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ON THE EPINEPHRINE — INDUCED FALL OF BLOOD EOSINOPHILS.

Action of diethylstilbestrol and the adrenolytics: 2-benzylimidazoline hydrochloride (Priscol), yohimbine and pyperidin-methyl-benzodioxane (933 F)

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It has been known for a long time that epinephrine injections cause an increase in the adrenal weight (1). This fact has been interpreted as a consequence of the non-specific stressing action of adrenaline (2) or of the physiological stimulation of the anterior pituitary (3). It is also known that prolonged treatment with high doses of stilbestrol causes a marked increase of the adrenal glands. Skelton, Fortier and Selye (4) observed that 1 mg of stilbestrol administered daily to rats during 10 days caused a fall of the adrenal cholesterol and ascorbic acid to 1/4 and 1/10 respectively of the control values. The production of cortical hormones by the adrenal glands is always associated with a decrease in its content of cholesterol and ascorbic acid (5). We felt that an assay of the activity of the adrenals of rats treated with diethyl-stilbestrol might throw some light on the understanding of the adrenal physiology.

This paper is both an attempt to block by adrenolytics the epinephrine-induced increase in production of cortical hormones (Experiment I), and a study of the activity of adrenal glands depleted by prolonged treatment with diethylstilbestrol (Experiment II). Since the judgement of activity of the adrenal was assessed by counting eosinophilic leucocytes, involving the analysis of data with Poisson's distribution, to which most biologists are not familiar, details of statistical analysis will also be given.

Experiment I.

INFLUENCE OF ADRENOLYTICS ON THE EPINEPHRINE-INDUCED FALL OF EOSINOPHILS.

MATERIAL AND METHODS.

Rats — 64 male white rats weighing from 120 to 160 g were distributed at random into the latin square presented in table I, the letters of which are explained in table II.

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TABLE I

Latin square used in the experiment designed to study the influence of adrenolytics on the epinephrine-induced eosinophil fall *

Order of treatment / Date	1	2	3	4	5	6	7	8
6.12	A	E	D	B	H	F	G	C
6.19	H	C	G	E	A	B	F	D
6.20	B	F	A	G	C	E	D	H
6.22	C	D	F	A	G	H	E	B
6.23	D	A	H	C	F	G	B	E
6.28	F	B	C	D	E	A	H	G
6.30	G	H	E	F	B	D	C	A
7.3	E	G	B	H	D	C	A	F

* For the meaning of the letters, see table II

TABLE II

Meaning of the letters of the latin square presented in table I

Inhibitors	Epinephrine Without(*)	With
Without (*) ..	A	B
Priscol	C	D
Yohimbine...	E	F
933 F	G	H

(*) *Without* — 0.9% sodium chloride was administered instead of the inhibitors or of epinephrine.

Drugs — Parke-Davis & Co. adrenaline hydrochloride, Specia's piperidin-methyl-benzodioxane (933 F), Riedel-Häen's yohimbine hydrochloride, and Ciba's "Priscol" solution.

Treatments — All animals received 2 subcutaneous injections, the second 30 minutes after the first, the latter being in a volume of 0.5 ml / 100 g of body weight while the former in 0.2 ml per 100 g. The dosages used were the following: epinephrine — 100γ, yohimbine and 933 F — 5 mg/Kg body weight and Priscol — 10 mg / Kg of body weight. All animals were bled twice from the tail for eosinophil counting, the first bleeding being done 20 minutes after the first injection and the second 4 hours after the second injection. The 64 rats, therefore, constituted the following groups of eight rats:

Group I received only 0.9% sodium chloride injections and served as absolute control. Group II received 0.9% sodium chloride in the first injection and epinephrine in the second one. Group III served as control for Priscol injections, receiving Priscol in the first injection and 0.9% sodium chloride in the second. Group IV received Priscol in the first injection and epinephrine in the second. Group V served as control for yohimbine injection, receiving yohimbine in the first injection and 0.9% sodium chloride in the second. Group VI received yohimbine in the first and epinephrine in the second injection. Group VII served as control for the 933 F, receiving 933 F in the first injection and 0.9% sodium chloride in the second. Group VIII received 933 F in the first injection and epinephrine in the second.

The eosinophilic countings were made in 1/10 dilution of the blood in Dunger's fluid in which the concentration of acetone was increased to 15% according to a modification for rat blood (6). The countings were made in a hematimetric chamber of 0.1 mm of height in which all the eosinophils present in 16 mm^2 were counted.

RESULTS

Table III presents the results for the eosinophil countings observed 20 minutes after the first injection in all animals, and table IV presents the final values observed.

TABLE III

Initial values for eosinophils in 0.16 cu. mm. of rat blood

Group	20 minutes after injection (*) of	Eosinophils in rat n.º							
		1	2	3	4	5	6	7	8
I	0.9% NaCl.	19	19	42	41	25	40	4	20
II	0.9% NaCl.	18	17	60	70	24	96	8	36
III	Priscol.	25	23	18	13	10	31	8	23
IV	Priscol.	15	9	41	40	47	18	17	21
V	Yohimbine.	21	32	6	95	57	64	5	21
VI	Yohimbine.	61	11	4	9	13	20	26	33
VII	933 F	34	13	36	17	37	43	38	5
VIII	933 F	31	13	28	48	19	15	19	64

(*) subcutaneous in a volume of 0.5 ml/100 g of rat

TABLE IV

Final values for eosinophils in 0.16 cu. mm. of rat blood

Group	Treatment	Eosinophils in rat n.° 1	2	3	4	5	6	7	8
I	Two injections of 0.9% NaCl. . .	18	19	27	27	23	51	1	15
II	0.9% NaCl + Epinephrine. . . .	2	3	29	20	11	47	2	13
III	Priscol + 0.9% NaCl	18	7	3	2	7	15	7	7
IV	Priscol + Epinephrine	2	6	3	11	19	10	2	2
V	Yohimbine + 0.9% NaCl.	9	7	0	33	18	33	2	0
VI	Yohimbine + Epinephrine	25	5	4	6	0	11	5	9
VII	933 F + 0 9% NaCl	20	23	31	6	17	15	35	7
VIII	933 F + Epinephrine	25	11	12	13	11	15	4	18

The analysis of this kind of data is commonly done by calculating the percentage of fall in each animal. But this method frequently causes distortion and should be avoided (7,8). It is more correct to use the final value only, interpolating each group for the general average of initial values, by covariance analysis.

These data were submitted to covariance analysis after the transformation $\sqrt{4x + 1}$,(*) x being the different values found.

The result of this analysis can be seen in table V. Inspection of the latter shows that neither days (rows of the latin square of table I), or order of

TABLE V

Covariance analysis of results presented in table IV after the transformation $\sqrt{4x + 1}$

Source of variation	Degrees of freedom	Sums of squares and products x^2	xy	y^2	justed squares	Degrees of freedom	Variance	
Rows (days). . .	7	156.97	110.16	115.29	40.00	7	5.714	
Columns.	7	73.63	61.21	65.91	24.54	7	3.505	
Treatments . .	7	75.07	27.87	124.59	117.35	7	16.764	***
Error	42	498.88	294.06	295.92	122.59	41	2.990	
Total.	63	804.55	493.30	601.71				

(***) highly significant

(*) The transformation $\sqrt{4x + 1}$ is used to transform the Poisson distribution into a normal one.

treatment, influenced the final countings. But the treatments caused a highly significant difference in the responses. Fig. I presents the 80% confidence limits (*) of the averages of the eight groups.

It can be seen that Priscol and yohimbine alone produce a fall of eosinophils and none of them inhibits the epinephrine-induced fall of eosinophils. On the other hand inspection of fig. I seems to indicate that a certain protection was provided by 933 F.

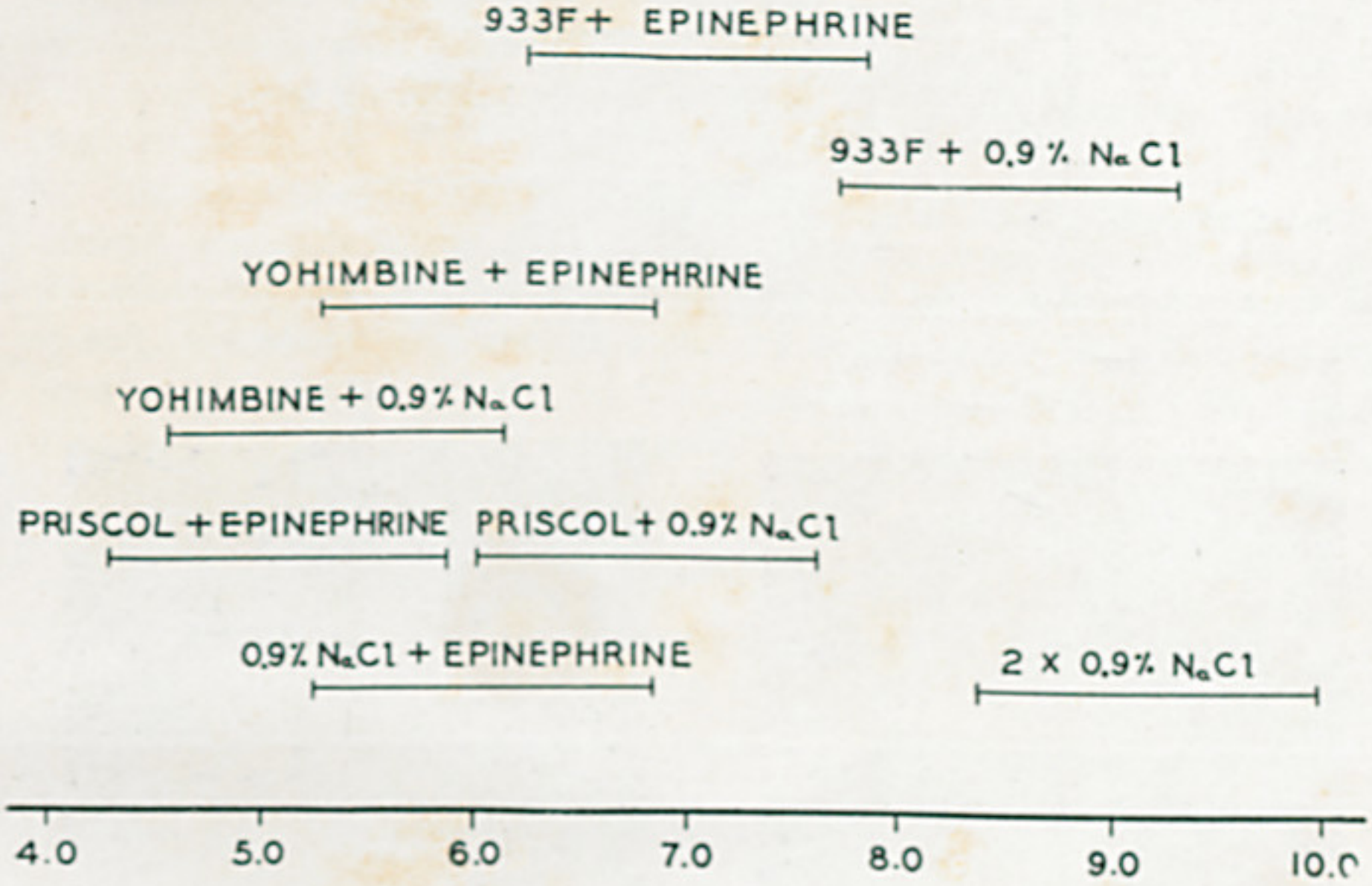


FIG. 1

80% confidence limits of final values of blood eosinophils of rats. Averages corrected by covariance analysis with initial values after the transformation $\sqrt{4x + 1}$. For interpreta ion see footnote of p. 119.

Therefore we repeated the experiment comparing the action of adrenaline on eosinophils in animals injected with saline with that of the same drug in animals injected with 20 mg of 933 F per Kg of body weight. The result of this experiment is presented as confidence limits of the corrected averages in table VI. It should be observed that the final values are about the double

(*) The use of 80% confidence limits of a set of averages is very convenient to detect statistically significant differences among them. It can be demonstrated that the probability of a difference being due to chance is at most 0.07 when the superior 80% confidence limit of the smaller average just touches the inferior confidence limit of the larger one (9).

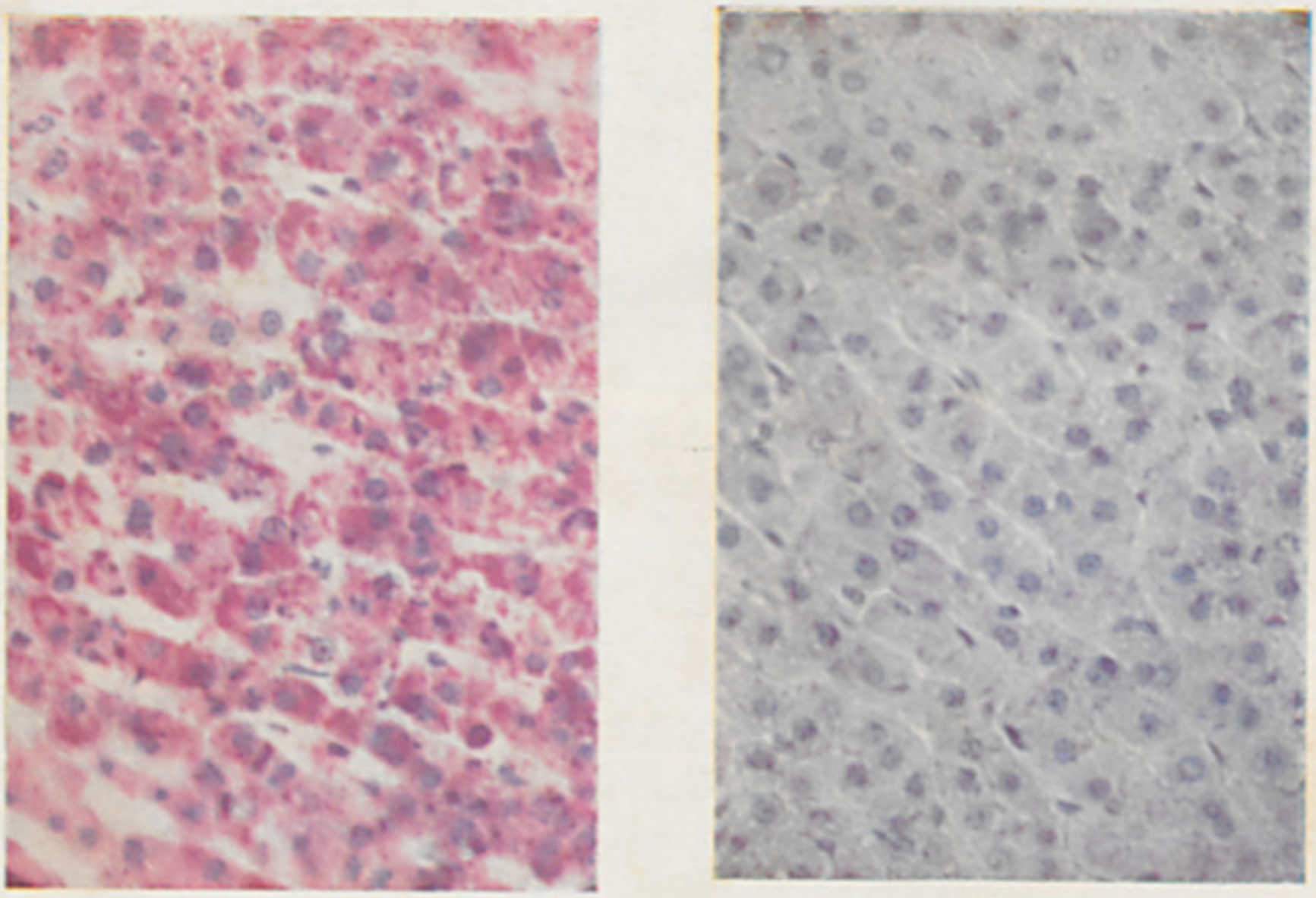
TABLE VI

Corrected confidense limits of average final eosinophil counts of animals treated with epinephrine plus 0.9% NaCl or 933 F

Treatment	Corrected (*) 80% confidence limits	
	inferior	superior
Saline + Epinephrine	8.850	12.030
933 F + Epinephrine	9.225	12.405

(*) By covariance analysis with the initial eosinophil counting.

of those presented by fig. I. This is due to the fact that the eosinophils present in 32 mm² were counted instead of 16 mm². It can be seen that no protection was afforded.



A B

FIG. 2

Sudanophilic material of the adrenal of a normal rat (A) and of a rat treated With diethylstilbestrol (B).

Experiment II.

ACTIVITY OF ADRENAL GLANDS DEPLETED BY PROLONGED TREATMENT WITH DIETHYLSTILBESTROL.

MATERIAL AND METHODS

24 male white rats, weighing from 150 to 200 g were distributed at random into 3 groups. Group I received 1 mg of diethylstilbestrol in 0.2 ml of ethyl-arachidate subcutaneously. Group II served as normal control receiving 0.2 ml of ethylarachidate subcutaneously. Group III served as adrenalectomized control receiving the same treatment as group II. These injections were given daily for 14 days.

On the fiftheenth day of treatment the eosinophils of each animal were counted, and each animal received 100 γ of epinephrine dissolved in 0.2 ml per hundred grams of body weight, 4 hours later the animals were bled again and the eosinophils were counted. After that the animals were killed and at autopsy the adrenlas and thymus were removed and weighed. Histological sections of the adrenals were made and colored for adrenal lipids by Sudan III.

RESULTS

Observation of microscopic sections colored by Sudan shows a complete disappearance of sudanophilic material of the adrenals of rats treated with stilbestrol. This can be seen in a typical case presented in fig. 2.

Table VII presents other pertinent results.

TABLE VII

Adrenals, and thymus weights and adrenal activity (*) of rats treated with diethyl-stilbestrol

Treatment	Adrenals	Thymus	Final eosinophils(***)
	80 % confidence limits		
Diethyl-stilbestrol then epinephrine	37.1 — 42.9 (**)	47.9 — 87.3 (**)	12 6 — 15.6
Oil, then epinephrine .	30.1 — 36.5	168.0 — 210.9	12.0 — 15.3
Adr- Ect- T. (****) + oil, then epinephrine.	———	251.7 — 294.6	18.5 — 21.7

(*) Epinephrine-induced eosinophil fall.
(**) Absolute weight in mg.
(***) After the transformation $\sqrt{4x + 1}$ and correction by covariance anlysis.
(****) Total adrenalectomy

It can be observed, as already known, that treatment with diethylstilbestrol caused a significant increase in the weight of adrenal glands and a thymus atrophy.

As to the responsiveness of adrenals to epinephrine it can be seen that the stilbestrol-treated rats, with complete exhaustion of the sudanophilic material of the adrenal, presented final eosinophil countings equal to those of normal controls.

DISCUSSION

Previous work showed that dibenamine (10) and dihydroergocornine (11) do not block the increase in the adrenal cortex activity produced by epinephrine injections. We can see now that Priscol, yohimbine and 933 F are also unable to block this epinephrine action. As a matter of fact, in the experiment herein reported it was observed that Priscol and yohimbine produce by themselves an increase of cortical hormones secretion as detected by the eosinophil fall. This action is similar to that described for dibenamine (10).

More work is therefore needed to find an adrenolytic blocking the epinephrine-induced fall of blood eosinophils. It is to be expected that such an adrenolytic would clarify the mechanism involved in the activation of the hypophysis by adrenaline. Still more important, it might also throw light on the mechanism involved in the activation of the hypophysis by stress in general.

Our results show also that rats treated with stilbestrol for fourteen days do not present an exhaustion of the adrenals caused by overproduction of cortical hormones. This is in contradistinction to the hypothesis of Mc Phail and Read (12), which attributed the death of mice treated with stilbestrol to cortical insufficiency. Vogt's (13) comparison of the action of stilbestrol on the adrenal with the action of antithyroid substances on thyroid is also not substanciated by our results. It seems therefore, that stilbestrol, while keeping the adrenal working at high level (4) does not exhaust its capacity of responding to a superimposed stimulus, as adrenaline.

SUMMARY

1. Priscol, yohimbine and 933 F, in the amounts used, do not inhibit the fall of blood eosinophils caused by epinephrine.

2. Priscol and yohimbine cause by themselves a fall of blood eosinophils.

3. Prolonged treatment of rats with diethylstilbestrol does not induce an exhaustion of the adrenal cortex in what concerns hormonal secretion, as detected by the test of eosinophil fall.

SUMÁRIO

1. Os adrenolíticos Priscol, ioimbina e 933 F nas quantidades empregadas nêste trabalho não inhibem a queda de eosinófilos do sangue provocada pela adrenalina.

2. O Priscol e a ioimbina por si sós determinam uma queda dos eosinófilos sanguíneos.

3. Pelo teste de queda de eosinófilos, verificou-se que tratamento prolongado de ratos com dietilstilbestrol não causa exaustão das adrenais no que diz respeito à sua atividade cortical.

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