Method F.—The coupling reaction was achieved at a constant pH of 9.6 in an ammonium hydroxide–ammonium chloride buffer following the procedure recently described (19).

Method G.—Coupling was accomplished at constant pH of 5.0 in a citric acid–disodium phosphate buffer; this procedure is described in *Reference 21.*

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Effects of Pentobarbital, Acetylsalicylic Acid, and Reserpine on Blood Pressure and Survival of Rats Subjected to Experimental Stress

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Sodium pentobarbital, 20 mg./Kg. *per os* daily, and acetylsalicylic acid (ASA), 100 mg./Kg. *per os* daily, failed to prevent the development of hypertension in rats subjected to experimental stress. ASA enhanced the lethal effects of the stressors and potentiated the effects of the stress conditions on gastric mucosa. Reserpine phosphate, 0.1 mg./Kg. (base) i.p. daily, administered after the animals had been subjected to the stress conditions for 6 weeks, did lower the blood pressure to control levels.

THE DEVELOPMENT of hypertension in animals exposed to experimental stress has been reported by numerous investigators (1–5). Buckley *et al.* (5) found that reserpine phosphate, 0.1 mg./Kg. i.p., and chlorpromazine hydrochloride, 4 mg./Kg. i.p., administered 1 hr. prior to subjecting rats to a 4-hr. variable stress program not only failed to decrease the pressor effects induced by the stressors over a 27-week period but also appeared to potentiate the lethal effects of the stressors. This present study was undertaken to investigate the effects of pentobarbital and acetylsalicylic acid (ASA) on animals subjected to chronic variable stress programs, and the effects of reserpine phosphate[1] administered to the experimental animals after physiological effects of stress exposure were evident.

EXPERIMENTAL

The stress chambers utilized were semisoundproof rooms designed by the Industrial Acoustics Co., New York, N. Y. (5). The stress program consisted of (*a*) flashing 150-w. spotlights (installed in each corner of the cage) which were on for $^1/_4$ sec. and off for $^3/_4$ sec. (in alternate pairs); (*b*) audiogenic stimulation at 5-min. intervals for 0.5-min. periods produced by amplifying a tape recording of noxious sound so that the intensity was approximately 100 decibels at the center of the cage; and (*c*) oscillation at the rate of 140/min. A simple conditioned avoidance response program utilizing automatic pole climbing units (4) was also utilized in the stress program. The cycle was initiated every 2.75 min. and consisted of a low tone for 15 sec., followed by the delivery of an electric shock (3 ma.) to the grid

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[1] Kindly supplied by Dr. William E. Wagner, Ciba Pharmaceutical Co., Summit, N. J.

floor for 30 sec. or until the animal leaped onto the Plexiglas pole. Control blood pressures were obtained twice weekly in both studies, utilizing a photoelectric tensometer (Metro Industries, Long Island, N. Y.) and the animals acclimated to this apparatus over a 4-week pre-experimental training period.

Study 1.—Fifty-nine male Wistar rats, weighing approximately 175 Gm., were randomly divided into 6 groups. The groups to be stressed contained 15 rats each and received the following treatment: group *A*, distilled water, 1 ml./Kg.; group *B*, sodium pentobarbital, 20 mg./Kg.; and group *C*, acetylsalicylic acid (ASA), 100 mg./Kg. Three unstressed control groups were divided as follows: group *D*, 5 animals treated with distilled water, 1 ml./Kg.; group *E*, 5 rats receiving sodium pentobarbital, 20 mg./Kg.; and group *F*, 4 rats receiving ASA, 100 mg./Kg. The animals receiving pentobarbital and distilled water were treated by oral intubation 0.5 hr. prior to subjecting the animals to the 4-hr. stress program. Acetylsalicylic acid was suspended in 1% carboxymethylcellulose and was administered by gastric intubation 1 hr. prior to subjecting the animals to the stress.

The stressors were applied singly or in combination, and the stressor schedule altered daily to prevent acclimation to the stimuli. The animals were stressed 4 hr./day, 6 days/week, from weeks 1–17 and 6 hr./day, 6 days/week, from weeks 18–30. The blood pressure of each animal was obtained once a week prior to administering drugs and subjecting the animals to the stress chamber. The animals in the 3 stressed groups were subjected to ten 2.75-min. cycles in the avoidance response pole climbing apparatus once weekly starting with the twelfth week. These animals were not exposed to the stress chamber on that particular day and received water or drug treatment prior to being subjected to the avoidance-escape situation.

Study 2.—A second study was conducted utilizing 68 male albino Wistar rats, which were divided as follows: group *A*, 24 rats treated with 0.1 ml./Kg. of saline i.p.; group *B*, 25 rats treated with 0.1 ml./Kg. of saline i.p., weeks 1–6; and reserpine phosphate, 0.1 mg./Kg. (base) i.p., 0.5 hr. prior to the experiment, from week 7 on; group *C*, 9 rats treated with saline, 0.1 ml./Kg. i.p.; and group *D*, 10 rats treated with saline, week 1–6 and reserpine phosphate, 0.1 mg./Kg. (base) i.p., from week 7 on. The animals in groups *A* and *B* were subjected to 2 hr. of variable stress daily, 6 days/week; and starting with the tenth week were also subjected to the pole-climbing stress once weekly. Groups *C* and *D* were utilized as nonstressed controls. Blood pressures and body weights were obtained once weekly on all animals prior to treatment and subjection to stress.

RESULTS

Study 1.—The effects of the stressors on blood pressures of the experimental animals are summarized in Fig. 1. The blood pressures of the stressed animals rose steadily reaching maximum values during the sixteenth and seventeenth week (group *A*, 158 mm. Hg ± 3.08; group *B*, 154 mm. Hg ± 2.25; and group *C*, 154 mm. Hg ± 2.0), whereas the mean blood pressures of the nonstressed animals ranged from 118–124 mm. Hg. When these data were subjected to the Student *t* test, the differences between the mean blood pressures of the stressed group and the nonstressed control group were highly significant ($P < 0.01$).

Increasing the duration of stress from 4 to 6 hr. during the eighteenth week did not appear to induce a further increase in blood pressure. At the end of the thirtieth week, none of the stressed rats of group *A* were dead, whereas the mortality rate was 13% for the pentobarbital treated stressed rats and 67%

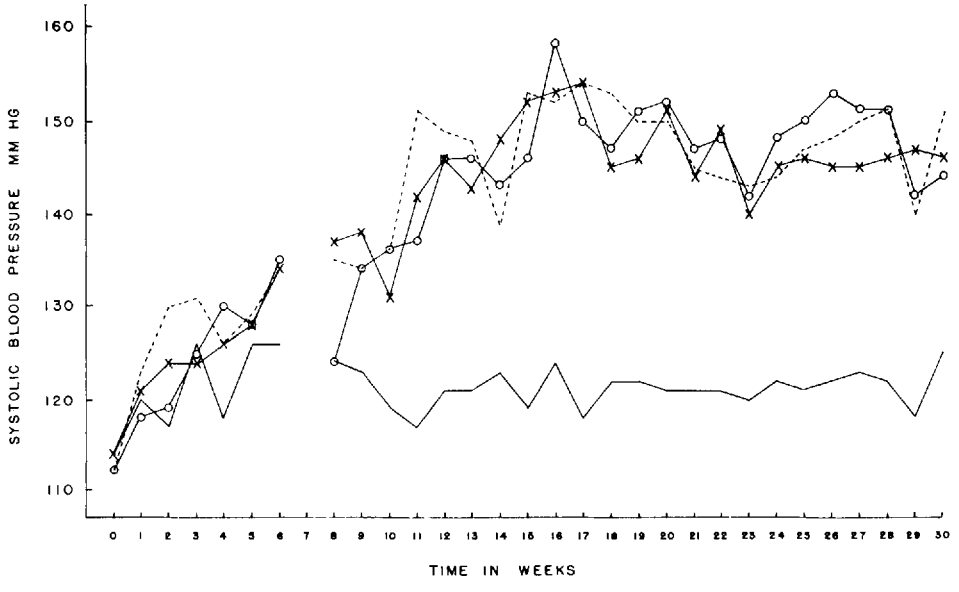

Fig. 1.—Effects of experimental stress on the systolic blood pressure of male Wistar rats in *Study 1*. Group *A*, distilled water, 1 ml./Kg., stressed; group *B*, pentobarbital, 20 mg./Kg., stressed; group *C*, ASA, 100 mg./Kg., stressed; group *D*, distilled water, 1 ml./Kg., nonstressed controls. Key: ○, group *A*; ×, group *B*; — — —, group *C*; ————, group *D*.

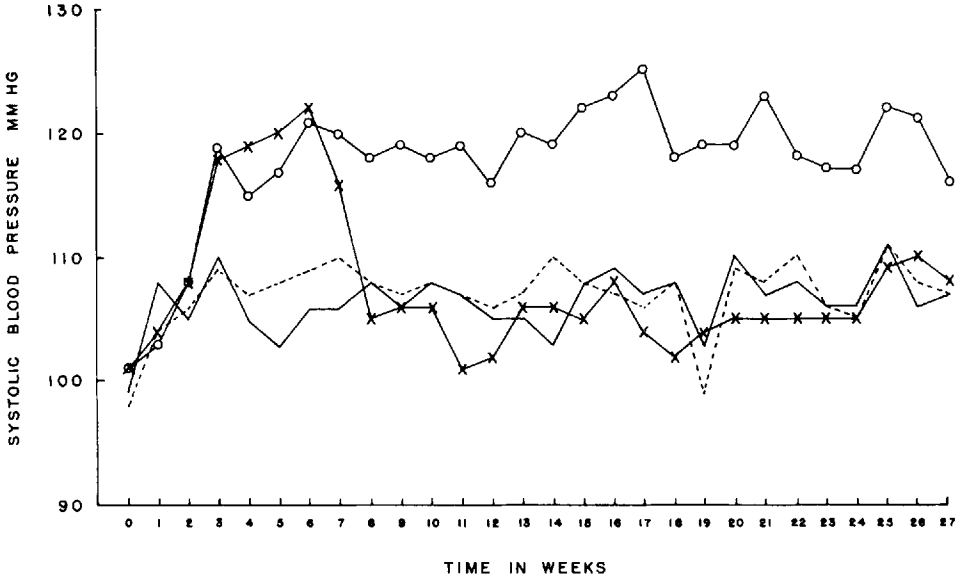

Fig. 2.—Effects of experimental stress on the systolic blood pressure of male Wistar rats in *Study 2*. Group *A*, saline, 0.1 ml./Kg., stressed; group *B*, saline, 0.1 ml./Kg., weeks 1–6, and reserpine PO_4, 0.1 mg./Kg., weeks 7–27, stressed; group *C*, saline, 0.1 ml./Kg., nonstressed controls; group *D*, saline, 0.1 ml./Kg., weeks 1–6, and reserpine PO_4, 0.1 mg./Kg., weeks 7–27, nonstressed controls. Key: ○, group *A*; ×, group *B*; — — —, group *C*; ———, group *D*.

for the ASA-treated stressed rats. The majority of deaths occurred at night between stress sessions. All of the animals in group *C* (ASA treated) which died during the stress session had marked ulceration of the stomach and small intestines with evident bleeding of the gastric mucosa. There was also a marked difference in the behavior of the various groups. The animals in each group were initially docile and very easy to handle; however, by the fifteenth week, the rats in group *A* (treated with distilled water) were extremely difficult to handle and demonstrated a high degree of activity. The animals in group *B* (pentobarbital treated) appeared to be more docile than those in group *A* but did show signs of irritability and became more aggressive during the latter portion of the study. The animals in group *C* (ASA treated) demonstrated the greatest change in that they were extremely difficult to handle and fought the feeding tube continuously. All of the animals in this group had pronounced diarrhea, excessive salivation, and appeared to be extremely sensitive to touch. The nonstressed controls remained fairly docile throughout the experimental period; however, group *F* which received daily administration of ASA appeared to be more excitable than the other two control groups, and most of the animals in this group had periods of diarrhea throughout the study.

Study 2.—The blood pressures of the animals in the two stressed groups gradually increased and by the sixth week of stress averaged 20 mm. Hg higher than initial blood pressures, whereas the blood pressures of the two control groups increased by less than 8 mm. Hg (Fig. 2). Reserpine phosphate, administered once daily starting with the seventh week produced mild diarrhea and ptosis in the animals of groups *B* and *D*. The mean blood pressure of the animals in group *B* dropped from 122 mm. Hg in week 6 to 105 mm. Hg in week 8, and ranged from 101–110 mm. Hg throughout the remainder of the experiment. The blood pressure of the saline-treated stressed group reached a maximum of 125 ± 2.9 mm. Hg during the seventeenth week of the experiment, whereas the reserpine-treated stressed animals in group *B* had a mean blood pressure of 104 ± 1.4 mm. Hg ($P < 0.05$). The number of deaths occurring in this study was relatively low in comparison to previous studies (4% in group *A*, 12% in group *B*, 11% in group *C*, and 20% in group *D*), and the hypertensive effect occurring during the chronic exposure to the stressors was also much less than has

Table I.—Conditioned-Avoidance Response in Male Albino Rats Subjected to Experimental Stress

Wk.	Response Latencies, sec. ± S.E. Group *A* Saline, 0.1 ml./Kg.	Group *B* Reserpine, 0.1 mg./Kg.
10	25.1 ± 1.57	36.0 ± 2.35
11	14.4 ± 1.72	25.9 ± 3.41
12	11.2 ± 1.11	27.8 ± 3.40
13	10.6 ± 1.43	29.1 ± 3.56
14	11.3 ± 1.27	28.4 ± 3.05
15	8.9 ± 1.34	29.1 ± 2.92
16	9.1 ± 0.92	24.9 ± 3.11
17	8.6 ± 1.09	20.9 ± 2.57
18	8.3 ± 1.21	19.6 ± 2.85
19	8.7 ± 0.75	19.7 ± 2.73
20	8.2 ± 0.96	20.4 ± 2.65
21	7.1 ± 0.97	19.7 ± 1.82
22	7.6 ± 1.56	15.6 ± 1.63
23	9.0 ± 1.36	15.6 ± 0.65
24	8.6 ± 1.15	14.4 ± 1.07
25	8.3 ± 1.06	14.7 ± 1.23
26	8.1 ± 0.99	15.1 ± 1.06

previously been obtained. The response latencies of the saline and reserpine-treated stressed animals in the avoidance-escape test are summarized in Table I. The response time of the reserpine-treated animals was always much greater than the saline-treated animals; however, there was a gradual decrease in response time over a 12-week period from 36 sec. to slightly over 15 sec.

DISCUSSION

The hypertensive effects induced by exposure to the stressors in the first study were similar to those previously obtained in this laboratory (3-5). The responses obtained in the second study, however, were relatively mild; and after reaching a peak in the seventeenth week, the animals appeared to be acclimated to the experimental conditions as demonstrated by a plateauing of the blood pressure over the next 10 weeks. Several additional studies have since been conducted, and this variation in the blood pressure response has also occurred. The physiological response of the animal to the experimental stressors appears to vary among samples obtained from the same strain and from an identical source at different times, but there is only a slight variation in the response of animals within a particular sample.

In the first study, pentobarbital and ASA failed to protect the experimental animals from the effects of the stressors. The stressors apparently potentiated the toxicity of ASA, an effect which was most apparent on the gastrointestinal tract. The oral administration of single doses of ASA (100–500 mg./Kg.) has been reported to cause small gastric mucosal erosions to fasted guinea pigs within 30 min. of dosing; however, they appeared to heal within 24 hr. (6). Many investigators have also associated ingestion of ASA with the development of gastric mucosal erosions in mice (7, 8). Selye (9) has reported that exposure of rats to one or more stressors will induce gastric ulceration. The intensity of the stressors utilized in this current study have been controlled so that gastric ulceration would not occur in untreated stressed rats. The data indicate that the effects of ASA on gastric mucosa are greatly enhanced by environmental stress. Selye (10) has suggested that stressors induce the discharge of ACTH which stimulates the release of both mineral corticoids and glucocorticoids from the adrenal cortex, and Timmer (11) has reported a marked increase in free plasma corticosterone of rats subjected to severe acute stress. Salicylates have also been reported to increase the plasma level of 17-hydroxycorticosteroids in rats (12), and it is, therefore, possible that this assumed increase in plasma corticosteroids may be responsible for the gastric ulceration. However, it appears more likely that the observed ulcerations were due to a combination of the local effect of the ASA on the gastric mucosa and an increase in free plasma corticosteroids.

Reserpine phosphate did reverse the effects of the experimental stressors on the blood pressure of rats (*Study 2*). These data are opposite to those obtained in a previous study (5) in which reserpine, 0.1 mg./Kg. i.p., not only failed to protect the rats from the pressor response induced by chronic stress but potentiated the lethal effects of the stressors. Epstein *et al.* (13) investigated the effects of reserpine on the stress of reduced barometric pressure in rats and also found that rather than protect the rats against the stress of altitude reserpine acted as an added stress factor. The experimental design in the previous study was different than the one presently being reported. In the present study, reserpine was not administered until the seventh week of stress to allow for the development of the stress reaction; whereas in the previous study, reserpine treatment was initiated immediately. These data suggest that the effects of reserpine and, for that matter, almost any compound may vary greatly, depending upon the time interval following the initiation of the stress program that treatment is started. However, it is also possible that the relatively mild physiological response to the stressors was responsible for the effect observed with reserpine.

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