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A CORRELATIVE STUDY BETWEEN ADRENAL FUNCTION AND THE DURATION AND INTENSITY OF AN EXPERIMENTALLY PRODUCED DISEASE OF ADAPTATION

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SUMMARY

The secretion of aldosterone, corticosterone and 18-hydroxy-DOC was studied following prolonged formaldehyde treatment in rats. Of these three steroids, aldosterone was the one whose secretion increased most and also the one whose increase was most significantly correlated to days of exposure. Corticosterone was also augmented and its increase correlated to days of treatment, but this correlation was not as distinct as in the case of aldosterone. Secretion data of both steroids as functions of days of exposure can be fitted to parabolae with minima around the 20th day. 18-Hydroxy-DOC secretions do not parallel those of the other two steroids. Renin plasma concentrations diminish in treated animals. All correlations mentioned were more pronounced in animals losing much body weight as a consequence of the treatment, than in those that lost little or no weight at all.

INTRODUCTION

FORMALDEHYDE injections, both as acute and extended treatments, have been widely used in the past to study the response of adrenals to environmental aggression[1, 2]. The treatment has raised adrenocortical hormone levels in arterial blood of dogs[1] and rats[3–5] and depleted ascorbic acid in adrenals of rat fetuses at the end of gestation[6, 7]. Removal of the hypothalamus has abolished at least the latter type of response[6]. The administration of cortisone plus morphine blocked the increase of corticoid levels in plasma of adult rats submitted to formaldehyde injections for up to twenty days[4]. A hypothalamic–hypophyseal mechanism thus plays a major role in formalin-induced adrenal alterations‡.

Formaldehyde injections *for more than 15 days* have many advantages for a study of adrenal function during stressful conditions. This was supported by the following preliminary observations:

a. Out of five types of stressful conditions employed (cold, ether, high frequency sound, lack of sleep, formaldehyde), the latter proved to be the one provoking the largest increases of adrenal weight.

b. After the above mentioned period, some formaldehyde-treated rats started to "adapt" to treatment while others gradually become "exhausted" and some of

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‡The question of whether this mechanism (CRF → ACTH → Adrenals) is the only one operating at all stages of treatment in adult rats is partially answered in this work but needs further investigation (see Discussion).

these (approximately 20%) died. Tentatively, a quantitative criterion for the adapted and exhausted conditions could be established, taking into account the algebraic increments (i.e. losses or gains) of body weight.

c. These algebraic increments proved to be negatively correlated to the increases of adrenal weight (see below).

These experiments and observations prompted us to choose formaldehyde injections for more than two weeks as a treatment intended to mimic extended exposure to aggression, with the purpose of studying not only overall changes in steroid secretions of treated animals, but also the correlation of these changes to the duration of the treatment and the intensity of their effects on the animals' body weight.

MATERIAL AND METHODS

Biological material. 58 male Wistar rats, 250–300 g initial weight, were used.

Treatment. The treated animals received daily intramuscular injections of a 10 per cent formaldehyde solution.

Body weight control. Animals, both controls and treated, were weighed on a torsion balance to the nearest gram, every other day.

Group selection and schedule of animals killed for steroid determinations. According to preliminary experiments (see the first series of results) the loss of body weight incurred by treated animals during the five days prior to killing was directly proportional to their adrenal weight. Consequently, whenever a lot was to be killed (every other day from the 15th day of treatment on) the loss of body weight in each treated animal during the last five days was calculated and plotted. The surviving treated animals were then divided into two equal subgroups according to this parameter; one subgroup (exactly half of the total group) comprised those animals that had experienced the heavier weight losses (exhausted animals), while the other subgroup was formed of the remaining animals, which had lost little or no weight at all (adapted animals). (The selective criterion is graphically explained in Fig. 2).

From the 15th day on, adrenal venous effluent from three animals was collected every day. These animals belonged respectively to the control group and the subgroups of exhausted and adapted animals.

Individuals to be killed were selected within each group and subgroup at random.

To avoid circadian variations, a time table for killing was strictly observed (11 a.m. to 1.20 p.m.). The control rat was always the second one to be killed, while exhausted and adapted rats were cannulated alternately at the beginning or at the end of the operation.

Blood collection for steroid determinations. Adrenal venous blood was collected during ten minutes according to the technique of Vogt[8]. Known amounts of [4-^{14}C] aldosterone and [4-^{14}C] corticosterone were added to the samples and these were extracted twice with five volumes of dichloromethane. The extracts were evaporated under nitrogen and spotted on paper chromatograms which were developed in a toluene-propylene glycol system[9].

Steroid determinations. From the chromatogram, three zones were eluted corresponding respectively to the radioactive aldosterone and corticosterone zones, and to an intermediate region which contained Porter–Silber positive material.

Corticosterone was determined by the method of Elliott *et al.* as modified by Péron[10]. Aldosterone was quantitated by the method of Kliman and Peterson [11] and 18-hydroxy-DOC* by the method of Porter and Silber as modified by Birmingham[12]. Losses were corrected for the first two determinations but not for the last one, by the addition of tracer amounts of the ^{14}C-labelled steroids.

Renin determinations. These were performed by the method of Boucher *et al.* [13]. Five (6) animals were killed on the 15th day of treatment, four (6) animals on the 20th, and five (7) animals on the 30th day of treatment. (Numbers between brackets indicate controls). Exhausted and adapted animals were selected as before. Of the fourteen treated rats employed, seven were of the exhausted and seven of the adapted type.

Statistical methods. Tuckey's test for multiple comparisons[14] has been employed to obtain the differences between groups in experiments dealing with steroid secretions. Student's 't' test has been used for the same purpose in experiments designed to determine renin secretions, and for establishing the significance of differences between the ascending slopes of the parabolae in Correlation B.

The least squares method has been utilized to fit secretion data to linear correlations and second order parabolae, this latter according to Ezekiel *et al.*[15]. The calculus involved in these procedures was processed by means of a PDP 8-1 computer (Digital Equipment Corporation, Maynard, Mass., U.S.A.).

RESULTS

Adrenal weights

Adrenal weights were determined on treated and control animals after 20 and 32 days of daily formaldehyde injection treatment and expressed in reference to the body weight of the animal before treatment started. Algebraic increments of body weight during the last five days prior to killing proved to be negatively correlated to adrenal weight in the group of treated animals. This correlation was designated Correlation A (Fig. 1). No correlation was obtained between adrenal weights and increments of body weight in the control group alone. Neither was adrenal size found to be correlated to the total body weight lost between the first and last days of treatment.

Steroid secretions

Table 1 summarizes steroid secretion values.

I. *Secretions per unit of initial body weight.* In exhausted as well as adapted animals†, secretions of aldosterone and corticosterone, but not those of 18-hydroxy-DOC, increased significantly. Aldosterone secretion increased more than corticosterone secretion.

II. *Secretions per unit of adrenal weight.* In exhausted animals, corticosterone and aldosterone secretions were augmented, the increases being again higher for aldosterone, while the secretion of 18-hydroxy-DOC per mg of gland diminished. In adapted animals, only the increases of aldosterone were significant.

* 18-hydroxy-DOC (18-OH-DOC): 20,21-dihydroxy-18,20-epoxy-4-pregnen-3-one.

†No data are available for 18-hydroxy-DOC secretions in the adapted subgroup.

Table 1. Steroid secretion rates

Aldosterone/initial body weight ng/kg/10 min				Corticosterone/initial body weight μg/kg/10 min				18-OH-DOC/ initial body weight μg/kg/10 min	
Controls(8)	Adapted(7)	Exhausted(9)	Total(16)	Controls(8)	Adapted(7)	Exhausted(8)	Total(15)	Controls(8)	Exhausted(7)
252·5 ± 26*	637·9 ± 79 + 153% $P < 0·0005$	755·2 ± 10·8 + 199% $P < 0·0005$	703·9 ± 70 + 178% $P < 0·0005$	95·8 ± 7·25	166·8 ± 26·4 + 74% $P < 0·01$	170·6 ± 12·7 + 78% $P < 0·005$	168·8 ± 13·5 + 76% $P < 0·0025$	16·8 ± 2·0	17·7 ± 2·1 not significant
Aldosterone/adrenal weight μg/g/10 min				Corticosterone/adrenal weight μg/g/10 min				18-OH-DOC/adrenal weight μg/g/10 min	
Controls(6)	Adapted(5)	Exhausted(7)	Total(12)	Controls(6)	Adapted(5)	Exhausted(6)	Total(11)	Controls(5)	Exhausted(6)
2·05 ± 0·27	3·69 ± 0·67 + 80% $P < 0·025$	3·88 ± 0·49 + 89% $P < 0·0025$	3·80 ± 0·38 + 85% $P < 0·005$	710 ± 80	1060 ± 280 not significant	890 ± 30 + 25·5% $P < 0·025$	970 ± 120 not significant	120·0 ± 10·0	90·4 ± 18·8 − 30% $P < 0·05$

*Means ± Standard error. Probabilities refer to differences between each group or subgroup and controls. 'Total' refers to all treated rats considered as one single group. Differences between 'adapted' and 'exhausted' rats and between either of these and 'totals' are not significant.

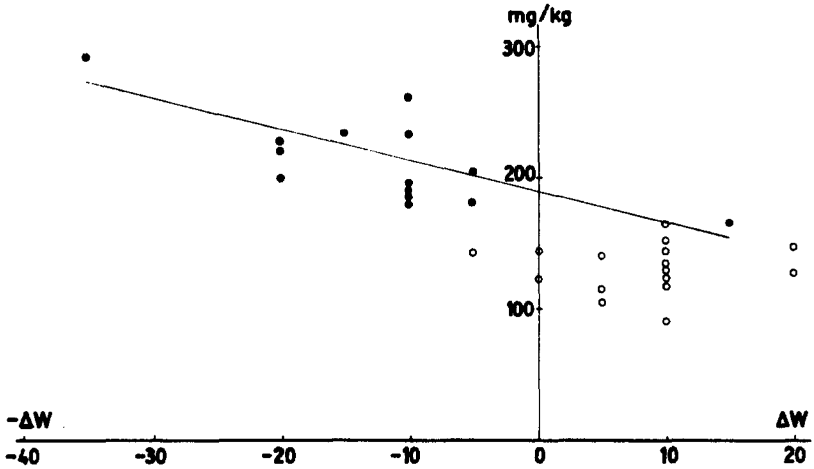

Fig. 1. Relationship between adrenal weights and algebraic increments of body weights. (Correlation A, in the text). (●): treated animals. (○): control animals. Abscissa (ΔW): algebraic increments of body weight (in g) in the animals during the last five days prior to sacrifice. Ordinate (mg/kg): adrenal weight per body weight. (———): adjusted values for a linear correlation between ΔW and mg/kg. The function for this correlation is:

$$y = 186{\cdot}9 - 2{\cdot}25x; \quad r = -0{\cdot}727; \quad P < 0{\cdot}01.$$

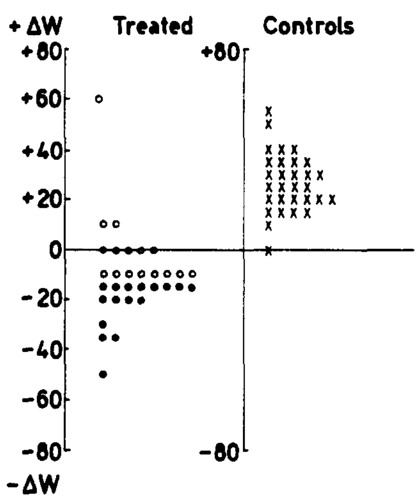

Fig. 2. Selective criteria applied for the division of treated animals into 'exhausted' and 'adapted' subgroups. Every day three rats had to be sacrificed (every other day from the 15th day of treatment on), ΔW, as defined in Fig. 1, were plotted on ordinates of graphs. The present graph corresponds to the 17th day of treatment. Treated rats were then divided into two subgroups according to the magnitude of the increments. Both subgroups contained an equal number of rats. The lower one was designated 'exhausted' subgroup, while the upper one was called 'adapted' subgroup. Each full circle (●) corresponds to one exhausted animal, and each empty circle (○), to an adapted one. Each (×) corresponds to a control. On each day of sacrifice, one control, one exhausted and one adapted rat were chosen at random.

Figures 3–8 analyze the relation between steroid secretions and days of treatment.

III. *Correlation between days of treatment and secretions per initial body weight* (Correlation B; Figs. 3–5). Both aldosterone and corticosterone can be fitted to parabolae having a minimum on or around the 20th day of treatment. The ascending slopes of the parabolae (above minima and above controls) are steeper for aldosterone than for corticosterone*. When the data for aldosterone were an-

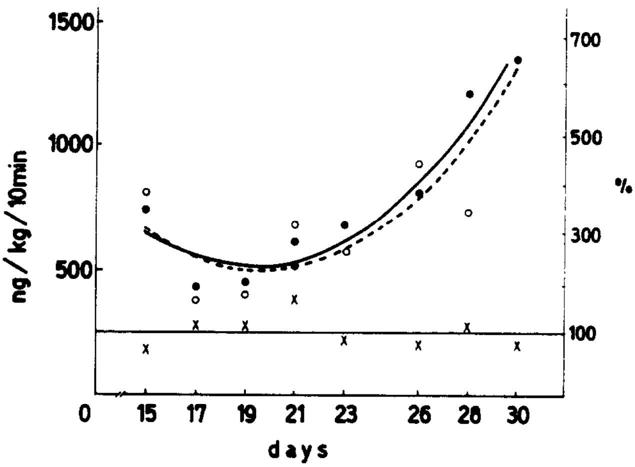

Fig. 3. Days of treatment and aldosterone secretion per initial body weight. Abscissa: days of treatment. Ordinate: aldosterone secretion in adrenal venous effluent, per kg of rat (initial weight), per ten minutes. Values for adapted animals (●), adjust better to a parabola (———) than to a straight line. This parabola corresponds to the function:

$$y = 3360 - 297x \times 7{\cdot}7x^2; \quad r = 0{\cdot}967; \quad P < 0{\cdot}001$$

Values for adapted animals (○) also adjust to a parabola not shown on this graph, whose $r = 0{\cdot}929$ with $P < 0{\cdot}05$. Values for all treated animals, considered as one single group, also adjust better to a parabola (---) than to a straight line; its function is:

$$y = 3389 - 295x + 7{\cdot}6x^2; \quad r = 0{\cdot}932; \quad P < 0{\cdot}001$$

(×) Individual control values; (———) Mean control value. Correlations in Figs. 3, 4 and 5 are designated Correlation B in the text.

*That the difference between both ascending slopes is significant has been proven as follows:

The secretion data from the 19th day on, i.e. only those corresponding to the ascending slopes of the parabolae, expressed as percentual increments over controls, were fitted to straight lines whose slopes (Mean ± Standard errors) had the following values:

I. $32{\cdot}73 \pm 3{\cdot}79$ ($r = 0{\cdot}969$, $P < 0{\cdot}01$), for aldosterone in exhausted animals (Fig. 3);
II. $7{\cdot}24 \pm 1{\cdot}70$ ($r = 0{\cdot}906$, $P < 0{\cdot}05$), for corticosterone in exhausted animals (Fig. 4);
III. $27{\cdot}72 \pm 4{\cdot}37$ ($r = 0{\cdot}895$, $P < 0{\cdot}001$), for aldosterone in all treated animals (Fig. 3);
IV. $4{\cdot}007 \pm 4{\cdot}60$ ($r = 0{\cdot}276$, P non significant), for corticosterone in all treated animals (Fig. 4).

The difference between I and II was significant ($t = 6{\cdot}25$, $P \leqslant 0{\cdot}0001$).

That the difference between III and IV was also highly probable could be inferred from the fact that III had a real significance while IV had none. But even assuming that IV were significant, a difference between III and IV would still be highly probable ($t = 3{\cdot}72$, $P < 0{\cdot}005$).

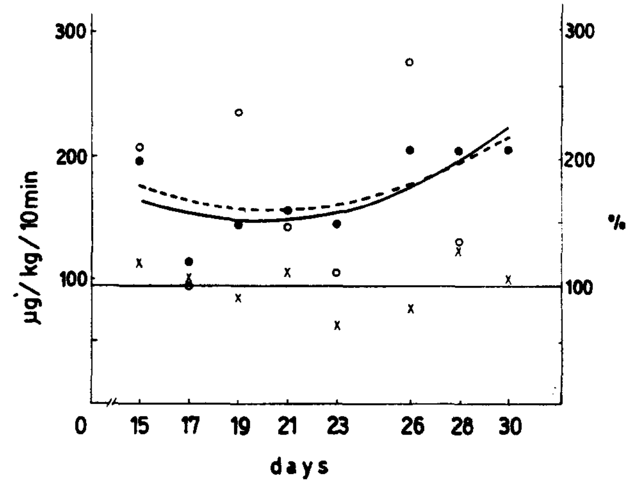

Fig. 4. Days of treatment and corticosterone secretion per initial body weight. Abscissa: days of treatment. Ordinate: corticosterone secretion in adrenal venous effluent, per kg of rat (initial weight), per ten minutes. Values for exhausted animals (●) adjust better to a parabola (———) than to a straight line. This parabola corresponds to the function:

$$y = 452 - 31x + 0{\cdot}75x^2; \quad r = 0{\cdot}997; \quad P < 0{\cdot}001$$

Values for adapted rats (○) do not adjust to any function. Values for all treated rats, considered as one single group, also adjust better to a parabola (---) than to a straight line; its function is:

$$y = 450 - 28x + 0{\cdot}70x^2; \quad r = 0{\cdot}972; \quad P < 0{\cdot}01$$

(×) Individual control values. (———) Mean control value.

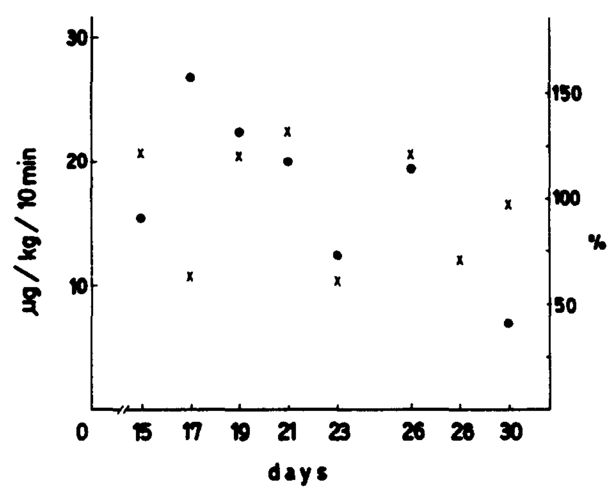

Fig. 5. Days of treatment and 18-OH-DOC secretion per initial body weight. Abscissa: days of treatment. Ordinate: 18-OH-DOC secretion in adrenal venous effluent, per kg of rat (initial weight), per ten minutes. (●) Values for exhausted animals. (×) Values for controls.

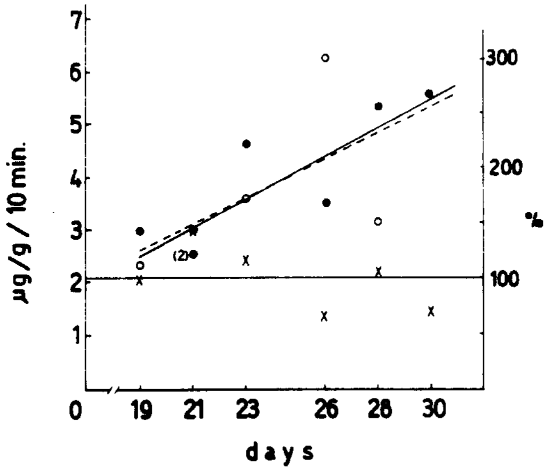

Fig. 6. Days of treatment and aldosterone secretion per adrenal weight. Abscissa: days of treatment. Ordinate: aldosterone secretion in adrenal venous effluent, per g of adrenal, per ten minutes. Values for exhausted animals (●) adjust to a positive linear correlation (———) whose function is:

$$y = -2{\cdot}64 + 0{\cdot}27x; \quad r = 0{\cdot}857; \quad P < 0{\cdot}05.$$

Values for adapted rats (○) do not adjust to any function. Values for all treated rats, considered as one single group, also adjust to a positive linear correlation (---); its function is:

$$y = -2{\cdot}23 + 0{\cdot}25x; \quad r = 0{\cdot}720; \quad P < 0{\cdot}05$$

(×) Individual control values. (———) Mean control value. Correlations in Figs. 6–8 are designated 'Correlation C' in the text.

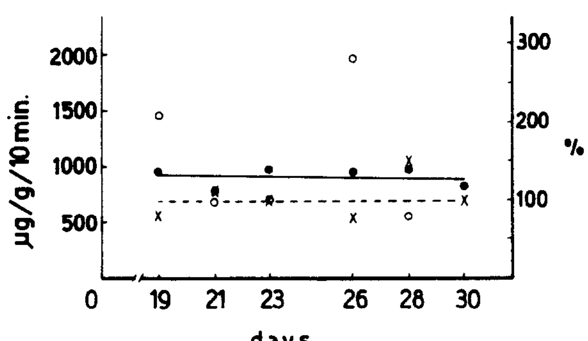

Fig. 7. Days of treatment and corticosterone secretion per adrenal weight. Abscissa: days of treatment. Ordinate: corticosterone secretion in adrenal venous effluent, per g of adrenal, per ten minutes. (●) Values for exhausted animals. (———) Mean value for exhausted animals. (○) Values for adapted animals. (×) Values for controls. (---) Mean value for controls.

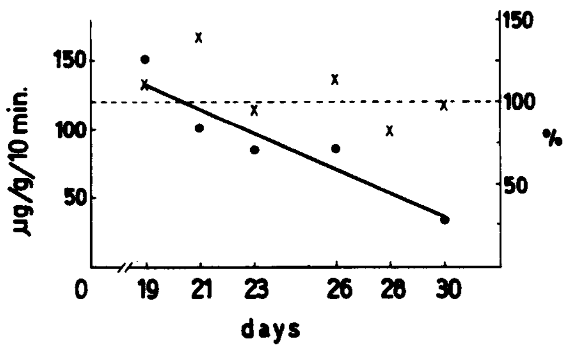

Fig. 8. Days of treatment and 18-OH-DOC secretion per adrenal weight. Abscissa: days of treatment. Ordinate: 18-OH-DOC secretion in adrenal venous effluent, per g of adrenal, per ten minutes. Values for exhausted animals (●) adjust to a negative correlation (———) whose function is:

$$y = 30 - 0{\cdot}9x; \quad r = -0{\cdot}934; \quad P < 0{\cdot}05$$

(×) Individual control values. (---) Mean control value.

alyzed, it was found that exhausted as well as adapted animals show Correlation B, but that the dispersion is greater for the latter subgroup. For corticosterone, exhausted animals demonstrate Correlation B, while adapted ones show no correlation whatsoever.

The interpretation of results for 18-hydroxy-DOC is hampered by the lower precision of the analytical method (see Discussion) and by the fact that only exhausted animals were analyzed. No significant values were obtained for this steroid, as far as Correlation B is concerned.

IV. *Correlation between days of treatment and secretions per adrenal weight* (Correlation C; Figs. 6–8). Aldosterone secretion values of exhausted rats show a positive linear Correlation C very clearly. This is also true for all stressed rats considered as a single group, but not for the adapted subgroup alone.

Corticosterone does not present Correlation C. Values for 18-hydroxy-DOC per mg of adrenal weight show a negative correlation to days of exposure.

Figure 9, finally, consists of a graph on which 'Aldo/B ratios' are plotted against days of exposure. (We define 'Aldo/B ratios' as the numbers obtained by dividing percentual values of aldosterone secretion per initial body weight by percentual values of corticosterone secretion per initial body weight. Percentual values are always expressed in reference to the average control values, considered to be equal to 100%).

It can be seen that the function described on this graph is also a parabola which again reaches a minimum on the 19th day of treatment. It is also worthwhile noting that the adjusted values of the parabola never descend below unity, thus indicating that the increases over controls of aldosterone secretion were always greater than those of corticosterone secretion†. Aldosterone increases were roughly 50% higher than the increments of corticosterone on the 15th day of treatment, 30% higher on the 19th day and 150% higher on the 30th day.

†Only one individual (non-adjusted) value, corresponding to the adapted rat killed on the 19th day, was below unity.

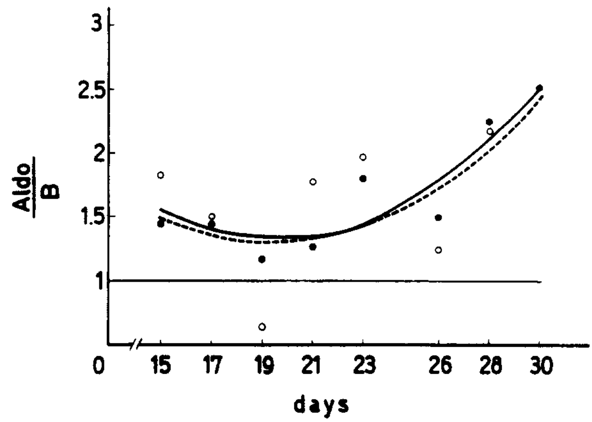

Fig. 9. Days of treatment and 'Aldo/B ratios'. Abscissa: days of treatment. Ordinate: 'Aldo/B ratios' as defined in 'Results'. Values for exhausted animals (●) adjust better to a parabola (———) than to a straight line. The function of this parabola is:

$$y = 4{\cdot}78 - 0{\cdot}37x + 0{\cdot}010x^2; \quad r = 0{\cdot}919; \quad P < 0{\cdot}001.$$

Values for adapted animals (○) do not adjust to any function. Values for all treated animals, considered as one single group, also adjust to a parabola (---) whose function is:

$$y = 5{\cdot}39 - 0{\cdot}41x + 0{\cdot}011x^2; \quad r = 0{\cdot}868; \quad P < 0{\cdot}001.$$

Renin concentrations

The results for renin, summarized in Table 2, indicate a significant decrease in plasma concentrations of this enzyme.

Table 2. Plasma renin concentrations

	15th day (μg/100 ml)	20th day (μg/100 ml)	30th day (μg/100 ml)	Total (μg/100 ml)
Controls	0·516 ± 0·057(6)	0·621 ± 0·140(6)	0·838 ± 0·231(7)	0·668 ± 0·098(19)
Treated	0·352 ± 0·057(5)	0·302 ± 0·016(4)	0·378 ± 0·146(5)	0·347 ± 0·053*(14)

'Day' refers to day of treatment. Values are given as means ± standard error *$P < 0{\cdot}02$. Numbers in parentheses refer to number of rats.

DISCUSSION

Alterations of steroid secretions

A certain number of variables have to be taken into account for a correct evaluation of the parameters discussed in this section:

The *methods of analysis* were more accurate for aldosterone and corticosterone than for 18-hydroxy-DOC, no internal standard having been available for the latter.

The decreases of body weight during the five days prior to sacrifice proved to be related to the increments of adrenal weight, but not to hormonal secretions. Neither were the latter in all cases proportional to the size of the animal's gland (see below). It was therefore thought convenient to *express results* separately per kg of *initial body weight* and per mg of *adrenal weight*. The importance of each of these ways of expression is in this case obvious: one of them represents a modified secretion, irrespective of changes in the size of the adrenals or the decrease of body weight, while the other takes into account the fact that the gland increases and compares hormone production to this magnitude.

The division of treated animals into 'exhausted' and 'adapted' subgroups according to the described criterion, was originally prompted by the necessity of obtaining statistically representative duplicates on each day of killing, and by the preliminary observation that body weight increments or losses were correlated to the adrenal weight and could thus be taken into account for establishing subgroups from which to choose representative individuals. Factors other than endocrinological might, however, be involved in the distinction between 'adapted' and 'exhausted' animals.

The differences between *adrenal steroid secretions* proved to lie in the *dispersion*, rather than in the *magnitude* of the values. Thus, a careful analysis of the data presented in Table 1 and Figs. 3–9 demonstrates that no differences exist between the magnitudes of hormonal secretions corresponding to adapted and exhausted animals, but that the standard errors, as a general rule, are higher for adapted rats. Moreover, very significant correlations exist between secretion values corresponding to exhausted rats and days of exposure, but these correlations are either less significant (the data showing a greater dispersion) or do not exist at all for values of the adapted subgroup. Stressed rats, considered as one single group, presented all of the correlations of the exhausted subgroup.

The following conclusions may be drawn from the steroid secretion data: Aldosterone was the steroid which increased most after prolonged formaldehyde treatment, and also the one whose increase was most significantly correlated to days of exposure. Corticosterone, under these conditions, was also augmented but the correlation with days of exposure was less pronounced. Secretion data of both steroids, as a function of days of treatment, can be fitted to parabolae with high values on the 15th and 30th days and a minimum around the 20th day of exposure.

'Aldo/B ratios', as defined earlier, represent the relationship between a biologically active 'metabolite' and its also biologically active 'precursor'. The increments of aldosterone values over controls were shown to be higher than those of corticosterone values, and the corresponding ratios again describe parabolae whose two branches descend towards the 20th day.

Recently, Vecsei and Kessler reported increases in *in vivo* conversions of corticosterone to aldosterone by adrenals of formaldehyde treated rats, which were too small to be significant [16]. All these rats had received the treatment for 14 days. Under the particular conditions employed by the authors, this period could well represent one of several minima obtained in 'aldo/B ratios' during the treatment.

Aldosterone and corticosterone secretions of exhausted animals increased more than their adrenal weights and these relative increments were again higher for aldosterone than for corticosterone.

The histological changes of the zona glomerulosa of treated animals are interesting to observe in this respect. This zone presents vacuoles whose size and number showed a clear relationship to days of exposure and aldosterone secretion values (Fig. 10). Aldosterone is known to be the only steroid which is produced uniquely by the glomerulosa, while practically all other steroids are produced in the three zones[17].

18-Hydroxy-DOC, on the other hand, showed a tendency to decrease in secretion as the treatment advanced*. It must be remembered that 18-hydroxy-DOC, like aldosterone, is a steroid whose methyl at C_{18} is oxidized, but that the former compound is mainly produced in the *fasciculata-reticularis*[18]. De Nicola[18] suggests a 'seesaw effect' between glomerulosa and *fasciculata-reticularis*, and this effect might well play a role in the present findings.

Furthermore, when our results are complemented with those of Vecsei[4], who studied earlier periods of formaldehyde stress in rats, a certain periodicity in individual corticoid secretions can be observed *during the whole duration* of exposure to aggression. It can be postulated that this periodicity might be partially responsible for the changing pathological syndromes observed by Selye[2] during the different stages of stress.

Renin concentration

From the beginning of these experiments on, the possibility could not be discarded that the high aldosterone values found were a consequence of an enhanced renin secretion. The latter could be due to systemic effects of the formaldehyde treatment (oedema, inflammation, cachexia), to an ACTH-mediated action on the release (synthesis?) of this enzyme, or to both phenomena at the same time.

Not only was renin not increased in treated animals, but its concentration was actually found to decrease after exposure to formaldehyde (Table 2). One explanation for this observation is that renin secretion diminishes as a result of a feedback mechanism caused by augmented aldosterone levels. This situation is known to occur in humans as a consequence of primary aldosteronism[19].

However, the problem *in rats* is somewhat complicated by methodological difficulties and because the role of the renin-angiotensin system as a stimulator of aldosterone secretion has not been clearly demonstrated in this species. This latter aspect has been discussed extensively in the past[20, 21, 22].

As mentioned above, a further difficulty in the interpretation of the differences found in renin determinations between the two groups is caused by the method employed: the amount of plasma utilized in this method is approximately 4 ml. This large volume requires the exclusion of the kidneys, in order to avoid an enhanced release of renin by these organs. The handling of the kidneys, on the other hand, might provoke an alteration of renin secretion which could be different for both groups and which could be misinterpreted as a 'real' effect present in treated animals.

In any case, the present results exclude the possibility that the increased aldosterone secretion was secondary to an augmented plasma angiotensin concentration.

This work describes the evolution of individual steroid secretions with the duration of a 'disease of adaptation'. An explanation for the phenomena observed is attempted but only partially obtained. Taking into account the combined evidence of our own results and those of others[4–7, 23], a dual mechanism, involv-

*Due to the drawbacks mentioned earlier, the data referring to 18-hydroxy-DOC have to be considered with caution. It is especially premature to state that *no* differences exist between 18-hydroxy-DOC secretion *per body weight* of treated and control animals and that the former are *not* correlated negatively to days of exposure (Table 1 and Fig. 3).

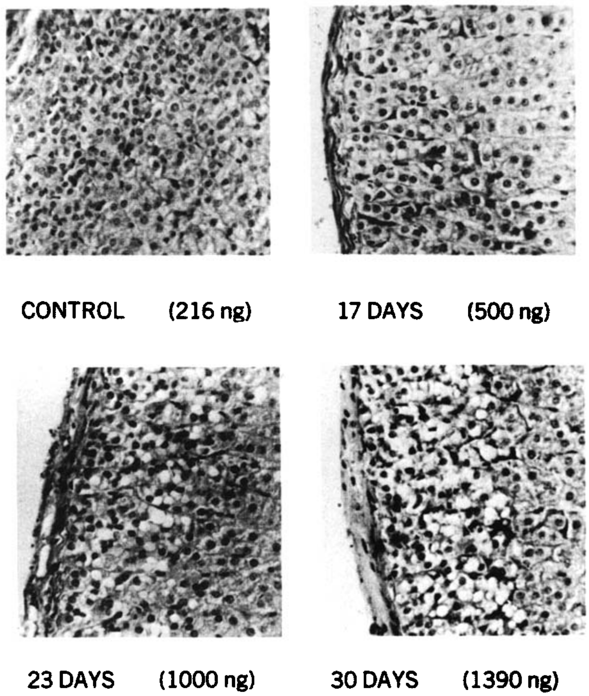

Fig. 10. Zona glomerulosa of one control and three treated rats. Bouin fixative and hematoxylin-eosin staining were employed. "Days" refer to days of treatment and 'ng', to millimicrograms of aldosterone in adrenal venous effluent. per kg (final body weight), per ten min. The same values, expressed per kg of initial body weight, would be: 252, 371, 679 and 1348, respectively.

ing the hypothalamic–hypophyseal-adrenal axis plus some other factor related to systemic effects that becomes evident at the very end of treatment might be responsible for the high aldosterone values observed. Particularly at the end of the experiment (when the aldosterone levels were highest), a hypothesis similar to the one proposed by Holzbauer and Vogt for bled dogs – in which high mineralocorticoid-glucocorticoid ratios are also obtained – might be advanced. According to this hypothesis, ACTH and the alternative factor could be able to 'replace each other, so that neither factor can be rightly regarded as *the* glomerulotrophic hormone' [23].

Experiments designed to explain these observations, as well as an attempt to generalize this pattern to all 'diseases of adaptation' [2], are presently being carried out in our laboratories. These experiments employ different stimuli and regular ACTH injections, as well as water and mineral ion determination in rats submitted to all these treatments.

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