normal serum seems most unlikely. The "endotoxin-detoxifying component" of normal blood found by Landy and associates(15) and active *in vitro,* can hardly explain our *in vivo* findings in view of the full lethality seen after simultaneous administration of serum and endotoxin extemporaneously mixed. The results suggest need for time for a recipient animal response, and failure with the *i.v.* route with the same 30 minute interval points to an intraperitoneal mechanism. Results with heat-treated serum, to yield a relatively protein-free filtrate, must be interpreted with caution. The protein coagulum formed may, despite washing, trap much non-protein. The results with tolerant donor serum suggest a non-protein moiety. Processing of normal and endotoxin-tolerant donor serum and plasma by the phenol-water partition method for lipopolysaccharide has not yielded endotoxin activity (unpublished experiments).

Summary. The broad protection against lethality of endotoxin obtained by passive transfer from endotoxin-tolerant donors is not mediated by adrenal cortical hormone, properdin, or phagocytosis-promoting factor. Limited protection by normal donor serum given *i.p.* shortly before, but not simultaneously with, inoculation of endotoxin has been demonstrated. The results are not explained by direct inactivation of endotoxin by serum. Heating removes this activity from normal, but not completely from tolerant, donor serum.

The skilled technical assistance of Miriam Graff is gratefully acknowledged.

1. Freedman, H. H., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 504.
2. ———, *J. Exp. Med.,* 1960, v111, 453.
3. ———, *Ann. N. Y. Acad. Sci.,* in press.
4. Wardlaw, A. C., Pillemer, L., *J. Exp. Med.,* 1956, v103, 553.
5. ———, *J. Immunol.,* 1959, v82, 313.
6. Silber, R. H., Porter, C. C., *J. Biol. Chem.,* 1954, v210, 923.
7. Tullis, J. L., In: *Blood Cells and Plasma Proteins,* Ed. J. L. Tullis, Acad. Press, New York, 1953.
8. Levitin, H., Kendrick, M. I., Kass, E. H., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 306.
9. Berry, L. J. Smythe, D. S., Young, L. G., *J. Exp. Med.,* 1959, v110, 389.
10. Tauber, H., Garson, W., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 886.
11. Pillemer, L., Schoenberg, M. D., Blum, L., Wurz, L., *Science,* 1955, v122, 545.
12. Landy, M., Pillemer, L., *J. Exp. Med.,* 1956, v103, 823.
13. Cluff, L. E., Bennett, I. L., Jr., *Johns Hopkins Hosp. Bull.,* 1957, v101, 281.
14. Zweifach, B. W., Benacerraf, B., Thomas, L., *J. Exp. Med.,* 1957, v106, 403.
15. Rosen, F. S., Skarnes, R. C., Landy, M., Shear, M. J., *ibid.,* 1958, v108, 701.

Received February 18, 1960. P.S.E.B.M., 1960, v103.

Effect of Tumor Cell Fractions upon Plasma Iron, Liver Catalase and Organ Weights of Rats. (25701)

RALPH F. KAMPSCHMIDT AND THOMAS A. MCCOY
Biomedical Division, Samuel Roberts Noble Fn., Ardmore, Okla.

A heat stable, water soluble, and alcohol precipitable extract from many different types of tumors contained a factor(s) which depresses liver catalase activity when injected into normal mice(1). Nakagawa and Nakagawa(2) reported that centrifugal fractionation of homogenates of Yoshida sarcoma and ascites hepatoma, yields an active supernatant fraction, which contained soluble protein and microsomes. The nuclear or mitochondrial fraction failed to show clear evidence of liver catalase depressing action. Our previous studies(3,4) indicated that an extract from Walker carcinosarcoma 256 or Flexner-Jobling carcinoma depresses hepatic catalase activity, lowered plasma iron and affected the weights of liver, spleen and thymus. Homogenates of the Walker tumor were, therefore, fractionated by centrifugation to separate the

TABLE I. Distribution of Factor which Depresses Plasma Iron of Normal Rats in Fractions Isolated from Homogenates of Walker Carcinosarcoma 256

Tumor fraction	Dry wt of injected material (mg) .5	1.	2.5	5.	10.
			Plasma iron μg %		
Sham*	268 ± 13†	273 ± 7	263 ± 27	270 ± 11	259 ± 17
Tumor homogenate			175 ± 14	131 ± 17	120 ± 14
N_W‡			203 ± 27	222 ± 24	179 ± 16
M_{W2}	152 ± 24	107 ± 21	77 ± 14	58 ± 5	66 ± 8
P_W			195 ± 30	216 ± 41	170 ± 9
S_2			251 ± 18	248 ± 31	204 ± 6

* Vehicle only.

† Each figure represents mean value for 4 rats ± stand. error (with duplicate determinations on each rat).

‡ For explanation of fraction designations, see text.

active substance(s) that produced these many diverse effects.

Methods. Homogenates of Walker carcinosarcoma 256 were fractionated in 0.25 M sucrose according to method outlined for liver by Schneider and Hogeboom(5). Fraction designations and centrifugation speeds used were as follows: NW-nuclear fraction (10 min at 700 X G), MW_2 – mitochondria fraction (10 min at 5000 X G), S_1 – supernatant and washings from mitochondrial fraction, P_W – submicroscopic particles (35 min at 100,000 X G), S_2 – supernatant and washing from submicroscopic particles. Particulates were suspended in 0.25 M sucrose and injected intraperitoneally into Holtzman rats weighing 160-180 g. Methods to determine plasma iron concentrations and liver catalase activity have been described(3,4). Determinations of plasma iron concentration were made 16 hours after administration of various fractions, whereas all other measurements were conducted 48 hours later.

Results. The effect of various fractions of the Walker tumor upon plasma iron of normal rats is shown in Table I. Mitochondria consistently produced the greatest depression in plasma iron. This fraction contained 8.7 ± 0.4% of total dry weight of the tumor. The mitochondrial fraction, even at 10-fold dilution, depressed plasma iron to essentially the same level as homogenate. It was therefore concluded that most of the activity was present in this fraction.

Since it was possible that only plasma iron was affected by injections of mitochondrial fractions, effects of tumor cell fractions upon liver, spleen, and thymus weights and liver catalase activity were determined (Table II). Only 25 mg of the various fractions was injected. It can be seen that the mitochondrial fraction significantly increased weights of liver and spleen and depressed thymus weight and catalase activity. While some of these effects were produced by other tumor fractions, mitochondria injections consistently produced the largest changes.

Discussion. It was shown that homogenates of tumor produce changes in organ weights, plasma iron and liver catalase at much lower concentrations than normal tissue homogenates(4). These changes, moreover, resembled those which occurred during early growth of the tumor. The present results again raise

TABLE II. Effects of Various Tumor Fractions upon Organ Weights and Liver Catalase Activity in Normal Rats.*

	Tumor fraction Sham	N_W	M_{W2}	S_1
No. rats	8	8	8	8
Liver†	1180 ± 30‡	1240 ± 30	1320 ± 20§	1200 ± 30
Spleen†	75 ± 2	134 ± 10§	144 ± 6§	114 ± 8§
Thymus†	64 ± 3	55 ± 4	42 ± 3§	60 ± 5
Liver catalase‖	5470 ±230	3730 ±240§	2700 ±180§	4250 ±240§

* Intraper. injections of 25 mg dry wt/rat. † mg dry wt/100 g body wt. ‡ Mean ± stand. error. § Significantly different from sham ($P < .01$). ‖ Catalase units(4).

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.