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# THE PRODUCTION OF HYPERTENSION IN MALE ALBINO RATS SUBJECTED TO EXPERIMENTAL STRESS*

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**Abstract**—Male albino rats were subjected to a chronic variable stress program consisting of flashing lights, audiogenic stimulation, and oscillation. Animals of each experimental group were exposed to 4-hr stress sessions 3, 5, or 7 times per week for 16 weeks. Half the animals were housed in complete darkness except during the stress session. The blood pressures of all stressed groups reached a maximum of 142–148 mm Hg within 8 weeks and were maintained at that level for 16 weeks of stress exposure. There was a maximal increase in urinary catecholamine levels after a single stress exposure and a return to control levels within 8 weeks of stress. Adrenal epinephrine was initially depleted after a single stress exposure, but returned to control levels by the 8th week, followed by a moderate overcompensation within the 16th week of chronic stress exposure. Adrenal and plasma corticosterone levels both markedly increased after a single stress exposure and returned to control levels by the 8th week of stress. Unlike the apparent catecholamine adaptation, plasma corticosterone levels again increased significantly by the 16th week of stress, whereas the adrenal steroid levels remained at normal levels. These results suggest that the adrenal glands had become more efficient in the rate of synthesis and release of steroids after chronic exposure to the stressors.

HUDAK AND BUCKLEY[1] successfully induced experimental hypertension by a combination of chronic stressors. The mean blood pressures of rats, exposed to a combination of flashing spotlights, noxious sounds, and motion, 5 days per week, reached a maximum of 152 mm Hg within 22 weeks. Buckley, *et al.*[2] further demonstrated the inability of daily intraperitoneal doses of chlorpromazine (4·0 mg/kg) and reserpine (0·1 mg/kg) to prevent the development of this hypertension.

This investigation is an extension of these studies, with special emphasis on the following objectives: (1) to determine the relative effectiveness of various stressor programs in producing experimental hypertension; (2) to determine the relative influence of different housing conditions on the development of hypertension by these stressors; (3) to determine if and when pituitary–adrenal adaptation occurs during stress application, and whether it comprises a causal–effect relationship with the stress; (4) to determine what role, if any, catecholamines play during physiological response to chronic exposure to variable stressors.

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## METHODS

*Experimental design*

Two hundred and ten male albino Sprague-Dawley rats (Charles River), weighing 75–90 g, approximately 30–45 days old on arrival, were used in these investigations.

*Experiment* 1. One hundred and fifty-nine rats were housed in our laboratories until they reached a weight of approximately 200 g. During this time the animals were numbered, body weights obtained periodically, and their systolic blood pressures taken on six different occasions via a photoelectric tensometer (Metro Industries, Inc.). Activity counts of each rat were estimated by an open-field technique. The rat was placed in a Plexiglas chamber (internal measurements, 25 cm wide × 52 cm deep × 29 cm high). Soft cardboard (Upjohn animal board, deotized) was used to cover the floor and was divided by lines into eight squares. The animal was observed for 3 min and an activity count recorded each time the rat crossed from one block to an adjacent block. Each animal received three trials, and the mean activity count was determined. There appeared to be a correlation between increased rate of weight gain and decreased activity, but no correlation with blood pressure. Animals with extreme activity scores and body-weight gains were discarded, and 120 animals with indices closest to the mean were selected for the study. Each rat was then randomly placed in one of ten experimental groups of twelve animals each (Table 1). All, except four experimental

TABLE 1. STRESS PROTOCOL

| Day of initiation of stress | Housing conditions | No. | Stress sessions* per week |
|---|---|---|---|
| Monday | Isolation | 12 | 0 |
| | Paired | 12 | 0 |
| Tuesday | Isolation | 12 | 5† |
| | Paired | 12 | 5 |
| Wednesday | Isolation | 12 | 7 |
| | Paired | 12 | 7 |
| Thursday | Isolation | 12 | 3 |
| | Paired | 12 | 3 |
| Friday | Saline‡ | 12 | 0 |
| | DOC-TMA§ | 12 | 0 |

* Each animal was exposed to 4 hr of the same variable stress program.

† These animals were also exposed to the pole-climbing apparatus once each week.

‡ These animals received 0·86% sodium chloride solution *ad lib.* once weekly with a second choice of water.

§ DOC-TMA, 6·25 mg (i.m.) three times per week. These animals also received saline once per week (0·86% sodium chloride *ad lib.*) with a second choice of water.

groups, were exposed to 3, 5, or 7 four-hr stress sessions per week. Each stress session was identical for each stressed group, and consisted of the following program, which was randomized throughout the study.

i. *Flashing lights.* Four lights were on for 1/4 sec and off for 3/4 sec, with two lights on simultaneously; i.e. the animals were in complete darkness for 1/2 sec out of every sec.

ii. *Audiogenic stimulation.* This was produced by tape recordings of noxious sounds (compressed air blasts, bells, buzzers, and tuning fork impulses) for 1/2-min periods

at 5-min intervals. The intensity of audiogenic stimulation at the center of the cage was approximately 100 decibels.

iii. *Cage oscillation.* This was maintained at the rate of 140/min. A control unit was constructed so that a variable 4-hr sequence could be automatically programmed to the stress room.

One group received 6·25 mg desoxycorticosterone trimethylacetate* (DOC-TMA), i.m., three times per week, in order to maintain a hypertensive state, and a control group received saline (0·86% NaCl). The DOC-TMA-treated and saline group were also offered normal saline *ad lib.* once per week. The 3-day stress groups were randomly presented weekly stress sessions, and sixteen different weekly schedules were utilized.

All animals were housed in standard metabolism cages 10 in. long × 7 in. wide × 7 in. high. Isolated animals were placed in identical cages in which black phenolic plastic was utilized to maintain the rats in total darkness. For convenience, each group was initiated into the study on a different day of the week, as shown in Table 1.

During the studies, blood pressures and body weights were obtained weekly, prior to stress exposure and at least 20 hr after the previous exposure. Urine was collected over a 16-hr period from each group once weekly in Erlenmeyer flasks containing 2 ml of 1 N sulfuric acid for catecholamine assays. After exactly 16 weeks of chronic stress exposure, all the animals including 5 control animals (housed 12 per cage), were sacrificed between 11 a.m. and 1 p.m. over a 5-day period. After sacrifice, blood was collected in 10-ml beakers previously rinsed with heparinized saline (500 u/ml). Adrenals were frozen in tubes immersed in an acetone–dry ice mixture and then placed in a freezer at −10° until assay. Plasma was then separated from whole blood by centrifugation at 2500 *g* for 20 min and then frozen at −10° until assay.

*Experiment* 2. The second study utilized ninety animals, which were subjected to a 3-day variable stress program of 4 hr per session. Blood pressures and body weights were determined weekly, and urine samples collected for the first 8 weeks. During the 8th week of stress, twelve experimental and twelve control animals were sacrificed, as previously described. The remainder of the animals continued on the stress program in order to duplicate the first experiment. An acute experiment was conducted in which six stressed animals and six controls were sacrificed immediately after a single stress exposure. Tissues were removed and preserved as previously described.

*Chemical assays*

*Urine catecholamine assays.* Catecholamine determinations were performed, by a combination of the methods of Euler and Lishajko[3] and Moore and Brody.[4] After the filtration of urine *in vacuo*, 500 mg EDTA and 2 ml of 1 M Tris buffer (pH 10·0) were added to each sample and the pH adjusted to 8·4 with 5 N sodium hydroxide. This mixture was then immediately poured over an alumina column containing 500 to 700 mg alumina.† Urine extracts were passed through the column (usual time 4 to 6 min) and washed with 5 ml of 0·2 M sodium acetate and 5 ml glass-distilled water. Excess water was removed by reduced pressure, and two 5-ml aliquots of 0·2 N acetic acid were then added to the column and stirred thoroughly with a glass rod before allowing each to pass through the column. Urinary norepinephrine (NE)

* Percorten, CIBA.

† British Drug House, chromatographic-grade alumina.

recovery averaged 82 per cent and the recovery of epinephrine (E) averaged 86 per cent. After the elution of catecholamines from the column, they were either assayed immediately, or frozen at −10° for no longer than 1 week. Standards ranging between 0·25 and 1·0 μg NE and 0·125 and 0·5 μg E, were also tested with each urine assay. Catecholamines were then assayed by a differential fluorescent method with potassium ferricyanide, as described by Moore and Brody.[4] Fluorescence was read 5 to 10 min after the addition of the alkaline–ascorbic acid solution, in a Turner fluorometer, model 110. The primary filter for activation was a 405 filter, and the fluorescent filter was 65A. The second filter combination consisted of two filters, 47A plus 2A, and the fluorescent filter was 2A15. For these assays, the high-sensitivity kit (Turner 110-865) was used. Catecholamine levels were expressed as ng/kg body weight/hr.

*Adrenal catecholamines.* Each left adrenal gland was homogenized with 10·0 ml of 5·0% tricholoracetic acid, in a hand homogenizer (A. H. Thomas). The adrenal extract was filtered and either immediately assayed or frozen at −10° for assay no more than 48 hr later. This extract (0·5 ml) was then diluted to 2·5 ml with distilled water and the catecholamines assayed as previously described. Daily catecholamine solutions were prepared in 5% tricholoracetic acid solution in concentrations of 1 μg of each ml, and 0·5 ml aliquots assayed as above. The high-sensitivity kit was not necessary, because of the high catecholamine levels.

*Adrenal and plasma steroids.* Corticosterone levels were estimated according to the method of Guillemin *et al.*[5] in the right adrenal or 0·5 ml of plasma. Since the fluorescence was measured on a Turner fluorometer, a greater quantity of fluorescent material was needed for assay, as compared to the Aminco-Bowman instrument used by Guillemin. Therefore, the fluorescent mixture was that described by Dixit;[6] namely, concentrated sulfuric acid, 2·4 parts to 1 part of a 50% alcohol–water mixture. In the final step of the assay procedure, 2·0 or 4·0 ml chloroform was thoroughly mixed with 4·0 ml of this acid mixture, 3·5 ml of which was used for final assay. The filter combination used was 47B for the activating wavelength and 2A 12-2 for the fluorescent wavelength. Recoveries from both of these tissues averaged 95–98 per cent.

*Statistics.* Statistical significance between groups was determined by the Student's *t* test.

## RESULTS

### *Effects of various chronic stressor programs on the blood pressure of male albino rats*

The stressors used in these experiments effectively produced experimental hypertension, whether they were presented three, five, or seven times a week (Table 2). The increase in pressure was approximately the same for both isolated and paired animals in every group, including the controls. The mean blood pressure elevations of the experimental groups subjected to chronic stress were significantly higher than the control groups ($P < 0{\cdot}01$), as observed in Table 2. After exposure to a single stress session the mean blood pressure of rats increased from 109 ± 1·1 to 114 ± 1·5 ($P < 0{\cdot}01$, n = 48). In addition to the data summarized in Table 2, the 3-day stress experiment was replicated and expanded to study the effects of chronic stress in animals housed twelve to a cage. In this experiment, the mean blood pressure increased to 144 ± 5·8 mm Hg (control = 111 ± 2·3 mm Hg, n = 12) by the 8th week of stress and remained at approximately this level for another 8 weeks of stress (mean blood pressure at end of 16th week = 146 ± 1·4 mm Hg). It was further observed

that if the stressors were discontinued after 12 weeks of stress (mean blood pressure = 141 ± 2·4 mm Hg), the blood pressure remained elevated and actually continued to rise, reaching a mean of 156 ± 1·5 mm Hg at the end of the 16th week (n = 12). Thus, this experimental hypertension does appear to be chronic and self-sustained once initiated.

TABLE 2. EFFECTS OF VARIOUS CHRONIC STRESSORS ON THE BLOOD PRESSURE OF MALE ALBINO RATS

| Group | Initial blood pressure* (mm Hg ± S.E.) | Final blood pressure† (mm Hg ± S.E.) | Change in blood pressure (mm Hg ± S.E.) |
|---|---|---|---|
| Control paired | 102·2 ± 1·0 | 112·7 ± 1·9 | +10·5 |
| Control isolated | 103·6 ± 1·1 | 117·7 ± 2·4 | +14·1 |
| 3-Day paired | 101·7 ± 1·6 | 147·2 ± 2·1 | +45·5 |
| 3-Day isolated | 103·3 ± 1·4 | 143·7 ± 2·3 | +40·4 |
| 5-Day paired | 104·0 ± 1·2 | 148·2 ± 3·1 | +44·2 |
| 5-Day isolated | 102·2 ± 0·9 | 142·8 ± 2·2 | +40·6 |
| 7-Day paired | 102·7 ± 1·2 | 142·3 ± 2·4 | +39·6 |
| 7-Day isolated | 104·8 ± 1·0 | 143·5 ± 2·6 | +38·7 |

No. = 12 for each group.

* All initial blood pressures represent the mean of the last four of six readings prior to the first stress session.

† All blood pressure values at the end of 16 weeks are significant from the respective control data at $P < 0{\cdot}01$ level of significance. All final blood pressure values were also significantly different from the initial readings ($P < 0{\cdot}01$).

No mortalities were recorded throughout this investigation, possibly because of preselection of animals. These experiments demonstrate the ability of the rats to adapt to stressors which were presented over long periods of time, even though the animals appear to have developed experimental hypertension.

*Urinary catecholamine levels and chronic stress.* Urinary catecholamine levels were quite variable on a day-to-day basis (Fig. 1); and, because of this variation, significant alterations in urinary catecholamine content were difficult to observe. The progressive increase in blood pressure and changes in urine catecholamines are depicted in Fig. 1. Control NE values varied greatly, whereas E levels remained fairly constant; however, at the time of maximal blood pressure changes, urinary catecholamine levels were approximately normal. Table 3 summarizes the effects of all the stress programs on daily urinary catecholamine levels in both paired and isolated conditions during the last 8 weeks of the study. There was relatively no day-to-day change in urinary catecholamine levels. However, the mean catecholamine level in each experimental group was lower than its respective control even though only one group demonstrated a significant difference. Therefore, there is very little indication from this portion of the study that alteration in catecholamine release is involved in the production or maintenance of this experimental hypertensive state caused by chronic stress exposure. One phenomenon that was observed throughout each experiment, and which is very clearly observable in Fig. 1, is that after one stress session, both NE and E levels increased significantly; however, these levels returned to normal limits within an 8-week period.

*Effects of various stressors on adrenal catecholamines.* These stressors, whether presented three, five, or seven times a week, had relatively little effect on adrenal catecholamine content after 16 weeks of stress. As observed in Table 4, a significant

increase was encountered only in the adrenal NE levels of paired rats subjected to the stress program 5 days per week and in the isolation control group ($P < 0.05$). Even though the stress groups did present evidence of higher adrenal E, little significance could be attached to these findings. Again, there were no significant indications of any correlation between the hypertensive state and adrenal catecholamine levels.

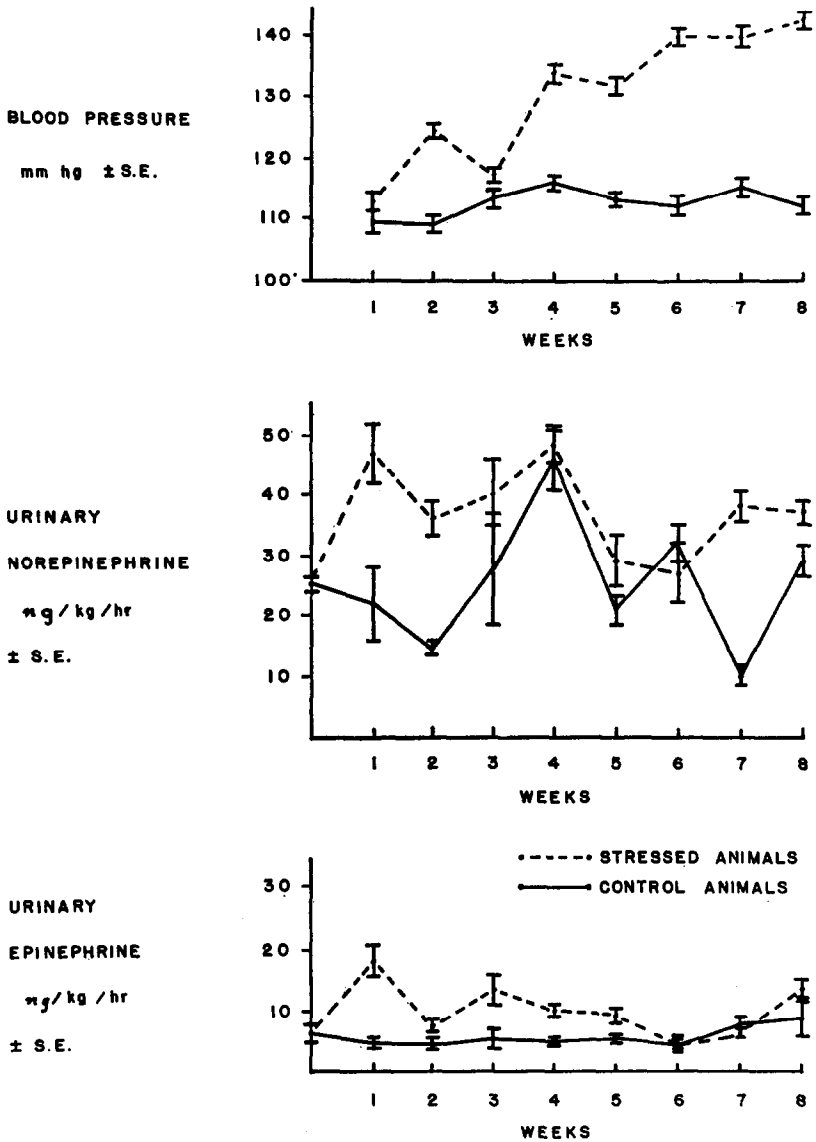


FIG. 1. Effects of a 3-day variable stress program on blood pressure and urinary catecholamine levels. Stressed animal blood pressure changes represent the means of twenty-four animals, whereas the urinary catecholamines represent the pooled means of twelve groups (two animals/group). Control values are represented by half the number of these animals. Weekly determinations represent the 1st day of the week; thus, 1st week represents one stress session.

*Effects of chronic stressors on the adrenal cortex.* In contrast to the previous findings concerning urinary and adrenal catecholamine levels, much significance can be attached to plasma corticosterone levels (Fig. 2). All the stress programs of this investigation produced significant increases in plasma corticosterone levels but did not significantly alter adrenal steroid content as compared to paired control animals

TABLE 3. EFFECTS OF VARIOUS CHRONIC STRESSOR PROGRAMS AND HOUSING AGGREGATION ON URINARY CATECHOLAMINE LEVELS (8TH TO 16TH WEEKS)

| | Catecholamine levels* (ng/kg/hr) | | | |
|---|---|---|---|---|
| | Paired | | Isolation | |
| Duration of stress | NE | E | NE | E |
| Control | 49·1 ± 5·8 | 17·8 ± 2·0 | 51·6 ± 4·2 | 18·9 ± 1·9 |
| 3-Day stress | 42·5 ± 1·8 | 16·4 ± 1·0 | 50·7 ± 2·1 | 16·0 ± 1·3 |
| 5-Day stress + CAR | 40·9 ± 5·3 | 14·4 ± 2·4 | 37·8 ± 2·8† $P < 0·01$ | 13·3 ± 1·8† $P < 00·5$ |
| 7-Day stress | 44·2 ± 3·8 | 15·0 ± 1·5 | 43·6 ± 3·8 | 15·0 ± 1·4 |

* Each value represents daily mean values of six pooled urine samples during the last 8 weeks of stress ± S.E.

† Significant from isolation-control animals.

TABLE 4. EFFECTS OF VARIOUS CHRONIC STRESSOR COMBINATIONS ON ADRENAL CATECHOLAMINES (16 WEEKS OF STRESS)*

| | Paired animals | | Isolated animals | |
|---|---|---|---|---|
| Experimental group | (μg NE/adrenal ± S.E.) | (μg E/adrenal ± S.E.) | (μg NE/adrenal ± S.E.) | (μg E/adrenal ± S.E.) |
| Control | 2·2 ± 0·5 (12)† | 12·9 ± 1·7 | 7·2 ± 2·0 $P$ 0·01 (11) | 14·6 ± 0·9 |
| 3-Day stress | 2·3 ± 0·8 (10) | 19·1 ± 3·4 | 4·1 ± 1·0 (12) | 17·0 ± 1·3 |
| 5-Day stress | 5·1 ± 1·1 $P < 0·05$ (10) | 17·3 ± 1·4 | 4·0 ± 0·9 (11) | 17·7 ± 2·0 |
| 7-Day stress | 3·9 ± 0·6 (12) | 15·0 ± 1·4 | 2·0 ± 0·3 (10) | 18·3 ± 1·4 $P < 0·05$ |
| Grouped controls | 4·4 ± 0·7 $P < 0·05$ (22) | 14·4 ± 0·7 | | |

* All E statistical comparisons were made with the paired control animals except where indicated in the text.

† Number of animals.

(Fig. 2). The data on 12 animals per cage were similar to those of the paired controls in that the adrenal steroid levels were 0·357 ± 0·041 μg/adrenal (n = 27) and mean plasma steroid levels, 16·6 ± 1·8 μg/100 ml (n = 18).

It was difficult to determine the relative severity of each stressor in terms of behavioral manifestations even though each animal was observed daily. However, in

terms of steroid production, the 7-day stress program was the most potent stimulator. Animals housed in isolation and exposed to daily stress exhibited significantly higher plasma corticosterone levels ($P < 0{\cdot}05$) than all other stress groups of fewer weekly exposures except the 5-day stress (paired housing). On the other hand, animals exposed to the same stress program, but housed in pairs, demonstrated significantly higher ($P < 0{\cdot}05$) steroid levels as compared to stressed animals housed in isolation.

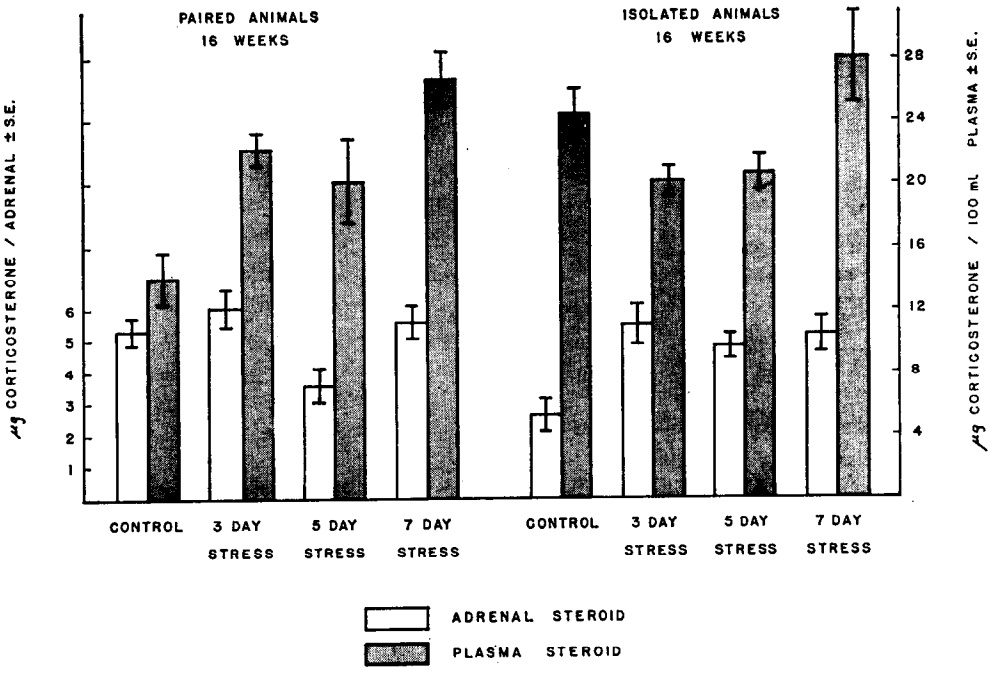


FIG. 2. Chronic effects of various stress programs on adrenal and plasma corticosterones. Each value represents the mean ± S.E. of ten to twelve animals.

Another interesting observation was the effects of simple isolation on adrenal and plasma corticosterone levels. Chronic isolation induced a significant increase in plasma steroid levels ($P < 0{\cdot}01$), while adrenal levels were significantly lower ($P < 0{\cdot}01$) than in animals housed in pairs. Thus, in these experiments chronic isolation should also be considered as a stress.

*Effects of desoxycorticosterone trimethylacetate-induced hypertension in rats.* Desoxycorticosterone trimethylacetate produced a significant increase in blood pressure. Even though there were relatively few effects observed on adrenal catecholamines and steroid levels (Table 5), both the adrenal E and plasma corticosterone levels were higher in the saline control group. It was also observed that the animals were hyperirritable and fought to obtain copious quantities of saline. There was also no evidence of an overproduction of catecholamines, as indicated by below normal urinary NE and E levels. However, the lower levels of urinary catecholamines may not give a true picture of sympathetic activity, since the amine might well be functionally inactivated by test binding rather than by deviations from the liberation sites and subsequent excretion.

TABLE 5. EFFECTS OF DOC-TMA-INDUCED HYPERTENSION

| Parameter (mm Hg) | Saline control, n = 12 | Saline + DOC-TMA, n = 12 |
|---|---|---|
| Final blood pressure (mm Hg ± S.E.) | 118·2 ± 2·4 | 144·0 ± 3·9* |
| Adrenal NE (μg/adrenal ± S.E.) | 3·5 ± 0·6 | 3·9 ± 0·6 |
| Adrenal E (μg/adrenal ± S.E.) | 16·7 ± 0·4 | 12·8 ± 1·4 |
| Adrenal corticosterone (μg/adrenal ± S.E.) | 0·649 ± 0·099 | 0·507 ± 0·063 |
| Plasma corticosterone (μg/100 ml ± S.E.) | 27·4 ± 2·9<br>$P < 0·01$* | 18·2 ± 2·2* |
| Urinary NE (ng/kg/hr ± S.E.) | 39·2 ± 3·40 | 30·5 ± 2·92†<br>$P < 0·01$† |
| Urinary E (ng/kg/hr ± S.E.) | 12·0 ± 1·82†<br>$P < 0·05$ | 9·7 ± 1·39†<br>$P < 0·01$ |

* Significant from saline controls.
† Significant from paired control animals (Table 3).

*Catecholamine and steroid adaptation during a 3-day chronic variable stress program*

In the first experiment, it was observed that experimental hypertension could be induced regardless of the number of exposures per week to a variable stress program. Because of the efficiency of the 3-day stress program and the possibilities of its use in place of a 7-day program, it was studied in greater detail. In addition, an acute-stress experiment was also conducted.

Urinary catecholamine levels increased significantly after a single stress (Fig. 1). Urinary NE increased from 21·9 ± 4·8 ng/kg/hr to 47·7 ± 4·5 ($P < 0·01$), whereas urinary E increased from 4·3 ± 0·32 to 17·1 ± 2·7 ng/kg/hr ($P < 0·01$). On the other hand, adrenal E was reduced from 12·3 ± 1·1 to 2·5 ± 0·3 μg/adrenal ($P < 0·01$). Only a slight alteration was observed in adrenal NE. The decrease in adrenal E is reflected almost quantitatively in the increase in urinary E levels, indicating the value of using urine levels as a reliable index of adrenal function. Adrenal E adaptation is evident by the 24th stress session, or 8th week, as it returned to new control levels (Fig. 3). There also appears to be an adrenal E overcompensation occurring by the 48th stress session.

The data on adrenal steroids were similar. After a single stress session, adrenal corticosterone content almost doubled but returned to normal levels within 8 weeks of chronic stress. Similar to adrenal E changes, there also appeared to be a minor overcompensation occurring by the 16th week of stress. Plasma corticosterone increased from 14·0 ± 1·8 to 30·4 ± 3·8 (mean ± S.E.; $P < 0·01$) after a single stress session. Adaptation occurred by the 24th stress session, and significantly increased levels were again demonstrated by the 48th stress session, or 16 weeks of stress.

## DISCUSSION

The overall objective of this study was to learn more about the production of experimental hypertension by means of variable "psychological" stressors which have been reported to produce experimental hypertension. It was theorized that, by manipulating the stress program, different degrees of hypertension would develop and be accompanied by alterations in adrenal catecholamine and steroid levels. A second

attempt to separate the effects of the various programs was accomplished by superimposing an isolation stress. However, regardless of the program and housing environment, the same degree of hypertension was obtained with relatively few endocrine differences. Blood pressures reached approximately the same maximum (142 to 148 mm Hg, Table 2) for all groups by the 16th week of stress. An analysis of

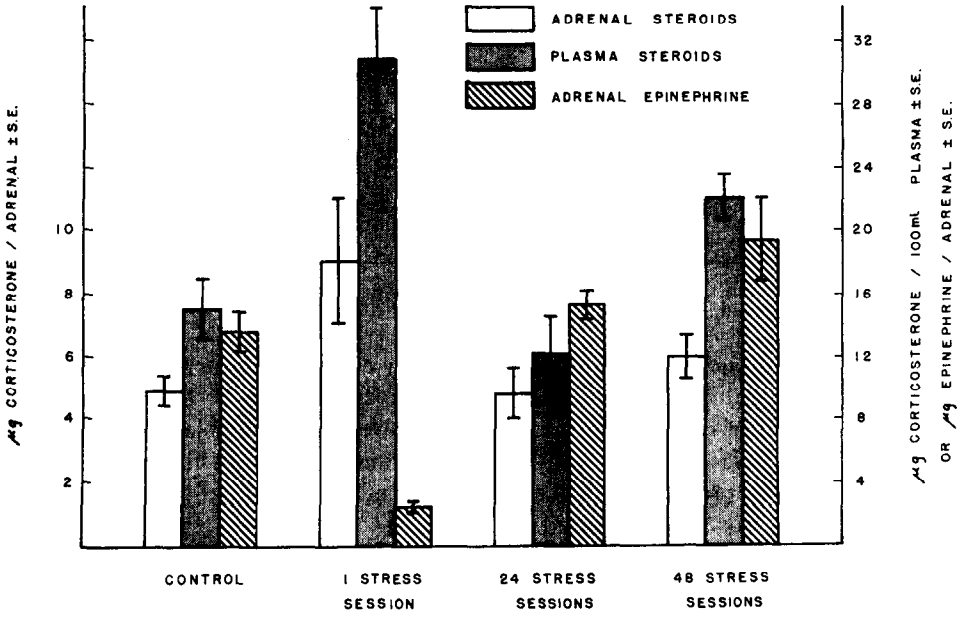


FIG. 3. Adrenal steroid and catecholamine adaptation after 16 weeks of a 3-day variable stress program. Each stressed group value represents the mean ± S.E. of five to twelve animals (one stress session, n = 5; twenty-four and forty-eight stress sessions, n = 12). The control data represent a mean of twenty-two animals.

the profile of the development of this hypertension indicates that the maximal pressure is achieved by the 8th week, and persists through the 16th week. These effects are identical, regardless of group or housing environment, giving little indication of any graded effect. Thus the relationship between this chronic stress and the development of hypertension appears to be all or none. In these experiments, a 3-day stress program appeared to be highly effective. Since each animal did not know when it would be stressed in the random design, the 3-day stress may have been equivalent to a 5- or 7-day stress program insofar as it produced elements of fear and anxiety, which may be a primary cause of the experimental hypertension.

Since it has been postulated that catecholamines may play a role in experimental hypertension,[7] urinary levels of these amines were also studied. The data presented (Fig. 1, Table 3) demonstrate an apparent adaptation of the sympathetic nervous system to the stressors. There were significant increases in both urinary NE and E after a single stress exposure, with a return to the normal range by the 8th week of chronic stress. Adrenal E presented a similar adaptive picture (Fig. 3, Table 4). After initial depletion, adaptation occurred within 8 weeks, with an overcompensation by the 16th week of stress.

In contrast to sympathetic adaptation, the adrenal cortex continues to function maximally even through the 16 weeks of stress, as indicated by the high plasma steroid levels (Fig. 2). On the other hand, adaptation is apparent when one compares adrenal steroids following a single stress (Fig. 3) with levels following 16 weeks of stress. In the last experiment, the increases in adrenal corticosterone levels were not significant but are considered to be a true effect because of the well-documented experiments of Stockham.[8] Selye[9] views the adaptation to chronic stress as dependent upon the pituitary–adrenal axis. In relation to pituitary–adrenal mechanisms in chronic stress, one may postulate that there is an initial stimulation of the system, with subsequent return to relatively normal limits during the phase of adaptation. The data of the present experiment do not support this hypothesis, since the pituitary–adrenal axis appeared to be functioning maximally throughout the duration of the 16 weeks of chronic stress (Fig. 2). The lack of the concurrent increase of adrenal steroids by the 16th week of stress suggests that the adrenal gland has become more efficient in the synthesis and release of corticosterone, thus providing higher plasma corticosterone levels.

Even though these experiments leave little doubt about the possible significance and involvement of the adrenal cortex in the ability of an animal to adapt to chronic stress, its role in this experimental hypertension is not clear. Both chronic isolation and the weekly administration of NaCl induced an elevation of plasma corticosterone levels (Fig. 1, Table 5), as was evident in all animals exposed to 16 weeks of a variable stress program. However, in contrast to chronic stress, neither of these treatments elicited hypertension (Tables 2 and 5). No adequate explanation can be offered for these contradictory results from the data at hand. The concurrent decreases in adrenal corticosterone and increase in adrenal NE (Table 4) following 16 weeks of isolation, however, might be useful in the design of more specific experiments to answer this problem. It should be recognized that even though such evidence does not aid an interpretation of a direct role of the adrenocortical steroids in hypertension, it does not rule out the possibility of a secondary or indirect role.

These studies re-emphasize the importance of adrenal adaptation to subtle and dramatic changes in the environment of an animal. The role of the adrenal medulla appears to be more important in acute stress situations, whereas the adrenal cortex appears to play a more important role in adaptation during chronic exposure to stress. The data do not clarify the relative role of the sympathetic nervous system during adaptation; however, a study of the intracellular distribution of tissue catecholamine and circulatory catecholamines might present a somewhat different picture. Even though catecholamines do not appear to play a major role in the development of sustained experimental hypertension, they may serve a supportive role in conjunction with a second substance that might be released during a chronic stress situation. Rabb[7] has suggested that the adrenalcortical hormones might sensitize the vasculature to the catecholamines, thus potentiating the normal pressor responses to the circulating amines.

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