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Caffeine as an Intensifier of Stress-Induced Hormonal and Pathophysiologic Changes in Mice[1]

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HENRY, J. P. AND P. M. STEPHENS. *Caffeine as an intensifier of stress-induced hormonal and pathophysiologic changes in mice.* PHARMAC. BIOCHEM. BEHAV. 13(5) 719–727, 1980.—Psychosocially stressed male mice competing in a Henry-Stephens complex population cage develop hypertension, cardiovascular damage, and chronic interstitial nephritis. Their plasma renin, noradrenaline, corticosterone, and adrenal-catecholamine synthetic enzymes are increased and they die prematurely. Adding 3.3 mg of caffeine a day per kilogram of mouse body weight (the equivalent of 20 μg/ml decaffeinated coffee) to their drinking water significantly intensifies most of these changes. A dose of 90 mg/kg of caffeine (the equivalent of 560 μg/ml, i.e., brewed tea or coffee) further increases the effects. The drug-induced enhancement of competitive social stimulation of the neuroendocrine system resulted in a further increase of plasma renin and corticosterone levels as well as blood pressure and adrenal weight. These effects together with accelerated mortality and increased pathology indicate that chronic consumption of caffeinated liquids adds to the risks of psychosocial stress.

Psychosocial stress Plasma renin Population cage Chronic interstitial nephritis Corticosterone
Blood urea nitrogen Tea Coffee Caffeine

PREVIOUSLY published evidence shows that formerly isolated male mice subjected to psychosocial stress by living in social disorder in a Henry-Stephens complex population cage develop hypertension, cardiovascular damage, chronic interstitial nephritis, and accelerated mortality [8, 9, 10, 11, 12]. The evidence for nephritis includes higher blood urea nitrogen as well as pathophysiological changes in the kidneys [13]. Previous studies have demonstrated higher levels of plasma renin [18] and corticosterone [12] together with higher concentrations of the catecholamine-synthesizing enzymes, tyrosine hydroxylase and phenylethanolamine N-methyltransferase (PNMT), in the adrenals [10]. Current unpublished work also indicates higher levels of plasma noradrenaline. Thus there is evidence that psychosocial stress in mice promotes autonomic arousal with its neuroendocrine accompaniments and pathophysiological consequences [12].

It is known that caffeine increases plasma levels of catecholamines, renin, and free fatty acids [1,16]. This poses the question whether caffeine would act in synergism with psychosocial stimulation, increasing the intensity of the observed hormonal changes and, hence, the morbidity and mortality of variously stressed mice.

In addition to coffee and tea, we have studied the effects of high and low doses of caffeine in water on male mice intensely stressed by chronic social disorder. The results were contrasted with those from similarly stressed males that drank pure water. Peaceful male CBA/USC siblings drinking the same liquids served as minimal stress controls, but even they experienced some stressful stimulation. Although siblings do not bite each other, the stable social hierarchy they develop is disturbed by occasional minor confrontations, and this disturbance resulted in their having slightly higher levels of adrenal-medullary-catecholamine synthetic enzymes [10] and plasma renin [18] than breeder males or isolates. Furthermore, as Kvetňanský *et al.* [15] have shown, the plasma corticosterone and catecholamines of rodents living in polycarbonate boxes increase in response to the stimuli generated by the routine animal husbandry of the vivarium.

Our observations were in three categories in which we: Measured the acute responses of the neuroendocrine system, studying plasma renin, plasma corticosterone, adrenal weight, and blood pressure; Kept a count of monthly fatalities; Assessed the extent of renal failure, measuring blood urea nitrogen, and evaluted myocardial damage and renal failure by microscopic sections.

In these studies, the exposures to stress lasted three to five months, representing 12% to 20% of the lifespan of the CBA/USC mouse.

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METHOD

The mice were raised in our facility according to standard animal husbandry procedures [8, 11, 12]. Male siblings living in standard vivarium polycarbonate boxes (29×18×13 cm) served as controls for minimal stress. Weaned and separated by sex when 21–28 days old, they had remained together since then. Mice of the same sex raised together since birth form a stable social system with little rivalry, but those isolated at weaning and raised alone until 4 months old compete vigorously and cannot maintain social stability when combined with others. Competition is heightened by the addition of normal socially raised female siblings and by providing the specially designed Henry-Stephens complex population cage.

The population cage consists of six animal-holding boxes of standard dimensions connected by narrow tubing into a circle around a central feeding and drinking area. Designed to induce social interaction, the population cage features multiple entries to boxes to discourage the formation of territory; narrow connecting tubes provide a one-way passage and encourage confrontations; and centrally located food and water force proximity on rival males [8].

Population cages stocked with 16 males and 16 females undergoing chronic social stress and single boxes stocked with minimally stressed male siblings were placed together on sliding 5½-foot shelves, stacked in tiers of four. The mice received standard lab chow ad lib; their liquid was replaced three times a week and their bedding, a 2-in. layer of fluffy wood shavings, once a week.

The mice were 4 months old at the beginning of the series of experiments which usually lasted five months, but one series was terminated at three months because the males on a high dosage of caffeine in water had a high mortality, the end point being when about 20% died.

All males were identified by an ear punch code and were weighed and checked for health once a month. Their blood pressure and pulse rate were determined and the condition of fur and signs of scarring were noted. Blood pressure was measured by applying a tail cuff to a warmed conscious mouse restrained in a tube, a technique described previously [8]. The measurements were made in the morning from 9–12.

Since mice drink at 1–2 hr intervals and fluid was available during the preliminary warming and blood pressure measurement, it is probable that the males' plasma caffeine levels were unchanged from those prevailing while they were in the population cage. Blood samples were taken to measure corticosterone by a modified Glick procedure [14] and blood urea nitrogen by the Azostix method. We confirmed the accuracy of this color-matching technique for our study by comparing Azostix values with a routine chemical assay. The hearts, kidneys, and paired adrenals were weighed and placed in 10% Formalin for Hematoxylin and Eosin and Periodic Acid Schiff Studies, as previously described [9].

Tea, coffee, and decaffeinated coffee were freshly brewed, poured into eight 125 ml bottles, and placed in the central feeding area. Standard commercial brands were used: Regular Yuban Coffee (General Foods Corporation, Box 3114, White Plains, NY 10625); MJB Tea Bags (MJB Company, San Francisco, CA 94107); and Regular Brim Decaffeinated Coffee (Maxwell House Division, General Foods Corporation, White Plains, NY 10625).

The manufacturers' instructions were followed and the coffees were brought to the recommended strength by percolation for 10 min in a standard 10-cup coffee pot. Tea was prepared by gently agitating the tea bags while steeping them in just boiled water for 5 min. The caffeine content of the beverages was routinely determined by gas chromatography [4] and the average strengths were: brewed coffee—560 μg/ml or 140 ± 10 mg/250 cc cup; steeped tea—440 μg/ml or 110 ± 10 mg/250 cc cup; and brewed decaffeinated coffee—16 μg/ml or 4 ± 2 mg/250 cc cup. Persons, weighing 70 kg, who normally drink 4 cups of average strength coffee are getting 8 mg caffeine/kg/day. Their caffeine intake will vary proportionally when drinking tea or decaffeinated coffee. The effects of drinking these liquids were contrasted in highly stressed and minimally stressed males. Various dosages of pure caffeine (U.S.P.) in water were used to test for the possible effects of other constituents in tea and coffee. The different strengths were: 20 μg/ml=brewed decaffeinated coffee or 3.3 mg/kg/day/mouse; 560 μg/ml=brewed coffee and tea or 90 mg/kg/day/mouse; 800 μg/ml=extra strong brewed coffee or 132 mg/kg/day/mouse.

To resolve the question whether flavor affected the consumption of liquids, miminally stressed males could choose between pure water and caffeinated water. Although the weak caffeine flavor (20 μg/ml) was barely perceptible to human taste, they chose pure water. But they did not drink less caffeinated water when it was the only liquid offered. They drank (mean ± S.D.): 5.6 ± 0.7 cc caffeinated water daily; 5.4 ± 0.6 cc pure water daily.

Highly stressed males drinking caffeinated beverages consume about the same amount as those drinking pure water: 6.9 ± 1.6 cc coffee/mouse/day; 6.5 ± 1.8 cc decaffeinated coffee/mouse/day; and 7.3 ± 0.8 cc pure water/mouse/day. After three months, the body weights of highly stressed males drinking coffee, tea, decaffeinated coffee, and pure water were essentially the same: 33.5 ± 1.8 g coffee; 34.7 ± 2.5 g tea; 33.5 ± 2.2 g decaffeinated coffee; and 34.8 ± 1.8 g pure water. As we have observed previously, there was no significant difference between the body weights of highly stressed and minimally stressed males [11].

For statistical comparison we used a two-way Analysis of Variance with replication, as described by Sokal and Rohlf [17]. This test was chosen because the experimental design involved six groups of mice divided into two sets of three groups each. Minimally stressed males in boxes were placed on the 5½-foot shelves accommodating the highly stressed males in population cages. A different liquid was provided for each shelf: one receiving pure water; a second, a weak dose of caffeine (20 μg/ml) in decaffeinated coffee or water; and a third, a full strength dose of caffeine (560 μg/ml) in either coffee, tea, or water. The probability values for the two different levels of stress, the three different strengths of caffeine, and the interaction between them are presented in the legends of the appropriate figures.

Tukey's *a posteriori* test of Honest Significant Difference (HSD), as described by Haber and Runyon [7] in their discussion of Analysis of Variance, was also calculated and is included in the appropriate legends. The renin study involved only four groups of mice and the study of monthly deaths and cardiac damage only two. These were treated with one-way Analysis of Variance.

Two series of tests were made. For the first one, we used caffeine in water to avoid all other constituents found in caffeinated liquids and reduce taste to a minimum. For the second, more extensive series, involving 16 population cages tested three at a time, we used brewed coffee, tea, and decaffeinated coffee as the principal liquids, although mice in two of the cages received extra strong and weak doses of caffeine

in water, respectively. The first series of tests ran three months and the second series, five months. Our observations on both series are described in three categories: Acute Neuroendocrine Responses, Monthly Mortality, and Pathophysiological Changes in the Heart and Kidneys.

RESULTS

Acute Neuroendocrine Responses

Evidence that the sympathetic adrenal-medullary system was aroused is shown in Fig. 1 which depicts plasma renin activity. Highly stressed and minimally stressed males drinking pure water and their counterparts drinking liquids with a high caffeine content were tested. When the data for all groups in this five-month study were compiled, there was a significant difference in renin between males taking high doses of caffeine and those taking pure water in both high stress and minimal stress environments ($p<0.001$), indicating that caffeine enhanced the intensity of the stress response.

The closely related variable of systemic arterial pressure showed a similar pattern. In the three-month study, there was the usual very significant rise in blood pressure of highly stressed males drinking pure water ($p<0.000$) compared with minimally stressed males also drinking it (Fig. 2). This value confirms previously published observations [8,11]. Caffeine-stress interaction also develops, however, and there was further significant increase in blood pressure when high doses of caffeine were given to both minimally stressed and highly stressed males ($p<0.001$). These data were confirmed in part by the five-month study of blood pressure. Figure 3 shows blood pressure readings for the final month. Highly stressed males have very significantly greater pressure ($p<0.000$) than minimally stressed males. However, they show no additional rise in blood pressure when they are given caffeine. But when the blood pressures of minimally stressed males getting caffeine and those getting pure water were compared, Tukey's *a posteriori* test of Honest Significant Difference (HSD) was positive. (The test requires 7.6 mm Hg to be significant at $p<0.05$.) Thus in minimally stressed males, the additional blood pressure rise on getting caffeine that was noted in the three-month study (Fig. 2) was also observed in the five-month study.

In addition to plasma renin and blood pressure, which indicated sympathetic adrenal-medullary activity, plasma corticosterone and adrenal weight indicated the extent of pituitary adrenal-cortical system arousal in these chronically stressed males [12].

As can be seen from Tukey's *a posteriori* test of HSD (Fig. 4), minimally stressed males getting high doses of caffeine in water had a significant increase in adrenal weight ($p<0.05$) which is further evidence of caffeine-stress interaction. Highly stressed males exposed to three months of social interaction in a population cage showed the expected significant increase in adrenal weight ($p<0.000$) compared to minimally stressed males. Although highly stressed males getting caffeine have somewhat larger adrenals, which suggests that caffeine-stress interaction may be occurring, the difference is not significant.

In a further five-month study, highly stressed males drinking the standard caffeinated liquids showed the expected and significant stress-induced increase in paired adrenal weights [12], increasing from a mean of 3.9 ± 0.2 mg for minimal stressed males to 5.8 ± 0.2 mg for highly stressed males ($p<0.000$). This time there was no evidence of caffeine-stress interaction.

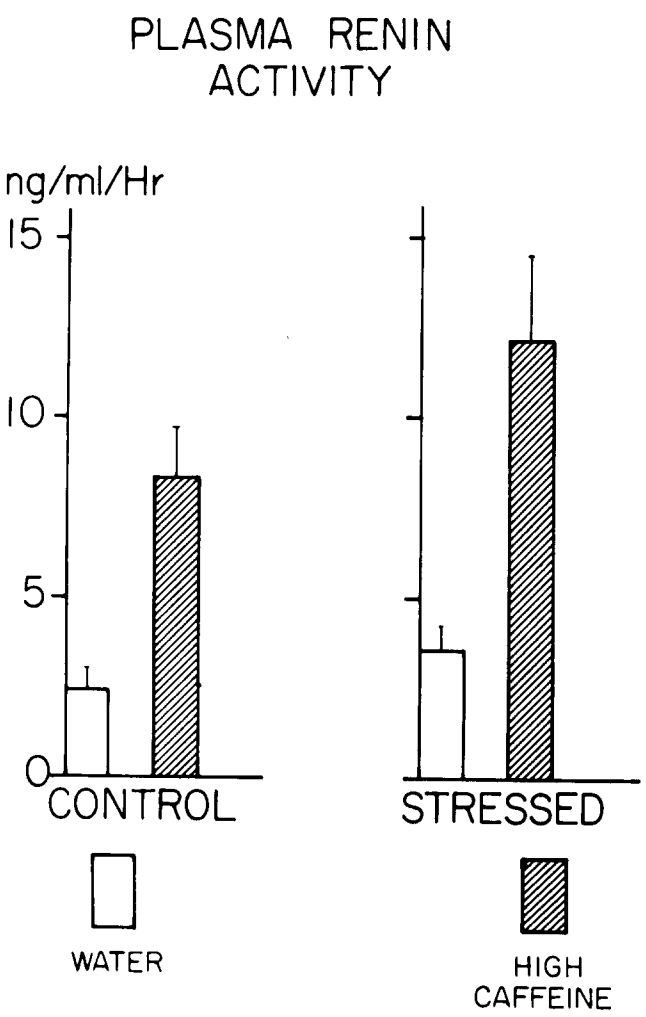

FIG. 1. Right: Mean plasma renin of male mice after five months of high psychosocial stress in two Henry-Stephens complex population cages. The error bars in this and subsequent histograms indicate one Standard Error of the Mean. Highly stressed males drank pure water in one cage; brewed coffee (560 μg caffeine/ml), in the other. Left: Data for minimally stressed males (sibling controls in boxes) drinking the same liquids. Tukey's Honest Significant Difference (HSD) 9.6 ng/ml/hr for $p<0.01$.

The corticosterone results were as follows: Minimally stressed males drinking caffeine in water had higher corticosterone than those drinking pure water, but the higher value did not reach significance. However, highly stressed males drinking pure water and those drinking a low dose of caffeine in water had significantly lower plasma corticosterone ($p<0.000$) than those drinking a high dose of caffeine in water (Fig. 5).

Summary—Acute responses involving both the sympathetic adrenal-medullary and the pituitary-adrenal cortical systems [12] were evaluated during the three- and five-month exposures to caffeinated liquids and stress. Plasma renin, blood pressure, adrenal weight, and plasma corticosterone were measured. The data supported our previous observations of the deleterious effects induced by high stress. In addition, measurements from minimal stress or high stress or both categories picked up a significant interaction ($p<0.05$)

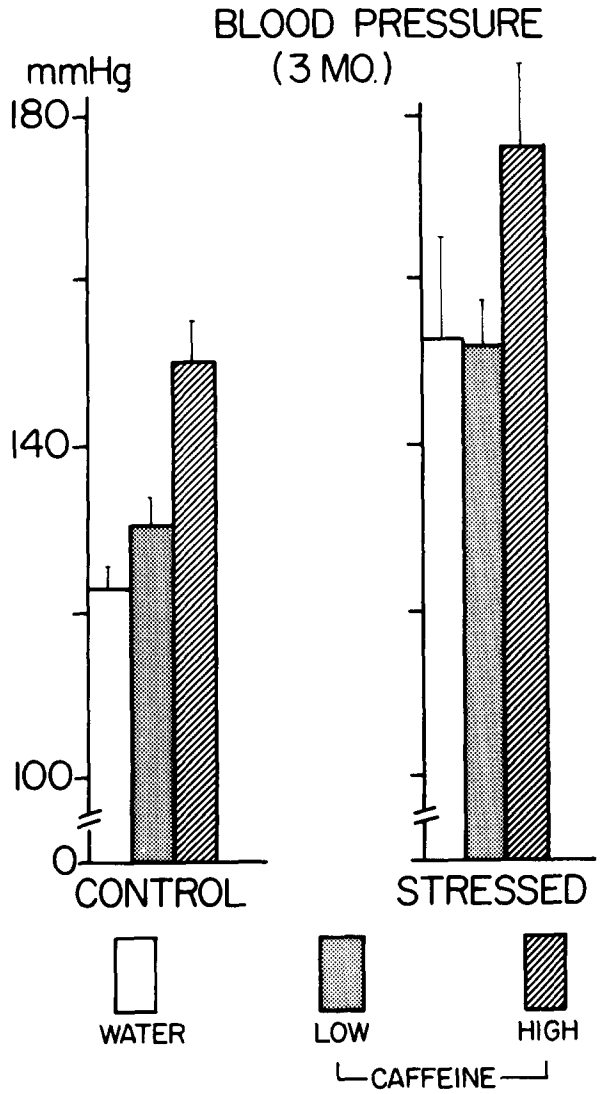

FIG. 2. Right: Mean systolic blood pressure of highly stressed males after three months of psychosocial stress in three population cages. Stressed males drank pure water in one cage; water containing 20 μg/ml caffeine ("decaffeinated coffee") in a second cage; and water containing 560 μg/ml caffeine ("brewed coffee") in a third. Left: Data for minimally stressed males in boxes drinking the same three liquids. The following probability data resulted from a two-way Analysis of Variance with replication: stress level $p<0.000$, caffeine level $p<0.001$, stress×caffeine interaction NS. Tukey's HSD 24 mm Hg for $p<0.05$. See also legend for Fig. 1.

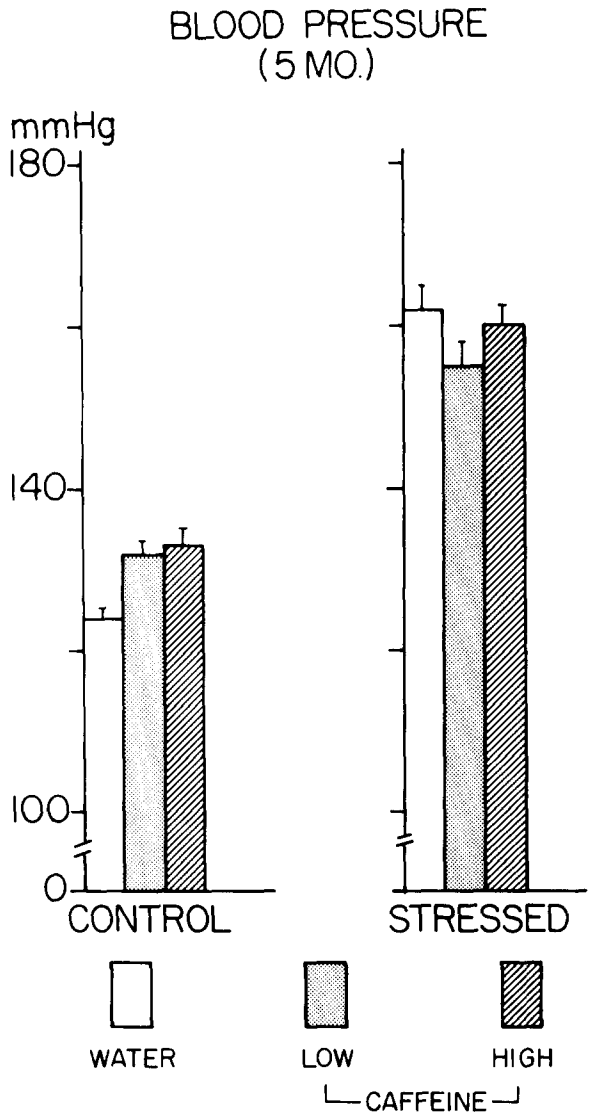

FIG. 3. Right: Mean systolic blood pressure of highly stressed males after five months of psychosocial stress in three population cages. Stressed males drank pure water in one cage; decaffeinated coffee in a second cage; and brewed coffee or tea in a third. Left: Data for minimally stressed males in boxes drinking the same three liquids. Stress level $p<0.000$, caffeine level NS, stress×caffeine interaction $p<0.01$. Tukey's HSD 7.6 mm Hg for $p<0.05$. See also legends for Figs. 1 and 2.

between stress and caffeine which indicated that males at the same level of stress receiving caffeine experience more intense neuroendocrine arousal than those receiving pure water.

Monthly Mortality

Figure 6 shows the sharp contrast in mortality between 96 highly stressed males drinking pure water and 144 drinking caffeinated liquids. The data include some from highly stressed males drinking pure water for nine months; not only are there fewer deaths each month, but they occur later in life. A one-way Analysis of Variance indicates that the difference in mortality between stressed males drinking pure water and those drinking caffeinated liquids is significant ($p<0.015$).

It is of further significance that there were no deaths of young, healthy, minimally-stressed male siblings during their five months stay in boxes while they were drinking pure water or water with a low dose of caffeine. By contrast, there was an unprecedented 11% mortality in those given a high dose of caffeine in water or in brewed tea or coffee. We have never observed such a heavy death rate in healthy, minimally stressed animals in more than 20 years of breeding

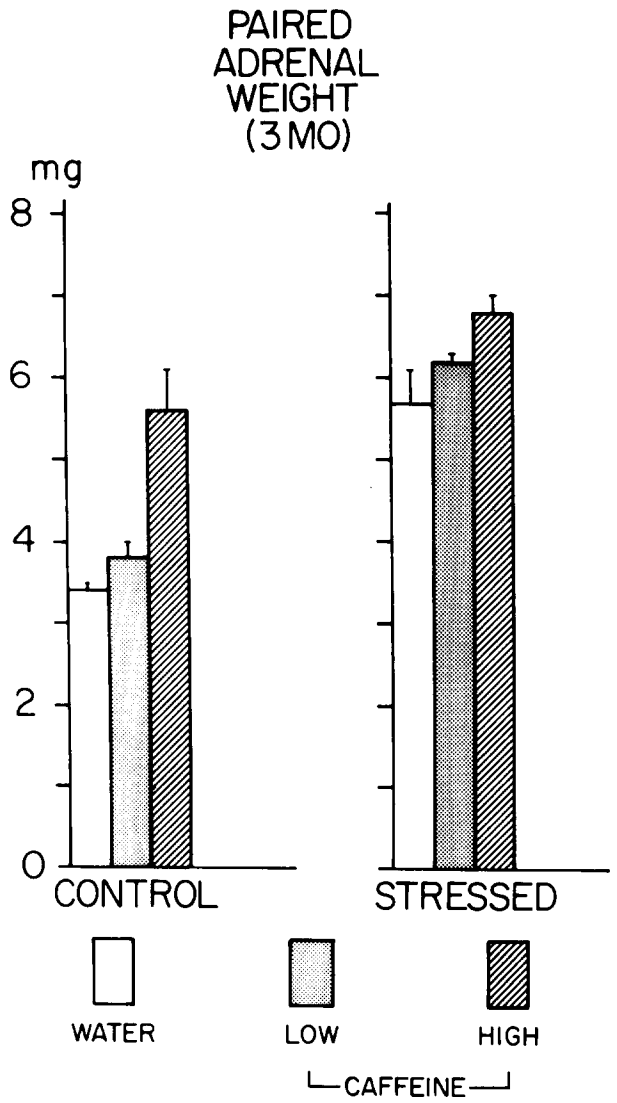

FIG. 4. Right: Mean paired-adrenal weights of highly stressed males exposed to three months of psychosocial stress in three population cages while drinking pure water or water containing a low dose or a high dose of caffeine. Left: Data for minimally stressed males in boxes drinking the same three liquids. Stress level $p<0.000$, caffeine level $p<0.000$, stress×caffeine interaction NS. Tukey's HSD 1.4 mg for $p<0.05$ [8]. See also legends for Figs. 1 and 2.

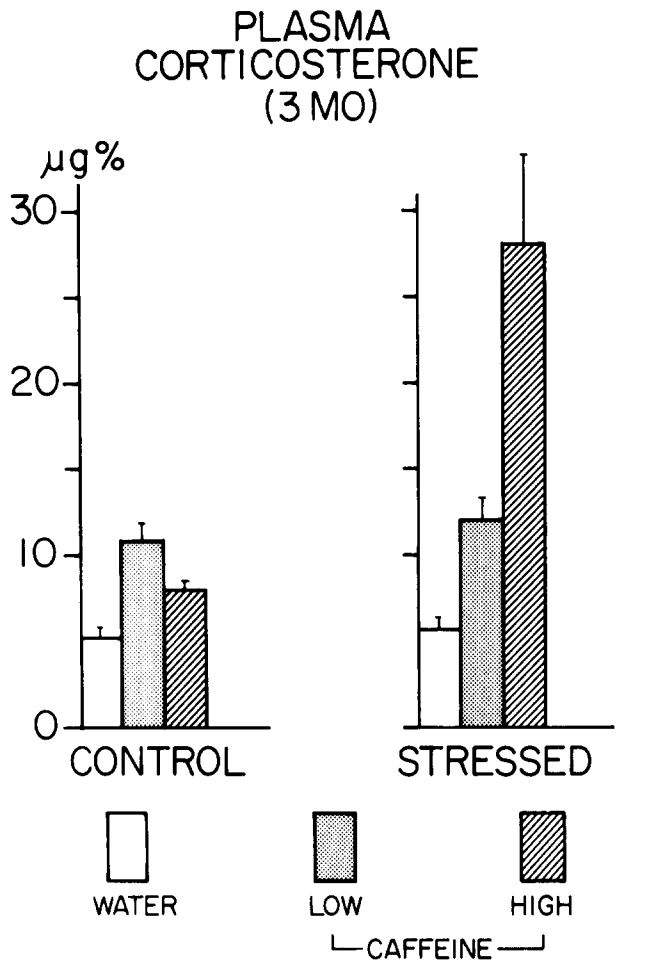

FIG. 5. Right: Mean plasma corticosterone of highly stressed males after three months of psychosocial stress in three population cages while drinking pure water or water containing a low dose or a high dose of caffeine. Left: Data for minimally stressed males in boxes drinking the same three liquids. Stress level $p<0.000$, caffeine level $p<0.000$, stress×caffeine interaction $p<0.000$. Tukey's HSD 9.9 μg% for $p<0.01$. See also legends for Figs. 1 and 2.

mice in our laboratory. The occurrence may have been related to the outbreak of fighting among these high-caffeine users. As we have noted earlier, the resulting social stress led to a rise in plasma renin (Fig. 1), systolic blood pressure (Fig. 2), and corticosterone (Fig. 5).

Summary—A significantly higher mortality for caffeine users was observed not only for highly stressed males in population cages (Fig. 6) but even for minimally stressed males in boxes (see text). Apparently the drug disturbed the behavior of these normally peaceful siblings. The ensuing vigorous fighting was paralleled by the neuroendocrine responses, mentioned earlier, and eventual death.

Pathophysiological Changes in the Heart and Kidneys

Progressive damage to the heart and kidneys develops in

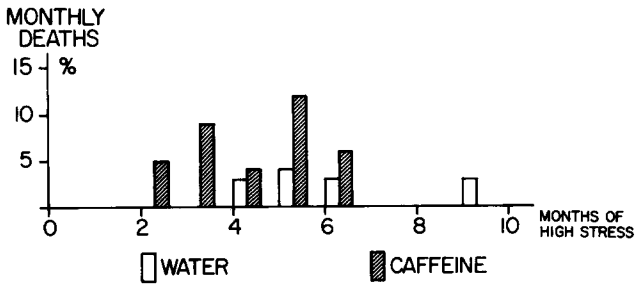

FIG. 6. Monthly deaths of highly stressed males (16/cage) competing for territory in Henry-Stephens complex population cages. Stressed males drinking pure water have fewer deaths occurring later than those drinking the various caffeinated liquids, including decaffeinated coffee ($p<0.015$). See also legends for Figs. 1 and 2.

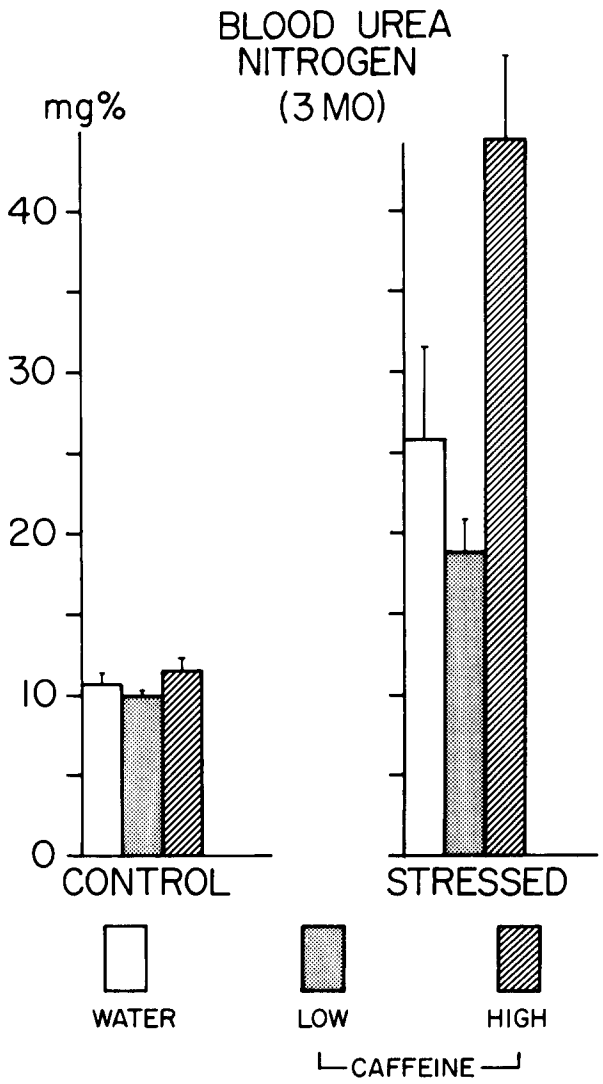

FIG. 7. Right: Mean blood urea nitrogen of highly stressed males after three months of psychosocial stress in three population cages while drinking pure water or water containing a low dose or a high dose of caffeine in water. Left: Data for minimally stressed males in boxes drinking the same three fluids. Stress level $p<0.000$, caffeine level $p<0.001$, stress×caffeine interaction $p<0.003$. Tukey's HSD 16.6 mg% for $p<0.05$. See also legends for Figs. 1 and 2.

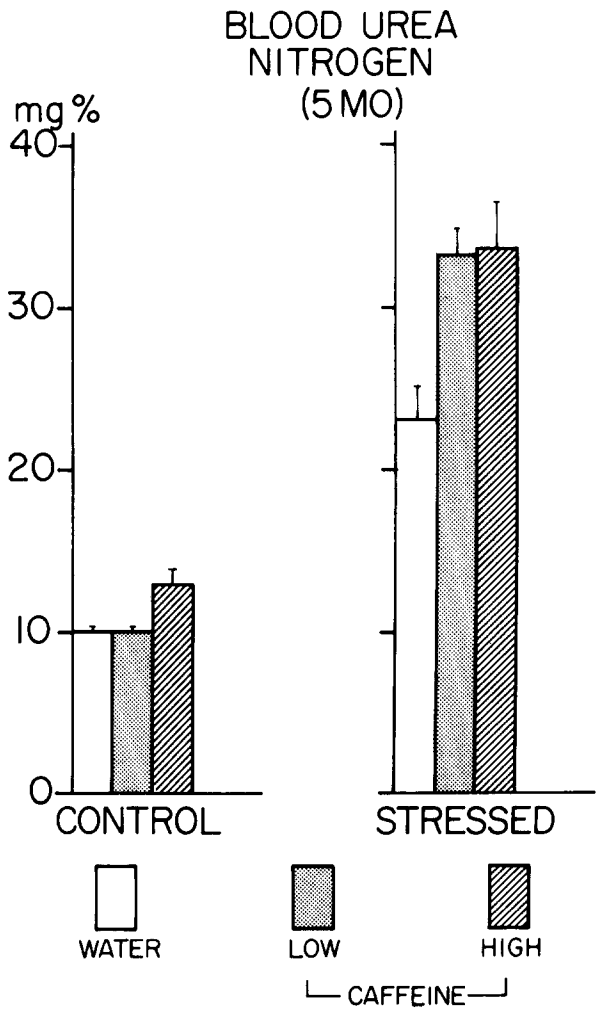

FIG. 8. Right: Mean blood urea nitrogen of highly stressed males after five months of psychosocial stress in three population cages while drinking pure water, decaffeinated coffee, and brewed coffee or tea. Left: Data for minimally stressed males in boxes drinking the same three liquids. Stress level $p<0.000$, caffeine level $p<0.001$, stress×caffeine interaction $p<0.01$. Tukey's HSD 9.2 mg% for $p<0.01$. See also legends for Figs. 1 and 2.

mice exposed to repeated episodes of psychosocial stress. The blood urea nitrogen of minimally stressed males taking caffeine in water for three months and those taking pure water showed the same low normal values (Fig. 7). This was to be expected because there is a minimum of stressful interaction among these socialized siblings in boxes.

In the three-month study, the blood urea nitrogen of highly stressed males attained higher values ($p<0.000$) than those of minimally stressed males while both were drinking pure water, thus indicating that the expected renal damage was in progress. Additionally there is evidence of a relevant interaction between stress and caffeine, for highly stressed males receving a high dose of caffeine in water for three months had significantly higher blood urea nitrogen ($p<0.05$) than those receiving a low dose of caffeine in water or only pure water (Fig. 7).

The blood urea nitrogen of minimally stressed males receiving decaffeinated coffee for five months was a normal 10 mg%, but it rose slightly, though not significantly, in those drinking brewed coffee and tea during this long period (Fig. 8). The rise corresponds to the unexpected 11% mortality of minimally stressed males whose unprecedented fighting and deaths were described in the preceding section.

The blood urea nitrogen of highly stressed males that had survived five months in the population cage showed the expected rise, averaging 23 mg% ($p<0.000$). In addition, the interaction between stress and caffeine is apparent, for the blood urea nitrogen of highly stressed males receiving either high or low doses of caffeine exceeds 30 mg%, a value contrasting sufficiently with that of stressed males receiving pure water to be significant ($p<0.01$).

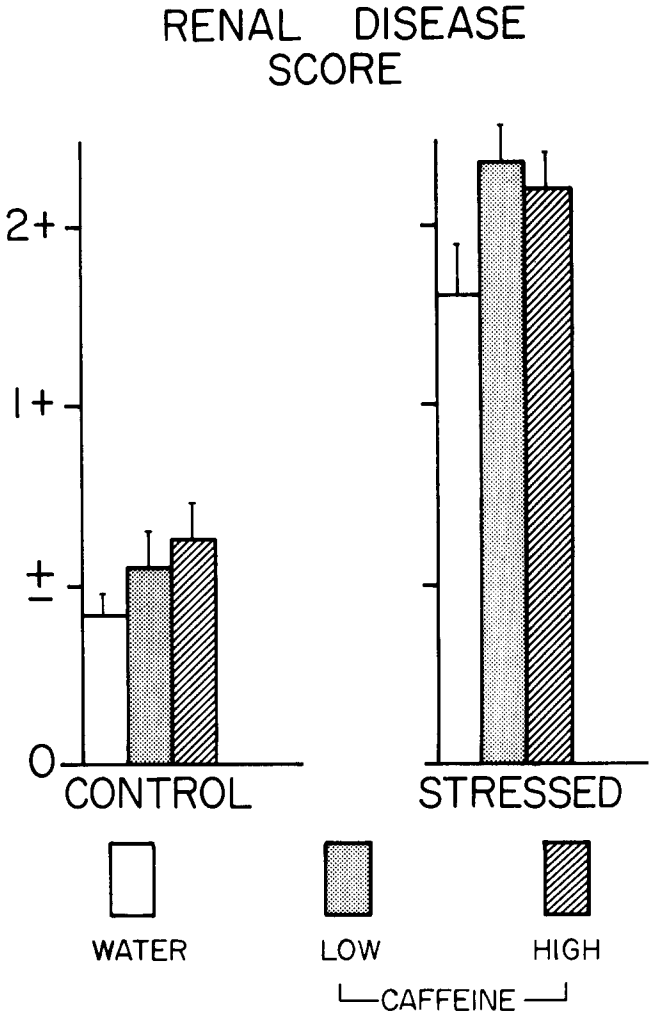

FIG. 9. Mean renal disease score of highly stressed males after five months of psychosocial stress in three population cages while drinking pure water, decaffeinated coffee, and brewed coffee or tea. Left: Data for minimally stressed males in boxes drinking the same three liquids. Stress level $p<0.000$, caffeine level $p<0.025$, stress×caffeine interaction NS. Tukey's HSD 0.8 units for $p<0.05$. See also legends for Figs. 1 and 2.

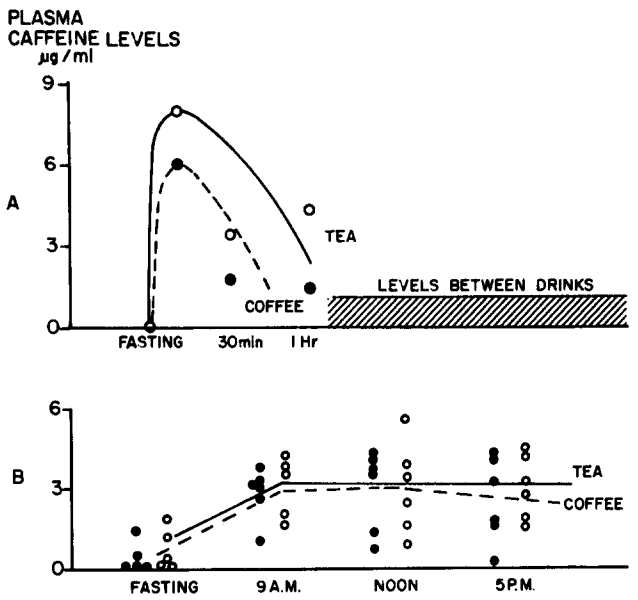

FIG. 10. Plasma caffeine levels for highly stressed male mice exposed to psychosocial stress in a Henry-Stephens complex population cage (A) and for members of a medical clinic (B). In the animal study, abscissa is for time and shows ½-hour and 1-hour plasma caffeine levels after stressed males drank large amounts of brewed coffee (closed circles) and tea (open circles). Initially, the plasma caffeine in mice was below resolution (1.0 μg/ml). In the human study, abscissa represents plasma caffeine of members of the medical clinic when fasting (before having their first cup of coffee or tea of the day) and at 9 a.m., noon, and 5 p.m. The solid line shows the average caffeine level for tea, the broken line, for coffee normally drunk by members. Open and closed circles represent individual values.

The changes in hearts were far less striking. Previously we have found myocardial damage with areas of fibrosis in highly stressed males living in population cages [11]. However, this takes a long time, and by five months, the longest period of observation in these studies, only minimal changes can be expected. We have confirmed there was a small amount of fibrosis which was significantly increased in the highly stressed compared to the minimally stressed males ($p<0.04$).

In contrast to the myocardium at five months, there was considerable histological evidence of renal damage in highly stressed males. The mean score was 2+ out of a possible 4+. This differs sharply from the minimally stressed males whose kidneys scored a near normal ± ($p<0.000$) (Fig. 9). In addition, interaction between stress and caffeine could be demonstrated. When data were combined for minimally stressed and highly stressed males, the difference associated with use of caffeine attains $p<0.025$.

Summary—Caffeine was associated with increased severity of pathophysiological changes in the kidneys of highly stressed males in population cages. Male controls in boxes receiving a high dose of caffeine are often no longer minimally stressed because they have begun to fight, and they develop minor changes. The data are compatible with the observed increase of mortality and neuroendocrine activity of stressed mice receiving caffeine.

Finally, the plasma caffeine of mice receiving brewed coffee and tea was of the same order as that of the staff of a busy medical clinic who drank their normal daily amounts of the same brands of coffee and tea prepared in the same way as for mice (see legend for Fig. 10).

The accelerated metabolic rate of the mouse brings the plasma caffeine level down so rapidly that the half-life is less than 30 min compared with that of humans which ranges up to 4 hours (Fig. 10). This rapid metabolism combined with a normally low consumption of liquid during daylight hours resulted in inadequate plasma caffeine for measurement. Hence, the minimally stressed males in boxes (controls) were primed to drink greater than normal amounts of liquid by having it withheld overnight. Just after slaking their thirst, their plasma caffeine rose briefly to a measurable 6–9 μg/ml, only to return to baseline by the end of an hour. As Fig. 10 shows, humans drinking the same liquids have a plasma caffeine level of the same order of magnitude as these mice which have recently drunk tea or coffee. Therefore, the

doses used were realistic from the viewpoint of applying these results to man.

DISCUSSION

Others have shown that large doses of caffeine are not necessary to produce significant effects. In the operant work of Webb and Levine [19] on differential reinforcement of low rate responding (DRL) in mice, the threshold for an effect in response to an intraperitoneal injection of caffeine was 3 mg/kg, which is similar to that noted in our study.

Bovet-Nitti and Messeri [2] report in their population growth studies of mice receiving caffeine that there were more deaths among the original mice, a delay in births, and a decrease in the total number of animals. These results were first seen after the mice had received an oral dose of caffeine of only 12 mg/kg/day. Seeking an explanation for this great sensitivity to caffeine of their socially ordered colonies, they cited Chance's observation that the toxic effects of central stimulating agents, such as amphetamine and adrenaline, are greater in groups of mice because of the ensuing social interaction [3]. Chance found that the toxicity of adrenaline is doubled in groups of mice, and Robertson *et al.* [16] have shown that caffeine doubles the adrenaline in man.

Minimally stressed mice daily drink liquid equivalent to 12 liters/70 kg man, or 4 dozen 250 cc cups. Their tenfold greater consumption of liquid than that of man partially compensates for the fact that decaffeination of coffee has reduced its caffeine content to 3% of that found in regular brewed coffee. For, at this rate of consumption, a liquid containing the same amount of caffeine as that in brewed decaffeinated coffee (20 μg/ml) will provide a mouse with the daily equivalent of 3 mg caffeine/kg body weight. This represents about two 8 oz cups of brewed coffee for a 70 kg man, which makes it a fair-sized amount of caffeine, considering that Gilbert *et al.* [6] believe physical dependence on caffeine begins in the range of 6–9 mg/kg. The 3 mg/kg dose of caffeine may be approaching a ceiling for exacerbation of the intensity of social interaction among mice in a population cage, and the thirtyfold further increase provided by brewed coffee may have little more behavioral effect.

There is some evidence that decaffeinated coffee contains enough caffeine to affect man despite his smaller intake of liquid per kilogram of body weight. Bellet *et al.* [1] have demonstrated a modest rise of free fatty acids in human plasma in response to decaffeinated coffee. A significant portion of the normal population develops anxiety, emotional lability, speeded thought, and diuresis as the result of using caffeine [5]. About a third of these do so at low levels of the drug. This sensitivity can be so great that occasionally persons will develop symptoms as a result of drinking several cups of decaffeinated coffee, representing a dose of no more than 20 mg caffeine or 0.3 mg/kg (J. F. Greden, personal communication).

At the other end of the scale, we have observed that 1 of 10 siblings in boxes receiving high doses of caffeine die within five months; this may be explained by Chance's discovery of the lethal effects of stimulant drugs in aggregated mice compared to solitary mice. Deaths may be due to cardiac arrhythmias induced during episodes of fighting and violent excitement.

Taken together, the various lines of evidence indicate that caffeine can significantly increase morbidity and mortality in mammals exposed to chronic social stress.

SUMMARY

Psychosocial stress in competing male mice living in Henry-Stephens complex population cages leads to hypertension, cardiovascular damage, and chronic interstitial nephritis. Since the role of caffeine as a risk factor contributing to cardiorenal pathology is open to question, we decided to use males subjected to this type of stress for long term, prospective, group pharmacological studies [2].

We measured plasma renin, blood pressure, adrenal weight, plasma corticosterone, monthly deaths, and blood urea nitrogen. We also assessed histopathological scores for myocardial fibrosis and chronic interstitial nephritis. Males exposed to several months of high social stress in population cages received pure water and water containing high and low doses of caffeine, and the chronic effects of drinking these liquids were contrasted. The high dose was 90 mg caffeine/kg/day as standard brewed coffee and tea; the low dose, 3 mg caffeine/kg/day as decaffeinated coffee. The effects of these caffeinated fluids were also contrasted in siblings living peacefully in standard vivarium boxes in a minimal stress environment. Water containing various strengths of caffeine provided additional tests. Even the minimally stressed males on low doses of caffeine showed some functional changes. Their blood pressure and plasma corticosterone were somewhat higher, suggesting they responded to each other more vigorously. Deaths increased for highly stressed males receiving both low and high doses of caffeine in liquid compared with those receiving pure water.

The highly stressed survivors receiving caffeine had significantly raised blood urea nitrogen levels and higher renal damage scores. All in all, 20 sets of observations based on 8 different measurements, such as deaths, blood pressure, etc. were made—14 of these increased in mice receiving caffeinated liquids.

We conclude that caffeine has a detectable though negligible effect on compatible groups of mice and that its use leads to increased morbidity and mortality of mice living in stressfully competitive environments.

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