the question, however, whether or not a substance present in tumor, such as the presently reported active material in mitochondria, can enter the general circulation of host(1). Nakahara and Fukuoka found that freshly prepared extracts of tumor tissue contained a small amount of dialyzable substance which depresses liver catalase of normal mice(6). In our experiments permitting mitochondria to stand several hours in 0.25 M sucrose at 0°C and then centrifuging, resulted in high proportion of activity in the supernatant.

The discrepancy between present results and those previously reported by Nakagawa and Nakagawa(2) may be due to different tumors employed or possibly to leakage of active substance(s) from mitochondria. Nakagawa, *et al.*(7) to purify toxohormone, found a highly active nucleic acid fraction. Investigations by other groups indicated that the active material may be a polypeptide(8). It is very unlikely that the active material in present studies was in the nucleic acid fraction, since it was concentrated in mitochondria.

Summary. The factor(s) in Walker carcinosarcoma 256 tissue which caused a depression in plasma iron, liver catalase activity and alterations in organ weights when injected into normal rats was localized primarily in the mitochondrial fraction.

The authors express their appreciation to West Clabaugh and Faye Jones for technical assistance.

1. Nakahara, W., Fukuoka, F., *Advances in Cancer Research,* 1958, v5, 157.

2. Nakagawa, S., Nakagawa, S., *Proc. Japan Acad.,* 1956, v32, 298.

3. Kampschmidt, R. F., Adams, M. E., McCoy, T. A., *Cancer Research,* 1959, v19, 236.

4. Kampschmidt, R. F., Mayne, M. A., Goodwin, W. L., Clabaugh, W. A., *ibid.,* in press.

5. Schneider, W. C., Hogeboom, G. H., *J. Biol. Chem.,* 1950, v183, 123.

6. Nakahara, W., Fukuoka, F., *Gann,* 1954, v45, 67.

7. Nakagawa, S., Kosuge, T., Tokunaka, H., *ibid.,* 1955, v46, 585.

8. (a) Ono, T., Sugimura, T., Umeda, M., *ibid.,* 1955, v46, 617. (b) Fukucka, F., Nakahara, W., *ibid.,* 1953, v44, 1. (c) Greenfield, R. E., Meister, A., *J. Nat. Cancer Inst.,* 1951, v11, 997. (d) Sato, H., Yunoki, K., Kamimura, M., *Acta Med. Univ. Kagoshima,* 1958, v1, 60.

Received February 23, 1960. P.S.E.B.M., 1960, v103.

Norepinephrine Depletion as a Possible Mechanism of Action of Guanethidine (SU 5864), a New Hypotensive Agent. (25702)

ROSEMARY CASS,* RONALD KUNTZMAN AND BERNARD B. BRODIE
Lab. of Chemical Pharmacology, Nat. Heart Inst., U. S. Dept. of H. E. W., Bethesda, Md.

A recent report(1) described pharmacological properties of 2-(octahydro-1-azocinyl)-ethyl-guanidine sulfate (Su-5864, guanethidine). This compound produced a variety of sympatholytic effects of prolonged duration, including lowering of arterial pressure. The drug acted by making the peripheral sympathetic system unresponsive to stimuli. The unresponsiveness could not be attributed to interference wtih conduction of nerve impulses or transmission across ganglia, or to blocking the action of norepinephrine. It was suggested that guanethidine might interfere with release and/or normal distribution subsequent to release of the neurohumoral transmitter at the sympathetic neuromuscular junction. The present paper shows that guanethidine decreases level of norepinephrine in heart and spleen without lowering norepinephrine level in the brain.

Methods and Materials. Rabbits, New Zealand white, weighing about 2 kg, and mongrel cats, weighing 3 to 4 kg, were used in these studies. Rabbits were given guanethidine (Su-5864)† in a single dose of 12.5 mg/kg intravenously; cats were given a single dose of 15 mg/kg subcutaneously. Immediately after

* Present address: Dept. of Pharmacology, School of Pharmacy, University of London, London, England.

† We wish to thank Ciba Pharmaceutical Products for generous supply of Su-5864.

TABLE I. Norepinephrine (NE) Concentration in Rabbit Heart Various Times after Administration of Su-5864 (12.5 mg/kg I.V.).

Time, hr	No. of animals	Conc. of norepinephrine, μg/g
0	11	1.78 (1.31-2.31)
1	4	1.17 (.91-1.34)
2	2	.69 (.59- .78)
4	6	.39 (.35- .42)
18	3	.27 (.13- .48)
48	2	.85 (.80- .90)
72	2	1.13 (1.11-1.15)
120	2	1.35 (1.29-1.42)
168	2	1.98 (1.82-2.14)

Figures in parentheses represent range of values.

injection some rabbits showed signs of transient apnea and prostration. At various times after drug administration the animals were killed by intravenous injection of air or by chloroform. The tissues were analyzed for serotonin and norepinephrine by methods previously described(2,3).

Results. Norepinephrine content of rabbit heart declined progressively for about 4 hours after guanethidine administration, reaching a level 15% that of normal (Table I). The norepinephrine level remained at this low value for at least 18 hours, had partially recovered in 48 hours, and reached normal value in about 7 days. The norepinephrine level in spleen also declined about 60% in 18 hours. However, norepinephrine levels in brain or adrenal medulla were not affected. In addition, there was no decrease in level of serotonin in brain over a period of 18 hours.

Guanethidine in the cat caused a decrease in heart norepinephrine level by about 75% in 24 hours but did not lower norepinephrine level in brain or adrenal medulla.

Discussion. Guanethidine, like reserpine, produces a decline in content of tissue norepinephrine in the rabbit and cat. The catecholamine depletion observed after guanethidine (12.5 mg/kg) is slower however than after reserpine(4). The effect on norepinephrine could be due either to release of the amine or to blocking of its synthesis. Both of these possibilities are now being investigated.

The failure of guanethidine to lower content of brain norepinephrine is not surprising as the drug is unlikely to cross the blood-brain barrier readily, due to its extremely low lipid solubility (compound not extracted from buffer pH 7.4 into chloroform).

The data presented here suggest that guanethidine lowers blood pressure by producing chemical sympathectomy through depleting norepinephrine from peripheral nerve endings.

Summary. Guanethidine (Su-5864), a new hypotensive agent, depletes the norepinephrine level in the heart of rabbits and cats. It is suggested that guanethidine lowers blood pressure by producing chemical sympathectomy through depletion of norepinephrine from peripheral nerve endings.

1. Maxwell, R. A., Plummer, A. J., Schneider, F., Povalski, H., Daniel, A. I., *J. Pharm. Exp. Therap.*, 1960, v128, 22.

2. Shore, P. A., Olin, J. S., *ibid.*, 1958, v122, 295.

3. Bogdanski, D. F., Pletscher, A., Brodie, B. B., Udenfriend, S., *ibid.*, 1956, v117, 82.

4. Carlsson, A., Rosengren, E., Bertler, A., Nilson, J., in *Psychotropic Drugs*, 1957, Elsevier Publishing Co., Amsterdam, 363.

Received February 23, 1960. P.S.E.B.M., 1960, v103.

A Simple Method for Concentration of Live and Formaldehyde-Inactivated Poliovirus. (25703)

NATHAN GROSSOWICZ, ALBERT MERCADO AND NATAN GOLDBLUM
Dept. of Bacteriology, Hebrew University-Hadassah Medical School, Jerusalem, and Virus Laboratory, Ministry of Health, Tel Aviv-Yaffo, Israel

Salk type vaccine has been widely used for immunization against poliomyelitis. Since antigenicity of currently used vaccine preparations is not uniformly satisfactory(1), concentration of poliovirus suspensions to be transformed into vaccine or of formalinized vaccines may provide considerably more potent immunizing agents. Several methods for