F., *Am. J. Physiol.,* 1962, v202, 795.

3. Collins, D. A., Hamilton, A. S., *ibid.,* 1944, v140, 499.

4. Dexter, L., Frank, H. A., Haynes, F. W., Altschule, M. D., *J. Clin. Invest.,* 1943, v22, 847.

5. Ginsburg, M., Brown, L. M., in *The Neurohypophysis,* Ed. H. Heller, Butterworth Scientific Publications, London, 1957, p109.

6. Gross, F., Regoli, D., Schaechtelin, G., in *Hormones and the kidney,* Ed. P. C. Williams, Academic Press, London and New York, 1963, p293.

7. Huidobro, F., Braun-Menéndez, E., *Am. J. Physiol.,* 1942, v137, 47.

8. Paladini, A. C., Scornik, O. A., in *Hormones and the kidney,* Ed. P. C. Williams, Academic Press, London and New York, 1963, p265.

9. Regoli, D., Hess, R., Brunner, H., Peters, G., Gross, F., *Arch. int. Pharmacodyn.,* 1962, v140, 416.

10. Sapirstein, L. A., Ogden, E., Southard, F. D., Jr., PROC. SOC. EXP. BIOL. AND MED., 1941, v48, 505.

11. Schaechtelin, G., Regoli, D., Gross, F., *Am. J. Physiol.,* 1963, v205, 303.

12. ———, *ibid.,* in press.

13. Stamey, T. A., *Renovascular Hypertension,* Williams & Wilkins Co., Baltimore, 1963.

14. Tobian, L., *Circulation,* 1962, v25, 189.

Received March 4, 1964. P.S.E.B.M., 1964, v116.

Serum Manganese in Myocardial Infarction.* (29371)

THOMAS C. SPARKMAN† AND FERRIN B. MORELAND

Department of Biochemistry, Baylor University College of Medicine and Veterans Administration Hospital, Houston, Texas

That the serum manganese concentration rises following myocardial infarction was reported by Hedge, Griffith, and Butt(1), who concluded that this increase is more useful diagnostically than the elevation in serum glutamic-oxalacetic transaminase activity (SGOT). The present study was undertaken to determine the specificity of increased serum Mn for myocardial damage and to determine whether degree of serum Mn elevation is proportional to extent of damage.

Materials and methods. Serum was obtained from normal persons and from hospital patients with myocardial infarction or other disease.

Samples were also taken from dogs before and after the production of acute coronary occlusion. Adult mongrel dogs were anesthetized with morphine and nembutal and were given artificial respiration by intermittent positive pressure. An incision was made through the fourth intercostal space, the pericardium entered, and silk nooses placed loosely around the anterior descending branch of the left coronary artery 5-10 mm distal to the origin of the circumflex artery. The lumen of the artery was progressively narrowed until complete occlusion was effected after 20 minutes; the obstructing ligature was left intact. The pneumothorax was reduced by over-inflation of the lungs as the chest was closed. Electrocardiographic tracings and venous blood samples were taken before operation and at intervals following the occlusion. Samples of normal and infarcted cardiac tissue were removed at necropsy for manganese determinations. All infarctions were confirmed by sectioning and microscopic study.

Sera were dry ashed, with the aid of a little nitric acid, as suggested by Thiers(2) and analyzed by the method of Gates and Ellis(3). Attempts to simplify the method by using trichloroacetic acid precipitation instead of ashing were unsuccessful, whether or not (ethylenedinitrilo) tetraacetic acid was added. The use of ion exchange resin to remove Mn from serum and recover it by elution was also disappointing.

SGOT determinations were made by the method of Cabaud *et al*(4) (normal is below 40 units).

Results. The data from the dogs are recorded in Table I. Before operation the serum Mn averaged 3.7 μg% with a range of

* This work was supported in part by National Tobacco Industry Research Commission.

† Present address: Cape Girardeau, Mo.

TABLE I. Serum Manganese Concentration Following Ligation of Anterior Descending Coronary Artery in Dogs.

Dog	Time after ligation, hr	Serum Mn, μg %	SGOT, units
1	Pre-op control	5.1	19
	Post-op control	5.2	18
	6	10.8	21
	9	9.2	30
	12	5.9	67
2	Control	2.7	21
	5	2.9	19
	7	3.2	43
	9	3.1	36
	12	5.4	46
	16	5.7	61
3	Control	5.0	41
	7	7.4	250
4	Control	2.5	23
	7	2.7	51
	14	5.0	64
	19	7.6	85
	27	5.9	110
	40	7.6	135
	48	7.9	181
	72	8.0	145
5 (operative control, thoracotomy only)			
	Pre-op control	3.2	
	Post-op control	4.1	
	8	3.6	
	16	3.2	
	18	3.3	
	24	4.0	

2.5-5.1. After the ligation, the Mn rose to twice the control concentration in 6 to 14 hours in 3 of the dogs (Nos. 1, 2, and 4); the fourth dog showed a rise to twice the mean control value but had started from a high normal concentration. In the only dog carried for 72 hours, the Mn and SGOT were both still elevated at that time. The control dog (operated but not ligated) showed no elevation in serum Mn. The Mn rose before the SGOT in 1 dog, concurrently in 2, and later in 1. In all animals, electrocardiographic changes (ST segment elevation with reciprocal ST depression) were observed earlier than serum Mn increase. Examination revealed no correlation between the size of the infarction and the degree of serum Mn rise. The animals with the greatest serum manganese elevation (dogs 1 and 4) were observed at autopsy to have areas of infarcted tissue no larger than the other dogs. There was also no difference in the Mn content of infarcted and normal areas of heart muscle (Table II).

Eight replicate determinations on a pool of normal sera yielded values of 5.6, 4.4, 3.2, 3.2 4.0, 4.0, 3.3 and 3.7 μg%, for a mean and standard deviation of 3.9 ± 0.6 μg%.

Results on several hospitalized patients are shown in Table III. The greatest serum manganese elevation observed was in a patient with generalized arteriosclerosis and gangrene of the foot. Electrographic tracing and serum GOT determination were within normal limits on this patient, but serum GPT activity was markedly elevated.

TABLE II. Manganese Concentration of Dog Myocardium, μg/100 g.

	1	2
Normal	145	159
Infarcted	147	152

In 3 patients with acute myocardial infarction confirmed by electrographic changes, the serum manganese concentrations were 9.2, 5.4, and 3.2 μg%. All 3 of these patients had increased serum GOT activity. The patient with myocardial infarction and serum manganese within normal limits was senile and had been chronically ill (pulmonary emphysema and tuberculosis).

A serum manganese level twice the normal concentration was observed in a patient with infectious hepatitis.

Moderate serum manganese elevation was observed in a patient during acute alcoholic intoxication without evidence of organic disease.

Discussion. Production of myocardial infarction in dogs produces a rise in serum manganese to about twice the concentration before infarction. However, if the control concentration is in the low normal range, the elevation may be only to approximately the upper limit of normal, decreasing the diagnostic value. Also the manganese rise is usually not earlier than the SGOT rise and always later than changes in the ECG. In the human patients, the rise in serum Mn was not specific for myocardial infarction and was not found to be better than the SGOT.

TABLE III. Serum Manganese Concentrations in a Series of Hospitalized Males.

Patient	Age, yr	Diagnosis	Serum Mn, μg %	SGOT, units	SGPT, units	ECG
PW	65	Generalized arteriosclerosis, gangrene of right foot	12.0	16	610	
WP	43	Acute myocardial infarction, 30 hr duration	9.2	75		+
HFC	35	Infectious hepatitis	9.0	1000	1500	
WP	41	Acute intoxication, anxiety reaction	6.7	45		
JK	46	Acute myocardial infarction, 24 hr duration	5.4	72		+
HMH	72	Acute myocardial infarction, 48 hr duration; senile, chronically ill (pulm. emphysema, tuberculosis)	3.2	45		+
		Pooled normal serum	3.9			

TABLE IV. Manganese Content of Blood, μg %.

		Method	Serum	Plasma	RBC's	Whole blood	Range
Albritton(5)	1952			10			0 –25
Idem	1952				20		0 –48
Spector(6)	1956			8			0 –19
Bower(7)	1956	Activ.	1.8		2.3	2.4	
Cotzias(8)	1958					3	
Bondarev(9)	1960	Spec.				1000	
Cholak & Hubbard(10)	1960	"				4.6	2.2 – 7.9
Leonov & Gatsko(11)	1960	Color.				14.6	±.9
Borg *et al*(12)	1961	Activ.		.9		3.1	
Durgakeri & Bellare(13)	1961	Color.	37				14 –52
Idem	1961					33	29 –40
Hedge *et al*(1)	1961	Spec.	1.0				
Papavasiliou & Cotzias(14)	1961	Activ.	.25	.27			.21– .30
Idem	1961					1.2	.9 – 1.5
Miller & Yoe(15)	1962	Color.	7				4 –12
Idem	1962				28		10 –46
Present work		"	3.9				

Determination of Mn is certainly more difficult than that of SGOT.

The concentration of manganese in the serum of normal humans has been determined chemically, spectrographically, and by neutron activation. The results have varied widely, even in recent reports (Table IV).

The normal values which we obtained, 3.9 μg% for human subjects and 3.7 for dogs, are lower than other workers using colorimetric methods have obtained. They are in the range of the spectrographic methods (discounting the result of Bondarev; the original article could not be consulted so we could not verify that the result quoted in the abstract was the same as originally reported). The concentrations found by neutron activation analysis have all been lower. There has also been lack of agreement as to the relative concentrations of Mn in erythrocytes and in serum. Some workers have reported these to be about the same, others that the whole blood or cells contain about 4 times the serum concentration. These findings are independent of whether a colorimetric method or activation analysis was used. The present investigation does not help to clarify this problem since no whole blood determinations were made. Our heart tissue values are intermediate between the widely discrepant values of Tipton (see 16) and of Hedge *et al* (1), and also do not show any difference between normal and infarcted tissue as reported by the latter workers.

Summary. Chemical determinations of serum manganese in a series of dogs showed an increase following ligation of the anterior descending branch of the left coronary ar-

tery. However the rise was no more prompt than that of SGOT and occurred later than ECG changes. Extent of the Mn elevation did not correlate with size of the infarct. No difference in Mn content was observed between normal and infarcted areas of myocardium. In human patients, serum Mn was not always elevated following myocardial infarction and was also increased in other diseases. Normal values for the dogs averaged 3.7 μg%, for the humans, 3.9.

1. Hedge, B., Griffith, G. C., Butt, E. M., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 734.
2. Thiers, R. E., *Methods Biochem. Anal.*, 1957, v5, 278, 289.
3. Gates, E. M., Ellis, G. H., *J. Biol. Chem.*, 1947, v168, 537.
4. Cabaud, P., Leeper, R., Wróblewski, F., *Am. J. Clin. Path.*, 1956, v26, 1101.
5. Albritton, E. C., *Standard values in blood*, 1952.
6. Spector, W., *Handbook of biological data*, 1956.
7. Bower, H. J. M., *J. Nucl. Energy*, 1956, v3, 18.
8. Cotzias, G. C., *Physiol. Revs.*, 1958, v38, 503.
9. Bondarev, L. S., *Trudy Stalinsk. Med. Inst.*, 1960, v18, 97; *Referat. Zhur. Khim. Biol. Khim.*, 1961, ab. 11S1034; *Chem. Abstr.*, 1962, v56, 5305h.
10. Cholak, J., Hubbard, D. M., *Am. Ind. Hyg. Assn. J.*, 1960, v21, 356.
11. Leonov, V. A., Gatsko, G. G., *Doklady Akad. Nauk Belorus S. S. R.*, 1960, v4, 360; *Chem. Abstr.*, 1961, v55, 6653c.
12. Borg, D. C., Segel, R. E., Kienle, P., Campbell, L., *Intern. J. Appl. Radiation Isotopes*, 1961, v11, 10.
13. Durgakeri, U. S., Bellare, R. A., *J. Sci. Ind. Rsch. (India)*, 1961, v20 C, 314; *Chem. Abstr.* 1962, v56, 13186c.
14. Papavasiliou, P. S., Cotzias, G. C., *J. Biol. Chem.*, 1961, v236, 2365.
15. Miller, D. O., Yoe, J. H., *Anal. Chim. Acta*, 1962, v26, 224.
16. Anon., *Nutrition Rev.*, 1962, v20, 132.

Received March 4, 1964. P.S.E.B.M., 1964, v116.

Quantitative Effects of Alkyl Benzene Sulfonate (ABS) on KB Cells in Tissue Culture.* (29372)

MELVIN A. BENARDE AND ROBERT J. KUCHLER (Introduced by J. B. Allison)
Bio-Engineering Laboratory, Department of Civil Engineering, and Department of Bacteriology, Rutgers, The State University, New Brunswick, N. J.

During the past few years synthetic detergents have displaced soap as the cleanser of choice, both domestically and industrially. The most widely used are those containing as a major constituent sodium dodecyl benzene sulfonate (ABS), an anionic surfactant. Whereas soap is easily degraded by natural microbial activity, the synthetic detergents have proven almost completely refractory. As a result, they appear to be present to a considerable degree in ground water or as residues on inadequately rinsed utensils and cutlery. This has led to the suggestion that a potential hazard may occur from such consumption.

Classical toxicological techniques for evaluating efficacy of chemicals for human consumption are time-consuming and extremely costly. Methods that may signal untoward metabolic reactions in a short period could be extremely valuable as a screening device to indicate the need for additional testing by established pharmacological methods of those chemicals that exhibit cytopathological lesions in *in vitro* tests. Tissue culture may offer just such an accelerated, relatively inexpensive procedure.

The present study was undertaken to ascertain several measurable physiological responses in an established cell line to graded doses of ABS. For this purpose we have used KB cells which were originally isolated by Eagle(1) from a human epidermoid carcinoma.

Materials and methods. A replicate series of KB cell cultures was set up by pipetting 10 ml from a large cell suspension into 150 ml milk dilution bottles. A nutrient mixture

* Technical facilities for the conduct of this work were provided by U. S. Public Health grants.