

alone affected thermal preference. Slight indications of synergic effects of the 2 drugs were seen but not statistically verified. The persistence of preference for a warmer environment for several days after discontinuing reserpine injections might be due to a persistent drug effect or to a persistence of a behavior learned during the drug phase.

It is of interest that reserpine greatly depressed one adaptive behavioral reaction (nest building) to the hypothermic state presumably resulting from the doses used but promoted another (choice of a warm environment). This difference may be related to the marked differences in the nature of the behavioral responses tested and the methods of testing. The thermal preference test involved arousal of the animal by handling, which could be expected to last throughout the 4-minute test period; nest building required long persistent activity under conditions of minimal disturbance. Reserpine-treated animals were easily aroused and quite active while awake but readily fell into a deep sleep when not disturbed. The experiment does not exclude, however, the possibility of a differential drug effect on mediational mechanisms of the two behaviors. The implications for behavioral research with reserpine in terms of distinguishing between sedative effects of the drug and other more specific actions are apparent.

Summary. Effects of reserpine, chlorpromazine and combined drugs on nest building and thermal preference of rats are described. In the doses used, reserpine alone had a marked and persistent effect on these functions. It depressed nest building while promoting choice of a warm environment. The differential behavioral effect is thought to be related to the great differences in persistence of activity required for the two tests and the ability of reserpine-treated animals to maintain alertness for brief periods after handling.

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Total Plasma Ascorbic Acid as Index of Stress in the Rat. (22008)

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One of the manifestations of physiologic stress is an increase in the production by the adenohipophysis of adrenocorticotrophin (ACTH) (1). A temporary rise in the concentration of total plasma ascorbic acid and plasma ascorbic acid following administration of ACTH in man has been reported (2).

As a preliminary to further experiments, it was deemed desirable to compare the sensitivity of this index of stress and possible ACTH titer in the blood of albino rats with

other such indices.

Methods. Animals in one group were anesthetized with sodium-ethyl-methyl-butyl-barbiturate (Nembutal) for 2 hours. Those in Group 2 were anesthetized with Nembutal for 2 hours and during this time non-activated electrodes were kept in contact with the left cervical sympathetic trunk just posterior to the superior cervical ganglion. The third group was treated like Group 2 except that the electrodes were activated so that the sym-

TABLE I. Average % Increase or Decrease following Treatment Indicated.

Component of blood	Nembutal only	Nembutal with non-activated electrodes	Nembutal with stimulation
Ascorbic acid	+14.15 (13)*	+17.97 (18)	+23.86 (15)
Eosinophils	-10.39 (20)	-25.50 (20)	-42.89 (31)
Lymphocytes	-31.27 (17)	-37.55 (17)	-43.84 (29)
Neutrophils	+55.26 (18)	+66.67 (17)	+56.22 (30)

* Figures in parentheses = No. of animals.

pathetic trunk was receiving .05 to .07 volt impulses of 20 milli-second duration from a square wave stimulator 20 times every second. Prior to attachment of the electrodes, a section of the vagus nerve in the region being stimulated was removed from animals in Group 2 and 3 to avoid death from cardiac or respiratory failure during the 2-hour period of treatment. Stimulated animals that did not exhibit sustained expansion of the pupil on the activated side were discarded. Blood counts and total plasma ascorbic acid determinations were made before and after treatment. Blood was usually taken from the tail. It was taken from the heart in only 4 cases. These 4 were giving excellent results but enough blood could not be obtained from the tail. The leucocyte count of blood taken from the heart differs from that of blood taken from the tail (3). The Roe and Kuether method of determining plasma ascorbic acid was used (4). Diet and temperature were maintained relatively constant. The experiments were started at the same time every day. Rats used were young adult males, all about the same age and weight, from the E. A. Doisy

TABLE II. Percent of Animals Treated Showing an Increase or Decrease in Blood Components.

Component of blood	Nembutal only		Nembutal with non-activated electrodes		Nembutal with stimulation	
	Incr.	Deer.	Incr.	Deer.	Incr.	Deer.
Ascorbic acid	100		100		100	
Eosinophils	40	60	10	90	10	90
Lymphocytes	12	88	12	88	7	93
Neutrophils	100		100		93	7

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Results. The 3 types of treatment outlined above produce temporary manifestations of stress, and thereby possibly of ACTH titer in the blood, demonstrable by each of the criteria used. Such manifestations are eosinopenia, lymphopenia, neutrocytosis and an increase in the total plasma ascorbic acid. The average percent changes in the eosinophil and lymphocyte counts and in the plasma ascorbic acid determinations, indicate that animals with non-activated electrodes attached were subjected to greater stress than were those receiving Nembutal only, and that activation of the electrodes caused the most severe stress (Table I). The average percent increase in neutrophils was not as great in the stimulated animals as it was in animals having non-activated electrodes attached (Table I). There-

TABLE III. Levels of Significance (P) of Differences between Methods of Treatment Indicated.

Component of blood	Nembutal only and Nembutal with non-activated electrodes	Nembutal with non-activated electrodes and Nembutal with stimulation	Nembutal only and Nembutal with stimulation
Ascorbic acid	.343	.105	.046
Eosinophils	.018	.015	<.001
Lymphocytes	.370	.393	.083
Neutrophils	.087	—	.809

fore, the neutrophil count is probably not a reliable index of ACTH titer in the blood. This conclusion is upheld by other workers (5).

From Table II it is seen that during 2 hours of treatment, all the animals in each of the 3 groups show an increase in plasma ascorbic acid, whereas a few from each group paradoxically show eosinophilia and lymphocytosis.

Thus it would appear that plasma ascorbic acid is the most reliable index of stress even though statistical analysis of the overall results (Table III) points to eosinopenia as the most sensitive indicator.

Summary. The effect of electrical stimulation of the cervical sympathetic trunk on the number of eosinophils, lymphocytes and neu-

trophils and on the total plasma ascorbic acid in the blood of the rat, indicates that the amount of total plasma ascorbic acid rises temporarily in these animals following this type of stress and that it is a reliable index of the severity of such stress and possibly of ACTH production following such stimulation.

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Effect of Intrinsic Factor Concentrate on Vit. B₁₂ Absorption by Gastrectomized Rats. (22009)

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A recent attempt(1) to use normal rats to test the potency of an intrinsic factor preparation revealed only an inhibitory effect on the absorption of oral vit. B₁₂ by this test animal. Normal rats had been employed for this study which involved doses of 4.2 and 0.42 μ g of radioactive vitamin labeled with cobalt 60.

Normal rats do not develop a macrocytic anemia comparable to pernicious anemia, and may thus be considered *a priori* unsuitable for the evaluation of intrinsic factor potency. In humans, however, gastrectomized individuals (2,3) have been employed successfully to demonstrate intrinsic factor despite the absence of anemia or megaloblastic marrow. This suggested the possibility that gastrectomized rats would serve as appropriate test animals. Accordingly studies were undertaken to examine the effect of potent intrinsic factor concentrates upon the extent of absorption of radioactive vit. B₁₂ by gastrectomized rats. Furthermore, physiological doses of 200 m μ g and 50 m μ g of vitamin, and 2 different intrinsic factor concentrates, were investigated.

Methods. Adult rats of different strains weighing 200-300 g were used. Upon open ether anesthesia total gastrectomy was performed utilizing an end to end esophagoduodenostomy to restore intestinal continuity. Fine silk was used for sutures and ligatures

throughout. Total removal of the stomach was verified in each instance by histological preparation of a sagittal section through the removed organ. In general at least 10 days were allowed for recovery from the operation before transfer of the animals to the nutrition laboratory. During the first 10 days after operation, the animals were fed on a milk and bread diet and lost about 10% of pre-operative body weight. Subsequently, they were offered a basal soybean diet, and during test periods were given 10 g of this diet daily. **Materials.** Two commercially available intrinsic factor concentrates were employed. Preparation I was used only in preliminary study (Series A) with low specific activity vit. B₁₂-Co⁶⁰. Its clinical potency was not retested. Preparation II was of proven potency at 40 mg, as tested hematologically and by the Schilling method(4), and at 20 mg by the latter procedure. Its binding power was determined by the tracer-dialysis method(5) to be 250 m μ g/mg. The vit. B₁₂ labeled with Co⁶⁰ employed in the preliminary study had a specific activity of $\approx$ 55 μ c/mg; and the activity of the second preparation, used in 3 additional definitive studies, was $\approx$ 200 μ c/mg.

Method. Four series of experiments (A, B, C and D) were performed in such manner