ الدكتور

عبدالله بن محمد آل سعود

والسيد أمين المجلس الأستاذ الدكتور

عبدالله بن عبدالعزيز آل سعود

والسيد أمين الصندوق الأستاذ الدكتور

عبدالله بن عبدالعزيز آل سعود



## THE EFFECT OF SOME STRESS FACTORS ON BLOOD LEUCOCYTIC COUNT, GLUCOSE AND CORTICOIDS IN CHICKENS

( 4 Tables and 3 Figers )

By

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### SUMMARY

The effect of some stress factors on blood corticoids, leucocytic count, and glucose in chickens has been investigated.

1.—Blood corticoids in chickens of 52 and 58 days of age have been determined by using two different methods depending on competitive protein binding assay (CPB).

2.—Comparison between these two methods CPB method using <sup>3</sup>H-B corticosterone and the Cortipac CPB assay using Selenium labelled Cortisol, proved that the last method can be satisfactorily used

3.—Injection of ACTH significantly increased the level of corticoids, eosinophils and pseudoeosinophils. Variations in total leucocytic and blood glucose level were not significant.

4.—Under heat stress (40°C) significant increase of blood corticoids and significant decrease in the blood glucose, eosinophils, and pseudoeosinophils was evident. Death occurred after two hours.

5.—Deprivation of food and water caused significant increase in the blood corticoids after 12 hours. Other variations in the blood parameters were not significant.

6.—Vigorous exercise significantly increased blood corticoids after one hour and blood sugar after two hours. Leucocytes showed no significant change.

### INTRODUCTION

Poultry industry suffers great losses due to stress factors. In stress, blood corticoids are increased and the birds resistance is decreased (GROOS (1972), and BUCKLAND, BLAGRAVE ANand LAGUE (1974).

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During the last few decades a great deal of work has been focused on determination of plasma corticoidis as an index for stress. The use of different methods of investigation yielded variable results; (SILBER, BUSCH and OSLAPAS (1958), NEWCOMER (1957, 1958 & 1959 a, b), NAGRA, BAUM and MEYER (1960), URIST and DEUTSCH (1960), HENRY, CHARLES and MELVIN (1961), SIETGGEL and BEANE (1961), BREITENBACH (1962), SIEGEL and SIEGEL (1966), SEIGEL (1968), DUNN, ALVERSON, CARSON, ROGLER and BOHREN (1970), and FRANKEL (1970).

The aim of the present investigation is the study of some blood parameters during stress. Comparison of two methods for corticoid estimation is also attempted.

### MATERIAL AND METHODS

One hundred and eighty female chickens, 52 days of age were divided into 6 groups. The birds were reared in wire cages. The ambient temperature ranged between 18-20°C and the relative humidity was about 75%, food and water were offered *ad lib*. Two experiments were carried out on two successive weeks.

In each experiment two groups of birds were treated and the third was kept as control. In the first week the experimental groups were treated as follows. One group was injected I.M. in the breast muscles with ACTH "SANABO, WIEN" (20 I.U. per Kg body weight dissolved in physiological saline solution), the second group was subjected to acute heat stress (40°C). In the second experiment, one group was also kept as control, the second group was deprived of food and water, and the third group was subjected to vigorous exercise.

Blood samples were collected from the wing vein after 1/2, 1, 2, 3, 6, & 12, hours from the start of the experiment. Heparin was used as anticoagulant. At the mentioned intervals, samples were collected from 5 chickens of each group simultaneously.

For counting the total number of leucocytes, fresh blood samples were collected and the method of NATTA & HERRIJK (1946) was adopted. Acidophils and pseudoeosinophils were counted together by using the WISEMAN (1931) method. The heparinized samples were centrifuged in a cooled centrifuge (-4°C) at 4000 R.P.M. Part of the plasma was used for blood glucose estimations according to WERNER and WEILINGER (1970) and the rest was kept at -20°C for corticoids determination by two methods. These are the competitive protein binding (CPB) assay of corticoids in peripheral plasma using corticosterone labelled with <sup>3</sup>H-B MURPHY (1967) as



modified by BUCKLAND *et al.* (1974), and by the Cortipac CPB assay\*\* in which Selenium labeled cortisol was used. The last method is depending on the significant cross reaction between cortisol and corticosterone.

All data were subjected to unpaired "t" test or to correlation comparisons, SNEDECOR (1956).

## RESULTS AND DISCUSSION

Tables I, II and Fig. I indicate the data of mean plasma corticosterone, since corticoids in chickens are mainly corticosterone, FRANKEL (1966). In the control group of 52 days old chickens the range of corticosterone was between  $1.7 \pm 0.5$  and  $2.3 \pm 0.4$  ug/100 ml and the corticoids between  $2.3 \pm 0.6$  and  $3.7 \pm 0.9$  ug/100 ml. In the control group of 58 days old chickens the range of corticosterone was from  $1.0 \pm 0.5$  to  $1.9 \pm 0.7$  and corticoids ranged  $1.6 \pm 0.7$  to  $2.8 \pm 0.9$ . These results are in complete agreement with the findings of BROWN (1968), DUNN *et al.* (1970), and SIEGEL *et al.* (1972). Higher values of corticoids were reported by NEWCOMER (1959 a & b), URIST *et al.* (1960) and BREITENBACH (1962). Lower values of corticoids however, were reported by BUCKLAND *et al.* (1974). It was also evident from table I, II and Fig. I, that all stressors significantly increased the plasma corticoids level. In the group injected with ACTH corticoids were significantly increased for more than five times after 1/2 an hour and gradually decreased after one hour to attain their normal level after two hours. The above results are in accord with the findings of NEWCOMER, (1959), NAGRA *et al.* (1960), URIST *et al.* (1960) EREITENBACH (1962), SIEGET *et al.* (1960), FRANKEL (1970), BUCKLAND *et al.* (1974). During acute heat stress corticoids were significantly increased after half an hour and remained steady for 2 hours after which the birds were dead. Similar finding were reported by CHARLES *et al.* (1961), DUNN *et al.* (1970), BUCKLAND *et al.* (1974), EDEND and SIEGEL (1974), CALHOUN and HUSTON (1974). In the group subjected to starvation the level of blood corticoid was significantly increased after 12 hours. Similar results were reported by CHARLES *et al.* (1961), and BUCKLAND *et al.* (1974). In the group subjected to vigorous exercise there was a significant increase in the level of blood corticoids after one hour then returned gradually to their normal level within 3 hours.

Of particular interest is the fact that determination of corticoids in chicken blood by Cortipac CPB assay is very satisfactory. Comparison of results obtained by the former method and those obtained by the use of competitive protein binding assay in which  $^3\text{H-B}$  corticosterone was used, revealed that the correlation coefficient is more than 0.90. The first method is simple and time saving while the second is elaborate and time consuming.

In case of the variation of blood glucose level during fasting and after injection of ACTH, such variations are statistically not significant under the conditions of the experiment. These results are rather contradictory to the

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\*\* Cortipac kit for cortisol CPB assay "Radiochemical center Amersham"



findings of BELL (1961), SIEGLE of BEANE (1961), FREEMAN, CHUBB & PEARSON (1966), LANGSLOW, BULTER, HALES & PEARSON (1970.) However, such results are in complete agreement with those of URIST *et al.* (1960), who reported that there is no effect on the blood sugar. A significant increase in blood sugar level during vigorous exercise and decrease during heat stress were evident (tables III & IV, Fig II & III).

TABLE 1. Changes in plasma cort concentration 52 days old chickens.

Groups	Parameter	Sample		collection		after	
		30 m.	one h.	2 h.	3 h.	6 h.	12 h.
Control group (30 chickens)	Corticosterone ug/ 100 ml	1.9 ±0.9	2.1 ±0.7	2.3 ±0.4	2.1 ±0.8	2.2 ±1.5	1.7 ±0.5
	Corticoids in ug/ /100 ml "Cortipac kit"	2.8 ±1.1	2.7 ±0.6	3.6 ±0.3	3.7 ±0.9	3.6 ±1.1	2.3 ±0.6
ACTH group (30 chickens)	Corticosterone ug/100 ml	19.6** ±4.0	6.5** 0.6	2.8 ±0.4	3.3 ±2.4	2.8 ±0.8	2.2 ±0.8
	Corticoids ug/100 ml "Cortipac kit"	21.5** ±5.4	8.0** ±1.0	4.1 ±0.7	4.7 ±2.5	3.8 ±1.4	3.3 ±1.3
Heat stress group (30 chickens)	Corticosterone ug/100 ml	14.6** ±2.0	13.7** ±4.5	16.4** ±2.7	—	—	—
	Corticoids ug/100 ml "Cortipac kit"	15.9** ±2.1	15.0** ±2.9	18.2** ±2.1	—	—	—

Values are means ± standard error

\*\* significantly different from the control group at  $p < 0.01$ .

There is a significant rise in the eosinophil and pseudoeosinophils count in the group injected with ACTH after 3 hours (table III). These results are in complete agreement with those obtained by NEWCOMER (1959), JOHN (1962), SIEGEL (1968), SCHUKRO (1974). A decrease in eosinophil and pseudoeosinophil count was observed under the heat stress (table III).



TABLE 2. Changes in plasma corticoids concentration in 58 days old chickens.

Groups	Parameter	Sample		collection		after	
		30 m	one h.	2 h.	3 h.	6 h	12 h.
Control group (30 chickens)	Corticosterone ug/100ml	1.5 ±0.4	1.4 ±0.5	1.7 ±0.7	1.0 ±0.5	1.9 ±0.6	1.9 ±0.7
	Corticoids in ug/ 100 ml Cortipac kit**	2.4 ±0.5	2.5 ±0.9	2.8 ±0.9	1.6 ±0.7	2.6 ±0.5	2.6 ±0.5
Fasting group (30 Chickens)	Corticosterone ug/100ml	2.0 ±0.7	2.0 ±0.7	1.7 ±0.8	1.3 ±0.8	2.9 ±1.9	6.0* ±1.3
	Corticoids in ug/100 ml	—	2.8 ±0.9	2.8 ±1.5	2.6 ±1.9	5.1 ±2.8	11.* ±1.4
V. exercise group (30 chi- ckens)	Corticosterone ug/100 ml	1.8 ±0.6	10.0** ±2.4	6.7 ±2.9	1.6 ±0.6	1.9 ±0.7	2.4 ±1.1
	Corticoids in ug/100 ml	2.5 ±1.5	12.2** ±2.2	8.4 ±3.4	2.3 ±1.1	2.7 ±0.5	3.5 ±0.8

values are means  $\pm$  standard error

\*\* significantly different from the control at  $P < 0.01$

Considering the total leucocytic count there is no significant variation under all stressors used. This may be explained on basis of individual variations (tables III and IV).

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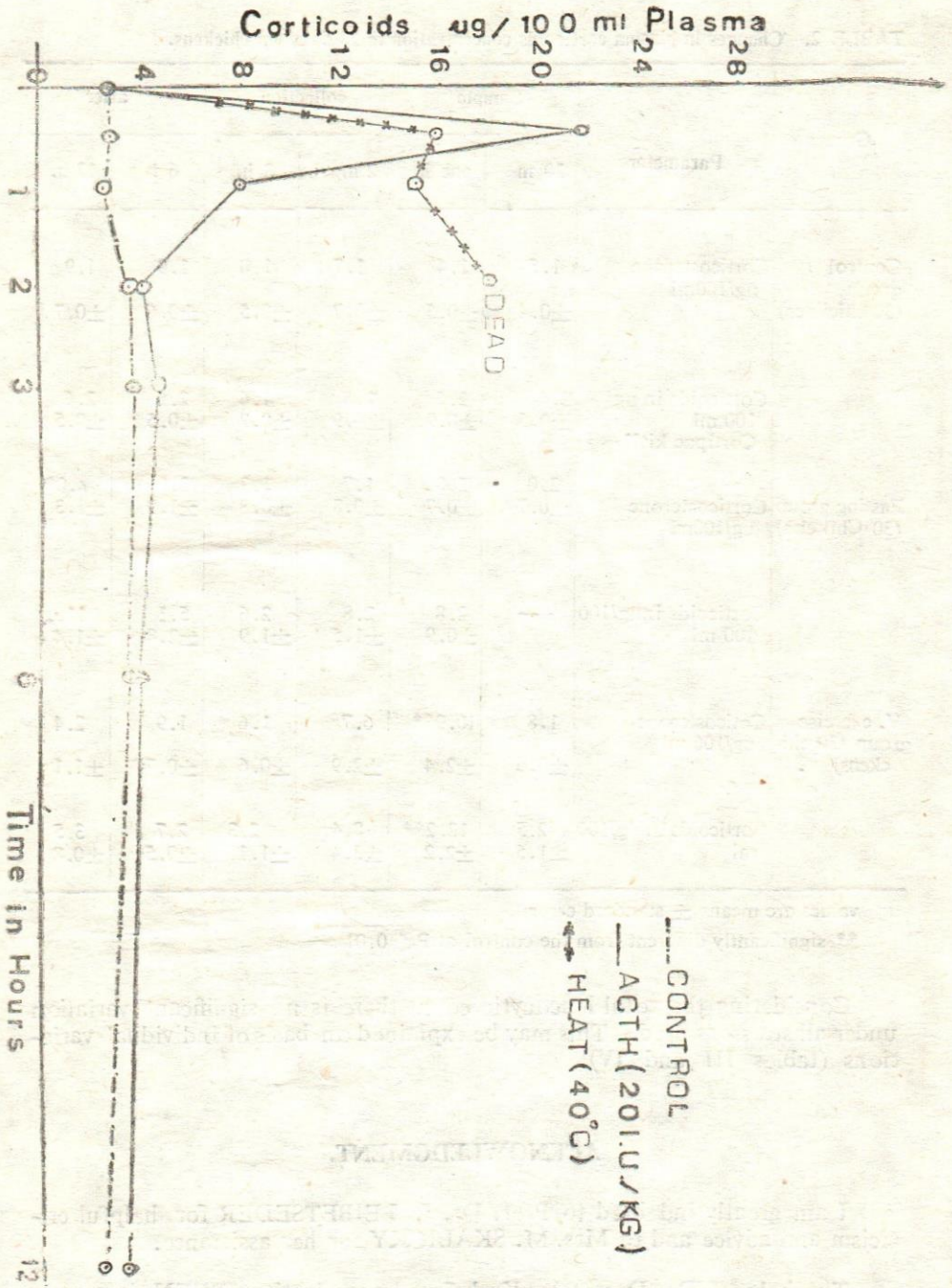


Fig. 1.—Changes in plasma corticoids concentration under the effect of different stress factors.



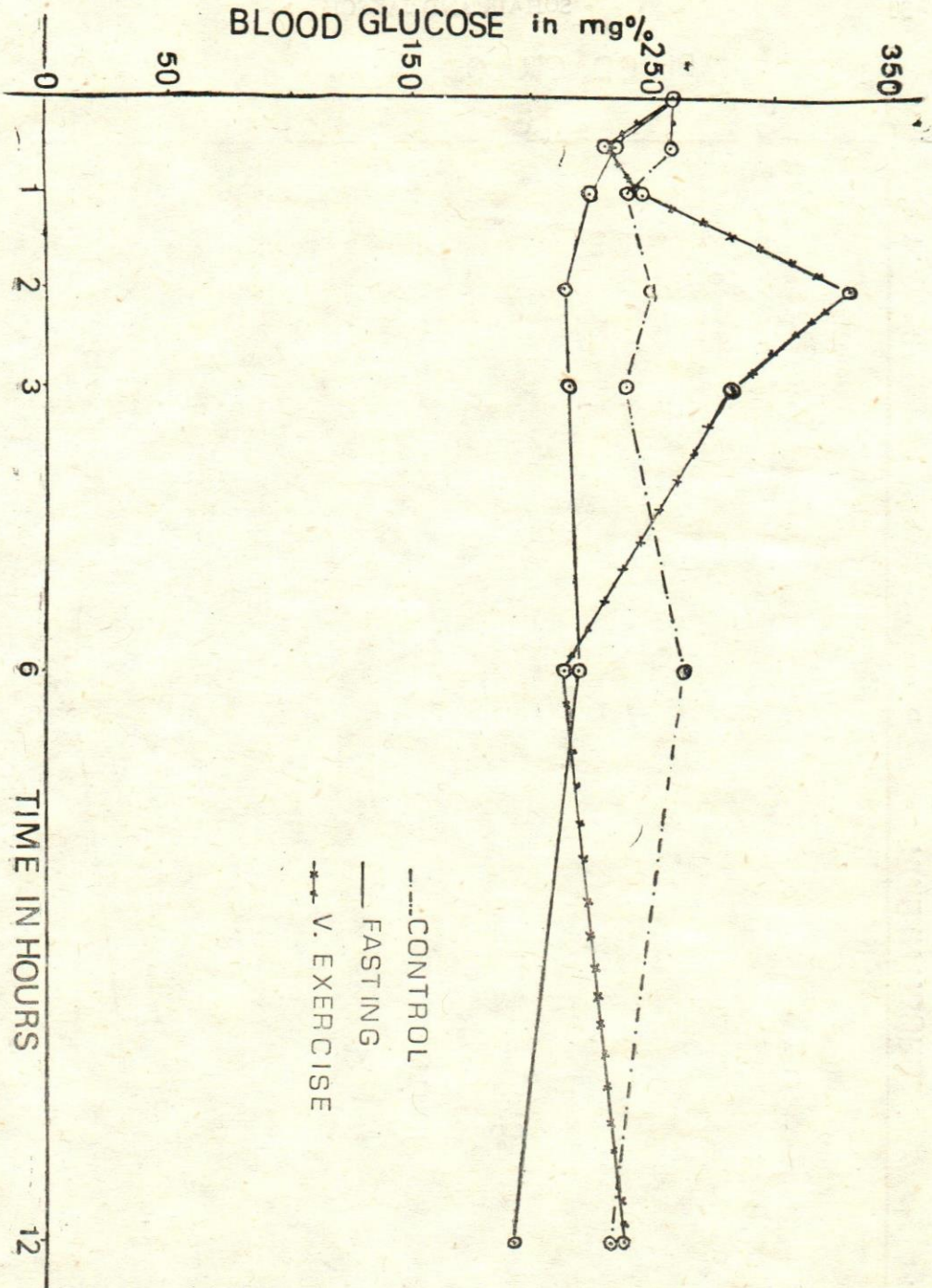


Fig. 2. Changes in plasma glucose level under the effect some stress factors.



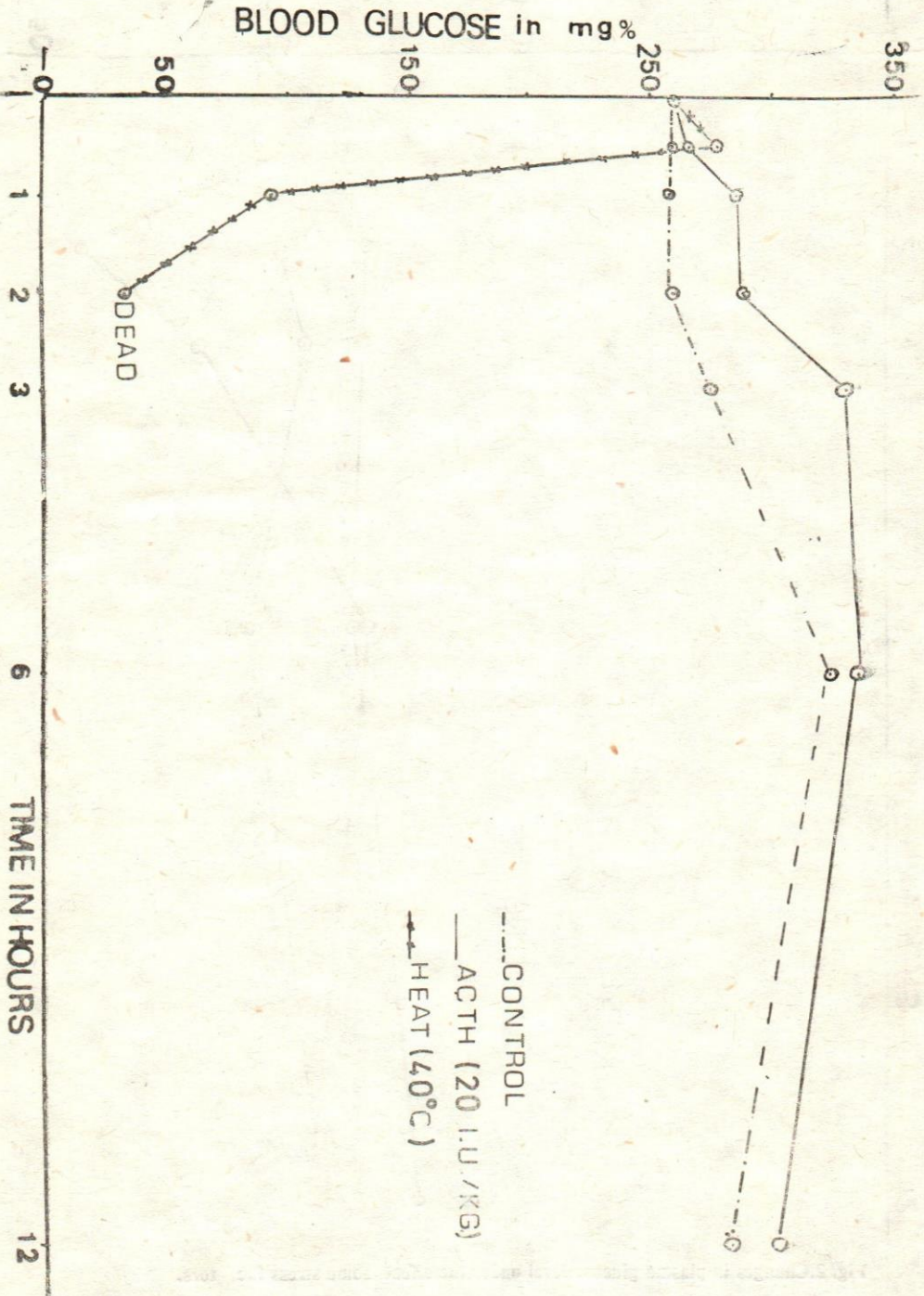


Fig. 3.—Changes in plasma glucose level under the effect of some stress factors



TABLE 3. Blood parameters in the first experiment.

Blood parameters	Groups	30	on hour	2 hours	3 hours	6 hours	12 hours
Blood glucose mg/ 100 ml of plasma	Group I	260 ± 13	258 ± 16	260 ± 15	275 ± 9	325 ± 17	285 ± 12
	Group II	264 ± 12	286 ± 13	287 ± 16**	331 ± 45	340 ± 19	303 ± 13
	Group III	277 ± 26	93 ± 31**	34 ± 11	—	—	—
Count of Eos + Pseu- doeosinophils per cmm . . . . .	Group I	4750	4310	3550	3900	4550	3867
	Group II	± 1139	± 397	± 731	± 718	± 773	± 1328
	Group III	± 3090	± 3100	± 5500	± 15530**	± 18200**	± 4050
Count of total leuco- cytes per cmm	Group I	± 842	± 468	± 938	± 2696	± 3322	± 1392
	Group II	± 2290*	± 800**	± 5650	—	—	—
	Group III	± 552	± 297	± 707	—	—	—
Count of total leuco- cytes per cmm	Group I	19120	15513	15075	17870	20050	22930
	Group II	± 8533	± 2932	± 2436	± 8065	± 3247	± 4109
	Group III	± 19350	± 14200	± 14400	± 21530	± 24040	± 21950
		± 1037	± 4061	± 3716	± 12713	± 4188	± 3980
		± 13788	± 6238	± 11900	—	—	—
		± 5921	± 1563	± 8051	—	—	—

Values are means ± standard error

\*\* significantly different from the control at  $P < 0.05$ \* significantly different from the control at  $P < 0.01$ 

N (number of chickens in each group) = 30

Group I (control), Group II (ACTH injected) and Group III subjected to heat.



TABLE 4.—Blood parameters in the second experiment.

Blood parameters	Groups	30'	one hour	2 hours	3 hours	6 hours	12 hours
Blood glucose mg/100 ml of plasma	Group I	258 ± 10	241 ± 14	250 ± 7	240 ± 25	264 ± 33	237 ± 16
	Group II	236 ± 16	227 ± 24	217 ± 24	218 ± 13	221 ± 15	199 ± 6
	Group III	233 ± 13	243 ± 26	331 ± 24*	284 ± 17	216 ± 5	238 ± 18
Count of Fos- + Pseudoesino phils per cmm	Group I	6990 ±1436	6625	± 6990	7020	7820	7813
	Group II	4210 ±1355	5250 1542	± 4110	3700	1977	357
	Group III	6013 ±2322	6375 3145	± 1905	1446	4910	5470
Count of total Leucocytes per cmm	Group I	23680 ±6240	22020 4647	± 23810	21250	31250	24325
	Group II	18310 ±1876	19800 3242	± 4120	3488	4393	2145
	Group III	16110 ±6387	20770 5825	± 18813	18825	21325	20350
				± 4542	1303	± 2716	± 8228
				± 25480	23800	28920	± 19683
				± 4104	5695	± 3684	± 2822

Values are means ± standard error.

\* Significantly different from the control at  $P < 0.01$

Number of chickens in each group = 30

Group I (control), Group II (fasting and Group III vigorous exercise).



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