cine level and spermidine/spermine ratio were elevated. At 48 h spermidine content and the spermidine/spermine ratio were enhanced, whereas the spermine concentration was lower than in the contralateral control kidney. At 72 h spermidine concentrations and spermidine/spermine ratios were further augmented. In the contralateral control kidney, the putrescine level was increased at 24 h, whereas spermine and total polyamine concentrations were elevated at 48 h after surgery, as compared with respective values of sham-operated rats.

Discussion. The enzyme ornithine decarboxylase (E.C. 4.1.1.17) is the first in the biosynthesis of polyamines, and is considered to be the site of regulation for the overall pathway. Its half-life is 11 min in the regenerating liver[12]. The increase in activity of ornithine decarboxylase seems to be part of a general response of tissues to stimulation, whether it be a stimulus to cell division or to increased activity of differentiated function[13]. Renal ornithine decarboxylase activity and polyamine levels have been shown to increase in response to various stimuli, such as after unilateral nephrectomy[14], different hormonal treatments[13,15-20] folic acid[21] suramin[22], beta endorphin or morphine[23] administration.

The results of the present study appear to demonstrate that, following unilateral ureteral obstruction, responses of polyamine metabolism are initiated rapidly in the occluded kidney. These responses are primarily characterized by a prevalance of spermidine over spermine, a condition otherwise found in rapidly proliferating tissues[24,25]. Since total polyamine concentrations were not augmented significantly in the obstructed kidney, and a redistribution occurred among polyamines, it is concluded that in addition to increased synthesis an interconversion of polyamines published for rat liver[26,27] might also contribute to the changes observed in the present study. The contralateral kidney exhibited a less apparent and a differential response-pattern. Therefore, we suggest that local factors might be involved in the polyamine alterations of the obstructed kidney, and support the view[4] that the early response of the obstructed kidney was not mediated by humoral factors.

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2 C.W. Tabor and H. Tabor, A. Rev. Biochem. *45*, 285 (1976).
3 J. Jänne, H. Pösö and A. Raina, Biochim. biophys. Acta *473*, 241 (1978).
4 L. Benitez and J.A. Shaka, Am. J. Path. *44*, 961 (1964).
5 J. Blondin, M.L. Purkerson, D. Rolf, A.C. Schoolwerth and S. Klahr, Proc. Soc. exp. Biol. Med. *150*, 71 (1975).
6 J.M. Kissane and R.H. Heptinstall, Lab. Invest. *13*, 539 (1964).
7 J.M. Kissane and R.H. Heptinstall, Lab. Invest. *13*, 547 (1964).
8 H.R. Jervis and E.P. Benditt, Lab. Invest. *28*, 456 (1973).
9 G. Emanuelli, G. Anfossi, G. Camussi, G. Calcamuggi, G. Gestonaro and G. Gatti, Experientia *35*, 729 (1979).
10 H. Inoue and A. Mizutani, Analyt. Biochem. *56*, 408 (1973).
11 N. Seiler, in: Polyamines in normal and neoplastic growth, p. 137. Ed. D.H. Russel. Raven Press, New York 1973.
12 D.H. Russel and S.H. Snyder, Molec. Pharmac. *5*, 253 (1969).
13 M.J. Thomson and J.F. Richards, Life Sci. *22*, 337 (1978).
14 J.T. Brandt, D.A. Pierce and N. Fausto, Biochim. biophys. Acta *279*, 184 (1972).
15 R. Sogani, S. Matsushita, J. Muller and M. Ruben, Biochim. biophys. Acta *279*, 377 (1972).
16 L.A. Holladay, J.H. Levine, W.E. Nicholson, D.N. Orth, W.D. Salmon, Jr, and D. Puett, Biochim. biophys. Acta *381*, 47 (1975).
17 W.E. Nicholson, J.H. Levine and D.N. Orth, Endocrinology *98*, 123 (1976).
18 A.C. Andersson, S. Henningsson and E. Rosengren, Experientia *31*, 1101 (1975).
19 G. Scalabrino and M.E. Ferioli, Endocrinology *99*, 1085 (1976).
20 S. Henningsson, L. Persson and E. Rosengren, Acta physiol. scand. *102*, 385 (1978).
21 A. Raina and J. Jänne, Fedn Proc. *29*, 1568 (1970).
22 L. Selmeci, E. Pósch, G. Simon and L. Hársing, Biochem. biophys. Res. Commun. *87*, 323 (1979).
23 M.K. Haddox and D.H. Russel, Life Sci. *25*, 615 (1979).
24 H.G. Williams-Ashman, G.L. Coppoc and G. Weber, Cancer Res. *32*, 1924 (1972).
25 D.H. Russel, in: Polyamines in normal and neoplastic growth, p. 1. Ed. D.H. Russel. Raven Press, New York 1973.
26 E. Hölttä, R. Sinervirta and J. Jänne, Biochem. biophys. Res. Commun. *54*, 350 (1973).
27 E. Hölttä, Biochemistry *16*, 91 (1977).

Effect of starvation and refeeding on catalase and superoxide dismutase activities in skeletal and cardiac muscles from 12-month-old rats[1,2]

C.J. Lammi-Keefe, P.V.J. Hegarty and P.B. Swan

Department of Food Science and Nutrition, University of Minnesota, St. Paul (Minnesota 55108, USA), 8 April 1980

Summary. Catalase and superoxide dismutase (SOD) activities were determined in muscles from 12-month-old rats after severe starvation and after subsequent refeeding. Catalase increased in most muscles after starvation and decreased after refeeding, while SOD remained unchanged.

Potentially toxic levels of hydrogen peroxide (H_2O_2) and superoxide radicals are controlled in aerobic organisms by catalase and superoxide dismutase (SOD) respectively. The biological significance of the increase in catalase in the rat gastrocnemius muscle after starvation[3] remains obscure. However, catalase is cited as a marker enzyme which increases with muscle breakdown[4]. In addition, it has been suggested that evaluation of biopsy material for catalase could be used as a marker enzyme for muscle wasting[3]. There is no comparative biochemical study of the activities of catalase and SOD for different rat skeletal muscles nor are there studies to examine how these enzymes in muscles respond to refeeding after starvation. It therefore seemed of interest to establish if catalase activity differs between muscles and if different muscles respond in a similar manner after starvation and starvation-refeeding, since this would be an important consideration in choosing muscles for biopsy. Since SOD and catalase function to regulate

Muscle weights and catalase activity of muscles from rats after starvation and refeeding

Muscle	Start (12 months old) Muscle weight (mg)[3]	Catalase[4]	Starvation[1,2] Muscle weight (mg)[3]	Catalase[4]	Refeeding Muscle weight (mg)[3]	Catalase[4]	Age control[1] (15 months old) Muscle weight (mg)[3]	Catalase[4]
Heart	1401.4±59.3 (3)[5]	59.1±8.7 (3)	1069.0±30.5 (4)	130.7±13.9[a] (4)	1379.5±58.0 (4)	59.0±4.1 (4)	1497.6±16.8 (3)	47.4±8.8 (3)
Sternomastoideus	462.6±16.2 (3)	14.1±7.4 (3)	391.8±20.1 (3)	30.8± 3.9[c] (3)	537.4±33.8 (2)	8.3±0.7 (2)	550.9±22.6 (2)	5.0±0.2 (2)
Biceps brachii	326.2±14.6 (3)	6.0±0.9 (3)	271.6±12.5 (3)	28.3± 4.3[a] (3)	341.3± 0.7 (2)	8.4±0.1 (2)	368.3±19.0 (2)	6.6±1.5 (2)
Plantaris	468.1±12.0 (3)	7.8±1.3 (3)	379.1±26.5 (3)	31.8± 0.7[a] (3)	514.9± 0.3 (2)	10.3±0.3 (2)	611.5±24.8 (2)	6.4±1.2 (2)
Soleus	167.5± 9.4 (3)	46.9 (1)	159.4± 7.8 (3)	49.7[d] (1)	176.5±13.4 (2)	56.2 (1)	185.3±17.5 (2)	44.1 (1)
Extensor digitorum longus	184.3± 3.9 (3)	6.2 (1)	153.4±10.4 (3)	32.4[d] (1)	198.7± 5.5 (2)	9.6 (1)	216.4± 4.6 (2)	6.3 (1)

[1] Significance calculated compared to 12-month-old animals using Student's t-test[7]; [2] Starvation to an average body weight loss resulted in average muscle weight losses of: heart (24%), sternomastoideus (15%), biceps brachii (17%), plantaris (19%), soleus (5%) and EDL (16%); [3] Mean±SE; [4] µg equivalents/g wet tissue weight (×10) (mean±SD); [5] Numbers in parentheses are number of samples assayed. [a] $p<0.001$; [b] $p<0.01$; [c] $p<0.05$; [d] statistics not done.

levels of superoxide radicals and H_2O_2, it was postulated that if catalase does increase in rat muscles with starvation, an increase in SOD might also be observed.

The purpose of this study was firstly, to establish catalase and SOD activities in 5 skeletal muscles and cardiac muscles of 12-month-old rats and secondly, to measure the response of these enzymes after total starvation and starvation-refeeding.

Materials and methods. Animals and experimental design. Male, Sprague-Dawley rats (ARS, Madison, Wisconsin) had access to Purina Lab Chow (Ralston Purina Company, St. Louis, Missouri) and tap water ad libitum. When the animals were 12 months old (545 g mean b.wt) the animals were divided into 4 groups. Control levels of enzymes were established by killing animals at 12 months of age and measuring enzyme levels. A 2nd group of animals was starved to an average body weight loss of 38.5% by the withdrawal of all food, and a 3rd group was similarly starved to an average body weight loss of 38.5% and refed the chow diet to the average mean body weight at the start of the experiment. A 4th group was fed the chow diet ad libitum and enzymes were measured in these animals when they were 15 months old.

Tissues. 1 fore-limb muscle: the biceps brachii; 3 hind-limb muscles: the soleus, plantaris, and extensor digitorum longus (EDL); 1 trunk muscle: the sternomastoideus; and cardiac muscle were studied. Muscles were dissected from one side of the animals. 1 muscle was used for each sample, except for the soleus and EDL in which case each sample was a pool of 2 muscles. Muscles were homogenized on ice in 1:10 (w/v) 0.01 M potassium phosphate buffer, pH 7.5 with a Brinkman PCU-2 Polytron. The homogenates were centrifuged 2 times in succession at 1000×g for 10 min. The final supernatants were assayed for SOD and catalase.

Enzymes. Catalase was assayed according to Baudhuin et al.[5]. Samples assayed for catalase were made 0.1% (w/v) with respect to bovine serum albumin and 1% (v/v) with respect to Triton X-100. Final reaction mixture concentrations were imidazole buffer (Sigma): 1×10^{-2} M, pH 7.2; bovine serum albumin: 0.1% (w/v); and hydrogen peroxide: 1.5×10^{-3} M. Incubations were made at 25 °C for 15 min. The remaining hydrogen peroxide was measured spectrophotometrically as the yellow 'peroxy titanium sulphate'. A standard curve was established using catalase from bovine liver (Sigma). Catalase activity was expressed as µg of equivalent bovine liver catalase per g of wet tissue weight. It was determined that the substrate concentration in the assay was optimal for the muscle enzyme and that the activity curve was linear when increasing volumes of supernatant were assayed.

Superoxide dismutase was assayed using the photochemical augmentation assay according to Misra and Fridovich[6]. Final reaction mixture concentrations were dianisidine: 2×10^{-4} M; riboflavin: 1.3×10^{-5} M; and potassium phosphate: 0.01 M, pH 7.5. The reaction mixtures were illuminated for 8 min and the oxidized dianisidine was measured spectrophotometrically. A standard curve was established using superoxide dismutase from bovine blood (Sigma). Superoxide dismutase activity was expressed as µg of equivalent bovine blood SOD per g of wet tissue weight. It was determined that the substrate concentration was optimal for the muscle enzyme and that the activity curve was linear when increasing volumes of supernatant were assayed.

Results and discussion. The 5 skeletal muscles and cardiac muscle from 12-month-old rats had different catalase activities (table). The lowest activities were in the biceps brachii, EDL, and plantaris. The highest activities were in the soleus and heart with an intermediate level in the sternomastoideus. This comparison demonstrates that the muscles within an animal differ with respect to catalase activity. Our findings in rats are in agreement with those of Stauber et al.[3] who reported differences in catalase activities between different muscle types in the chicken. Those authors attributed the higher catalase activity of red muscles, in contrast to white muscles, to the higher lipid content of red muscles. Recently catalase has been localized immunocytochemically in hamster skeletal muscles, and immunoreaction products were more numerous in type I aerobic fibres than in type II anaerobic fibres[8].

Catalase increased in all the muscles except the soleus when the animals were starved to an average body weight loss of 38.5% (table). On a percent basis, the greatest increases were in the muscles which had the lowest catalase activities in the controls, e.g., the EDL, biceps brachii, and plantaris. The increase in the soleus was negligible (6%). Catalase specific activity in the rat gastrocnemius muscle has previously been reported to increase after starvation[3]. In our experiment, after refeeding to the original average mean body weight, catalase activity decreased to approximate the catalase activity at the start of the experiment (table).

The starvation to a 38.5% body weight loss in the 12-month-old animals took 18 days and the refeeding to the original mean body weight took about 40 days. Upon refeeding, the rate of weight gain approximated the rate of weight loss until the weight was within approximately 5% of the starting mean body weight; the gain from this point was very slow. The animals were therefore 14 months old at the end of the refeeding period.

Enzyme levels were determined for age controls at 15 months of age. Catalase activity did not appear to change between 12 months and 15 months (table). The mean

concentration of superoxide dismutase in heart muscle (183 µg equivalents/g wet tissue weight) was twice that in each of the 5 skeletal muscles studied (53–88 µg equivalents/g wet tissue weight). The SOD activity of most muscles did not change after starvation and refeeding or between 12 and 15 months of age. Thus, while the functions of catalase and SOD are closely related, starvation did not affect them in the same manner.

The significance of the increase in catalase activities after starvation is not clear. Electron micrographs of selected muscles after starvation revealed no gross degeneration or disturbance of contractile proteins (unpublished observation). Catalase did not correlate with the percent decrease in muscle weight (table). Therefore, the use of catalase as a quantitative indicator of muscle weight loss is questionable. Mitochondria in skeletal muscles show extensive hypertrophy and elongation and an increase in number with fasting[9]. These changes were apparent as early as the 1st day of starvation and the alterations in muscle morphology disappeared shortly after refeeding a normal diet. The soleus is a muscle with more mitochondria and a higher oxidative capacity than white muscle[10]. This muscle also has a higher catalase level than white muscles, such as the EDL. Therefore, one is tempted to speculate that the increase in catalase with starvation is related to a possible increase in the number of mitochondria.

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2 Scientific Journal Series No. 11, 191, Minnesota Agricultural Experiment Station.
3 W.T. Stauber, J.W.C. Bird and B.A. Schottelius, Exp. Neurol. *55*, 381 (1977).
4 A.G.E. Pearse and M. Johnson in: Muscle Diseases, p. 25. Ed. J.H. Walton, N. Canal and G. Scarlato. Proc. int. Congr. Excerpta Medica, Amsterdam 1970.
5 P. Baudhuin, H. Beaufay, Y. Rahmen-Li, O.Z. Sellinger, R. Wattiaux, P. Jacques and C. de Duve, Biochem. J. *92*, 179 (1963).
6 H.P. Misra and I. Fridovich, Archs Biochem. Biophys. *181*, 308 (1977).
7 R.G.D. Steele and G.H. Torrie, Principles and Procedures of Statistics. McGraw-Hill Inc., New York 1960.
8 K.N. Christie and P.J. Stoward, J. Histochem. Cytochem. *27*, 814 (1979).
9 S.A. Adibi, B.A. Krzysik, E.L. Morse, P.M. Amin and E.R. Allen, J. Lab. clin. Med. *84*, 548 (1974).

Mitotic response and sister chromatid exchanges in lymphocytes cultured in sera from different sources[1]

A.F. McFee and M.N. Sherrill

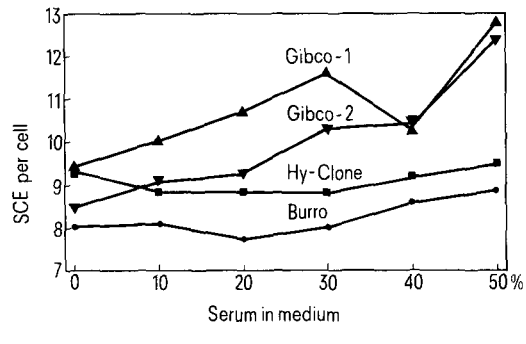
Comparative Animal Research Laboratory, 1299 Bethel Valley Road, Oak Ridge (TN 37830, USA), 20 September 1979

Summary. The numbers of sister chromatid exchanges in lymphocytes grown in varying concentrations of serum from different sources indicated that some sera contain a factor, probably introduced as a contaminant, which induces SCEs. Sera from 6 animals showed no evidence of a difference in baseline SCE levels due to the donor of the serum.

Sister chromatid exchanges (SCE) scored in cultured cells are recognized as a sensitive measure of the mutagenic action of various chemicals in mammalian systems (see review of Latt et al.[2]). The overall sensitivity of the system is almost certainly reduced by the many variables associated with the in vitro growth of cells, not the least of which is variation in composition of the growth medium. Identification of some of the contributing variables would help reduce the range of baseline SCE rates which are reported to vary, for example, from 6.9 to 15.1 per cell in human lymphocytes[3,4]. Fetal calf serum, which is almost universally used as a media component, has been blamed by Kato and Sandberg[5] for inducing some of the SCE seen in control cultures. They noted differences in SCE rates among Chinese hamster cells grown in media containing sera from different commercial sources; the rates were sharply reduced by heat inactivation of the sera. Although differences in collection and handling processes could obviously influence the SCE-inducing properties of sera, inherent differences in the sera of different donors could also contribute. Chatot et al.[6] recently noted that the growth of rat embryos cultured on sera from different donors varied significantly more than that of embryos grown on serum from a single donor.

In this report we present further data on sera from different sources which suggest that variations in processing procedures are of primary importance in determining the SCE-inducing potential of serum.

Materials and methods. Samples of serum collected at our laboratory from 6 adult burros (3 male, 3 female) were tested to see whether sera from different animals would vary in their ability to induce SCE. Lymphocytes from 2 human and 2 pig donors were simultaneously grown in media made with sera from each of the 6 animals. Sera used for the dose-response comparisons were 2 lots of fetal calf serum from Gibco (Grand Island, N.Y.), 1 lot of Hy-Clone fetal calf serum (Sterile Systems, Inc., Logan, Utah)



Sister chromatid exchanges in swine lymphocytes grown in media containing increasing concentrations of serum from different sources. Points are means of 25 cells from each of 2 animals.