C00-1602-29

Appendix VII

CHANGES IN CATALASE ACTIVITY IN RAT TESTICULAR PREPARATIONS INDUCED BY HYPOPHYSECTOMY AND X-IRRADIATION

Louis E. Buhrley and LeGrande C. Ellis

Department of Zoology and Division of Biochemistry

Utah State University, Logan, Utah 84321

DISCLAIMER

ABSTRACT

Catalase activity (E. C. 1.11.1.6) was measured in rat testes from control, hypophysectomized and irradiated rats (150 R) with standard manometric techniques. A comparison was made in the enzyme activity from minced testicular preparations and teased-tubular preparations for all groups of animals. Catalase activity was greater for all of the minced preparations than it was for the teased-tubular preparations, as has been previously observed in this laboratory for steroid biotransformations. Hypophysectomy markedly reduced total catalase activity of rat testicular tissue when expressed on a per animal basis, but it was increased by hypophysectomy due to a loss of germinal element of the testes. On this basis, the enzyme was assumed to be preferentially located in a more stable cell type in the seminiferous tubule which was not as severely affected by the withdrawal of gonadotropins (i. e., non-germinal cells). Irradiation increased catalase activity from 4-31 days, but decreased it from 35 through 50 days post-treatment, indicative of a physiological role of this enzyme for the testes.

INTRODUCTION

Hydrogen peroxide (H_2O_2) is formed as a by-product in some cells through specific metabolic reactions and by ionizing radiation (Alder, 1963; Pryor, 1970). One such system for the formation of H_2O_2 is the monoamine oxidase (MAO-E. C. 1.4.3.4) system where 5-hydroxytryptamine (5-HT) is transformed into 5-hydroxyindole acetic acid (5-HIAA) according to the following mechanism (Guilbault *et al.*, 1969; and Kapeller-Alder, 1970):

$$RCH_2CHNH_2 + O_2 \xrightarrow[MAO]{} RCH_2C{=}NH + H_2O_2$$

$$RCH_2C{=}NH + H_2O \xrightarrow[MAO]{} RCH\ CHO + NH_3$$

$$RCH_2CHO + NAD + H_2O \xrightarrow[\text{Aldehyde Dehydrogenase}]{} RCH_2COOH + NADH_2$$

This enzyme system has now been observed in the interstitial cells and walls of the seminiferous tubules of rat testes (for review, see Ellis *et al.*, 1972). The enzymic system is FSH dependent (Urry *et al.*, 1972) and is positively related to sexual development and senescence and aging (Ellis *et al.*, 1972). Furthermore, the enzyme is depressed by social stress in Uinta ground squirrels (Frehn *et al.*, 1972), indicative of its physiological role in the male gonad. 5-HT has been observed to be an inhibitor of androgen synthesis *in vitro* (Ellis, 1972) and is important in determining the testosterone/androstenedione ratios of the androgens with development of the testes.

In addition, H_2O_2 inhibits the conversion of androstenedione into testosterone, but increases the conversion of testosterone into high-polar compounds. It also increases lipid peroxidation in rat testicular

preparations (Ellis and Baptista, 1969). The oxidation of sulfhydryl groups (SH) to disulfur bridges (S-S) may account for this inhibitory action since SH-containing compounds have been shown to be radioprotective (Van de Vijver, 1969). Of significance is the fact that steroid biotransforming enzymes contain SH-groups that are important for biological activity (Ellis and Baptista, 1969; Inano *et al.*, 1967). It is possible that hydrogen peroxide may contribute to cellular damaged induced by irradiation (Thomson, 1963) and the amount of catalase within a cell may determine its sensitivity to irradiation. The following investigations were undertaken in order to ascertain the distribution of catalase (H_2O_2:H_2O_2 oxidoreductase, E. C. 1.11.1.6) in rat testes, if it is diminished after hypophysectomy (gonadotropin dependent), and if its activity is altered by irradiation.

METHODS

Male rats (Holtzman Strain) were maintained in the small animal colony with feed (Purina Lab Chow) and water given *ad libitum* until they were sacrificed by decapitation. The testes were quickly removed, trimmed and decapsulated. Minced preparations were prepared using razor blades. Seminiferous tubules were obtained by teasing the tubules from the surrounding interstitial tissue as described elsewhere (Ellis and Van Kampen, 1971).

Catalase activity was determined with a Gilson differential respirometer using standard manometric techniques (Chance and Maehly, 1955). Preliminary kinetic studies were undertaken, varying amount of tissue, incubation time, temperature and substrate concentration to assure

linearity of the assay. The following conditions were utilized in the assay: Fifty mg of minced tissue or seminiferous tubules; 2 ml phosphate buffer (pH 7.4); 0.1 ml hydrogen peroxide (0.15 N); 10 C, and 2 min incubation time.

Hypophysectomized rats were obtained commercially when three weeks of age and were sacrificed when 18 weeks of age. Twelve-week-old male rats were subjected to 150 R of whole-body irradiation. Three control and three irradiated animals were sacrificed serially over a period of 50 days.

RESULTS

The kinetic data for the assay method (Figs. 1, 2, 3) indicate that the assay is linear within the range of our experiments. A comparison of catalase activity from both minced and teased-tubular preparations from 6 male rats (Fig. 4) expressed on a per 100 mg of tissue basis indicates that the enzyme activity was more active in the minced preparations than for the seminiferous tubules, indicating that the enzyme is largely located in the interstitial tissue.

After hypophysectomy (Fig. 5), the enzyme activity increased when expressed on a per 100 mg of tissue basis. However, the total enzyme activity (calculated for both testes) was markedly reduced for the mince and tubule tissue when expressed on a per animal basis.

Irradiating rats with 150 R when 12 weeks of age (Fig. 6) and sacrificing them serially over a period of 50 days increased total catalase activity of minced preparations from 4 to 31 days after the treatment. From 38 to 50 days post-irradiation the enzyme activity was greater for

the control animals than was observed for the treated animals. No consistent changes were observed in catalase activity from teased-tubular preparations (data not shown).

DISCUSSION

Catalase is an iron-containing porphyrin enzyme that transforms hydrogen peroxide into water and molecular oxygen (Sumner, 1941). The distribution of catalase is ubiquitous in nature, but is found in abundance in mammalian and non-mammalian aerobic cells that contain a cytochrome system (Deisseroth and Dounce, 1970). Peroxidases are similar to catalase, but require a separate hydrogen donor for their activity, whereas catalases utilize another molecule of H_2O_2 as the hydrogen donor. Both enzymes are sometimes referred to as hydroperoxidases (Deisseroth and Dounce, 1970).

Catalase is located intracellularly (de Duve, 1960) and seems to be concentrated in the microbodies or peroxisomes (Baudhuin *et al.*, 1965). While catalase accounts for almost half of the protein content of peroxisomes (de Duve and Baudhuin, 1966), several other oxidative enzymes have also been demonstrated (de Duve *et al.*, 1960) in this body.

Peroxisomes are thought to be formed within the cell by the smooth endoplasmic reticulum (de Duve and Baudhuin, 1966). The intracellular distribution of radioactively-labeled catalase suggests that the enzyme is synthesized by the rough endoplasmic reticulum and then transferred to the peroxisome (Higashi and Peters, 1963). Biochemical and microscopic techniques indicate that catalase is localized in the sap within the peroxisome and that the characteristic dense core is associated with an

insoluble urate oxidase (de Duve, 1965; de Duve, 1969).

Hydrogen peroxide is produced in most cells through the enzymatic reduction of oxygen as a by-product of some metabolic reactions and is the major stable product formed by ionizing radiation of water (Adler, 1963; Pryor, 1970). In addition, the testis contains endogenous 5-HT and MAO (Ellis, 1972 and Ellis *et al.*, 1972) that is capable of forming H_2O_2 under physiological or pathological conditions.

Our data (Fig. 4 and Ellis *et al.*, 1972; Ellis and Baptista, 1969; Urry *et al.*, 1972) show that MAO, steroid biotransformation and catalase all have the same general distribution in the rat testis. Thus, catalase may serve a functional role in the male gonad to decompose H_2O_2 that may be formed during normal metabolic activity. This concept is strengthened by the observation (Fig. 6) that catalase activity increases after irradiation, but returns to normal activity after 38 days. The decrease in total activity after hypophysectomy indicates a gonadotropic dependence of catalase, but substrate induction cannot be ruled out at this point. The lack of correlation between catalase activity and the loss of testicular weight after irradiation, plus the fact that the activity increases when expressed on a per mg of tissue basis (Fig. 5), indicates that the enzyme is located in a stable cell type within the testis.

Both the amount of serotonin in the testis and the amount of enzyme present are related to sexual development of the rat testis (Ellis *et al.*, 1972). Hydrogen peroxide is highly injurious to several cell components and may cause some of the tissue damage attributed to the irradiation (Thomson, 1963; Heikkila and Cohen, 1971). Moreover, both irradiation and hydrogen peroxide can induce the formation of free radicals; compounds

with unpaired electrons which undergo reactions of hydrogen abstraction or chemical addition.

Vodicka (1969) has reported that catalase activity is present in the mouse testis and the present experiments show that catalase activity is present in the rat testis. Our data (Fig. 4) indicate that the bulk of catalase activity is largely found in the interstitial tissue. This is of particular interest, since a similar distribution for steroid biotransformations has previously been observed (Ellis and Berliner, 1969, and Ellis and Baptista 1969).

The physiological role of catalase within the cell is the subject of much speculation and debate (Deisseroth and Dounce, 1970; de Duve and Baudhuin, 1966). A logical role for catalase would be the removal of harmful peroxides generated metabolically and by the action of ionizing radiation on oxygenated water. As early as 1933 it was suggested that catalase might be able to modify the effects of ionizing radiation. In 1947, it was shown that the enzyme could prevent the death of sea-urchin sperm bathed in irradiated water (Thomson, 1963). Two studies in 1960 indicated a positive correlation between sensitivity to ionizing radiation and catalase activity: Shefner (1960) with work on *E. coli* and O'Brien (1960) with data from three genetically related yeast strains. Several reports have since failed to confirm these observations in other cells and tissues. Regenerating liver cells have a greatly reduced level of catalase activity, but this reduction in activity did not alter the radiosensitivity of the liver cells (Myers, 1960). Engel and Adler (1961) failed to find any correlation between radiation sensitivity and catalase activity in three strains of *E. coli*. Clayton and Howard (1962) observed the level of catalase activity in *Rhodopseudomonas spheroides* before and after x-irradiation. They reported no changes in the level of

catalase activity after irradiation and concluded that intracellular catalase offered little or no protection against the harmful effects of ionizing radiation.

A useful model for the study of catalase function in mammals has been provided by a strain of acatalasemic mice. The blood catalase is about 1% or 2% of the activity seen in normal mice and tissue catalase is also reduced. Feinstein and co-workers (1968) reported that these animals are no more sensitive to whole-body irradiation than normal animals. Tissue catalase can be inhibited by aminotriazole and when this drug is given to the acatalasemic mice, they are essentially devoid of catalase activity. When this experiment was performed, the sensitivity to irradiation in these mice was essentially the same as that of normal animals. Acatalasemia has also been reported in humans (Takahara, 1952). It is an autosomal recessive trait and apparently causes recurrent necrotic lesions of the nasal and oral cavities. It has been suggested that a small amount of residual catalase activity is present even in the homozygous individual and this may be sufficient to metabolize much of the hydrogen peroxide produced within the body (Aebi, 1965).

A recent study of catalase in three species of wild ducks indicated that, although the birds differed greatly in the LD_{50} for x-irradiation, they did not differ significantly in tissue catalase activity (Feinstein *et al.*, 1969.

Feinstein *et al.* (1968) provided speculation for the absence of any differences in radiation sensitivity between normal and acatalasemic mice. First, even when the animals are given aminotriazole, as much as 1% of the total body catalase activity of normal mice may remain. Since the rate of decomposition of hydrogen peroxide by catalase is so rapid, this residual

activity may be adequate to remove the hydrogen peroxide produced by the ionizing radiation. A second factor is the possibility that the peroxide produced by the radiation may be too far removed from the peroxisomes to be detoxified. Permeability barriers may exist which would prevent the peroxide from entering the peroxisomes.

Adler (1963) pointed out two additional factors which may explain the lack of correlation between radiation sensitivity and catalase activity. It is possible that the diffusion of hydrogen peroxide from the site of production to a sensitive target area may not be the major mechanism by which x-irradiation produces harmful effects in the cell. Also, catalase is not the only cellular mechanism for the decomposition and removal of hydrogen peroxide.

REFERENCES

Adler, H. I. (1963). Catalase, hydrogen perioxide, and ionizing radiation. Rad. Res. 19 (Suppl. 3):110-129.

Aebi, H., Cantz, M., and Suter, H. (1965). Cellular distribution of catalase activity in red cells of homozygous cases of acatalasia. Experientia. 21: 713-714.

Baudhuin, P., Beaufay, H., and de Duve, C. (1965). Combined biochemical and morphological study of the particulate fractions from rat liver. J. Cell. Biol. 26:219-243.

Chance, B., and Maehly, A. C. (1955). Assay of catalases and peroxidases. II. Method for crude cell extracts. Methods in Enzymology. 2:764-821.

Clayton, R. K., and Howard, I. A. (1962). Protein synthesis and viability in x-irradiated Rhodopseudomonas spheroides. Biochem. Biophys. Acts. 56:257-267.

de Duve, C. (1960). Intracellular location of enzymes. Nature. 187: 836-853.

de Duve, C. (1965). The separation and characterization of subcellular particles. Harvey Lectures. Ser 59:49-87.

de Duve, C. (1969). Evolution of the peroxisome. Ann. New York Acad. Sci. 168(2):369-381.

de Duve, C., Beaufey, H., Jacques, P., Rahman-Li, Y., Sellinger, O. Z., Wattiaux, R., and De Coninck, S. (1960). Intracellular localization of catalase and some oxidases in rat liver. Biochem. Biophys. Acta. 40:186-187.

de Duve, C., and Baudhuin, P. (1966). Peroxisomes (microbodies and related structures). Physiol. Rev. 46:323-357.

Deisseroth, A., and Dounce, A. L. (1970). Catalase: physical and chemical properties, mechanism of catalysis, and physiological role. Physiol. Rev. 50:319-375.

Ellis, L. C. (1970). The testis, A. P. Johnson, W. R. Gomes, N. L. Vandemark. Academic Press, New York. 739-785, Vol. III.

Ellis, L. C. (1972). Inhibition of rat testicular androgen synthesis in vitro by melatonin and serotonin. Endocrinology 90:17-28.

Ellis, L. C., and Baptista, M. H. (1969). A proposed mechanism for the differential sensitivity of the immature rat testis. Proc. Ninth Ann. Hanford Biol. Symp. 9:963-974.

Ellis, L. C., and Berliner, D. L. (1969). The Gonads. Appleton-Century Crofts, New York, 739- Vol. II.

Ellis, L. C., Barfuss, D. W., and Van Kampen, K. R. (1970). Modification of rat testicular response to x-irradiation by various lighting regimes: 1. The pineal gland and melatonin synthesis as a possible mediator of the response. Life Sci. 9(1):1011-1020.

Ellis, L. C., Jaussi, A. W., Baptista, M. H., and Urry, R. L. (1972). Correlation of age changes in monoamine oxidase activity and androgen synthesis by rat testicular minced and teased-tubular preparations in vitro. Endocrinology 90:194-202.

Engel, M. S., and Adler, H. I. (1961). Catalase activity, sensitivity to hydrogen peroxide, and radiation response in the Genus Escherichia. Rad. Res. 15:269-273.

Feinstein, R. N., Faulhaber, J. T., and Howard, J. B. (1968). Sensitivity of acatalasemic mice to acute and chronic irradiation and related conditions. Rad. Res. 35:341-349.

Feinstein, R. N., Howard, J. B., and Faulhaber, J. T. 1969). Catalase levels and radiation resistance in three species of wild duck. Int. J. Radiat. Biol. 15(4):341-346.

Frehn, J. L., Urry, R. L., Balph, D. F., and Ellis, L. C. (1972). Photoperiod and crowding effects on testicular serotonin metabolism and lack of effects on melatonin synthesis in Uinta ground squirrels (Spermophilus armatus). Jour. Exptl. Zool. in press.

Guilbault, G. C., Kuan, S. S. and Brignac, P. J., Jr. (1969). Fluorimetric determination of oxidative enzymes: analytical applications of the monoamine and diomine oxidase systems. Anal. Chem. Acta. 47:503-509.

Heikkila, R., and Cohen, G. (1971). Inhibition of biogenic amine uptake by hydrogen peroxide: A mechanism for the toxic effects of 6-hydroxydopamine. Science. 172:1257-1259.

Higashi, T., and Peters, T. Jr. (1963). Studies on rat liver catalase. II. Incorporation of ^{14}C-leucine into catalase of liver cell fractions in vivo. J. Biol. Chem. 238:3952-3956.

Inano, H., Nakano, H., Shikita, M. and Tamaoki, B. I. (1967). The influence of various factors upon testicular enzymes related to sterordogenesis. Biochem. Biophys. Acta 137:540-548.

Kapeller -Alder, R. (1970). Amine Oxidases and Methods for their Study. pp. 26-27, Wiley-Interscience, New York.

Myers, D. K. (1960). Catalase levels and radiosensitivity of liver cells. Can. J. Biochem. Physiol. 38:1489-1498.

O'Brien, R. T. (1960). Radiation sensitivity studies on respiration deficient yeast. Rad. Res. 12:471.

Pryor, W. A. (1970). Free radicals in biological systems. Sci. Am. 224: 70-83.

Shefner, A. M. (1960). Radiation resistance and hydrogen peroxide tolerance in Escherichia coli. Rad. Res. 12:471.

Sumner, J. B. (1941). The chemical nature of catalase. Advances in Enzymology. 1:163-176.

Takahara, S. (1952). Progressive oral gaugrene probably due to lack of catalase in the blood (acatalasaemia). Lancet. 2:1101-1109.

Thomson, J. F. (1963). Catalase, hydrogen peroxide, and ionizing radiation. Rad. Res. 19(Suppl.3):110-129.

Urry, R. L., Jaussi, A. W. and Ellis, L. C. (1972). A simple microradiometric technique for the rapid measurement of monoamine oxidase activity in rat testicular minced and teased-tubular preparations. Anal. Biochem. in press.

Van de Vijver, G. (1969). Metabolism of Tetrahymena pyriformis: V. protective effect of sulfhydryl substances against gamma-irradiation. Enzymologia. 36:375-385.

Vodicka, I. (1969). Catalase activity in kidney, spleen, and testes of homogenates of control and whole-body-irradiated male mice. Strahlentherapie. 137:730-736.

LEGENDS

Figure 1. Effect of incubation time on rat testicular catalase activity.

Figure 2. Effect of enzyme concentration on rat testicular catalase activity.

Figure 3. Effect of substrate concentration on rat testicular activity.

Figure 4. Comparison of catalase activity of minced and tubular preparations. The activity is expressed as microliters of oxygen involved per 100 mg of tissue during the first two minutes of incubation.

Figure 5. Catalase activity of minced and teased-tubular preparations for control and hypophysectomized animals expressed as microliters of oxygen per 100 mg of tissue per two minutes.

Figure 6. Catalase activity of minced and teased tubular preparations for control and hypophysectomized animals expressed as total activity for both testes.

Figure 7. Catalase activity of control and x-irradiated animals expressed as microliters of oxygen per animal. Number of days post-irradiation is listed on the abscissa.

Figure 1

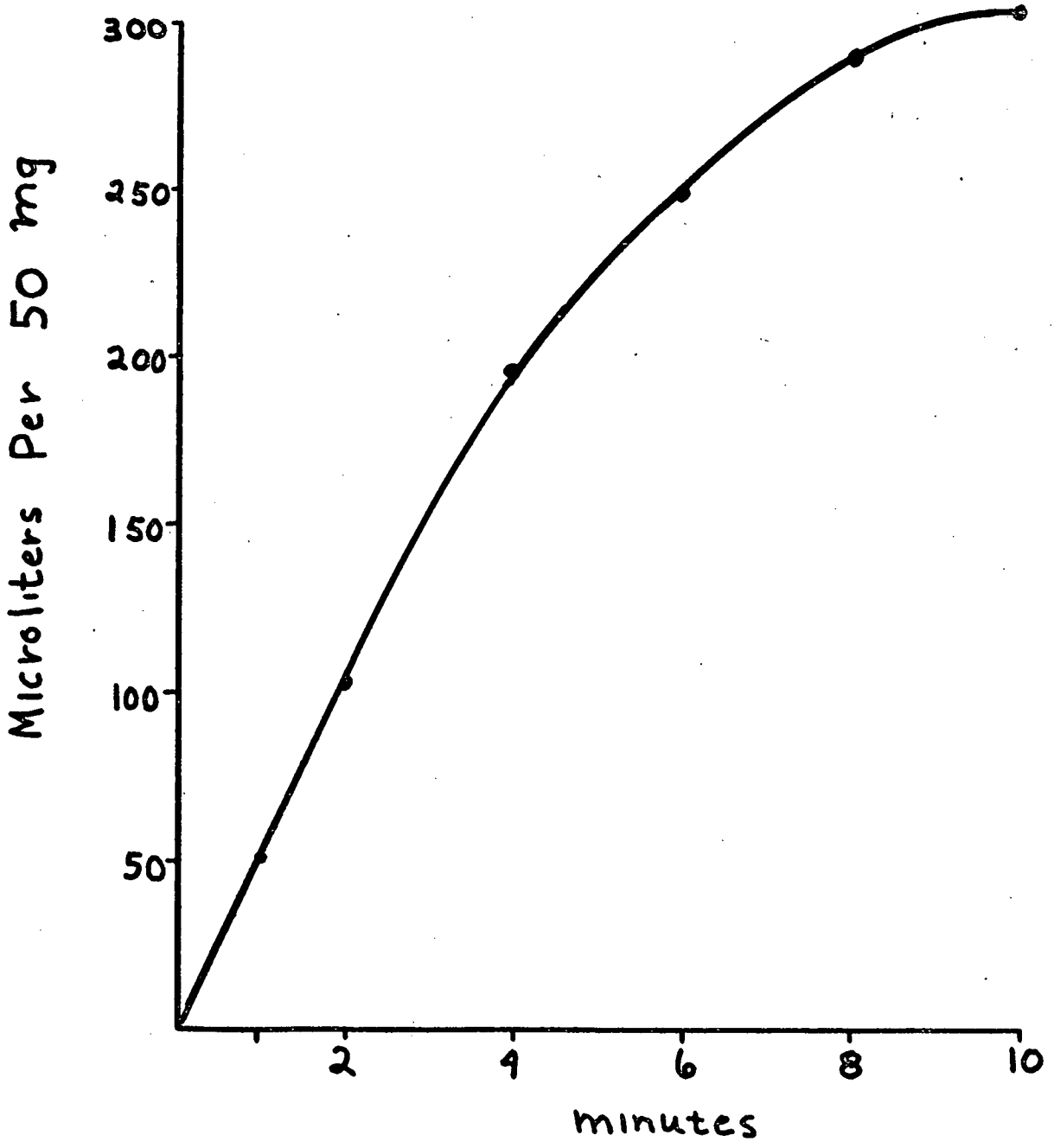

Figure 2

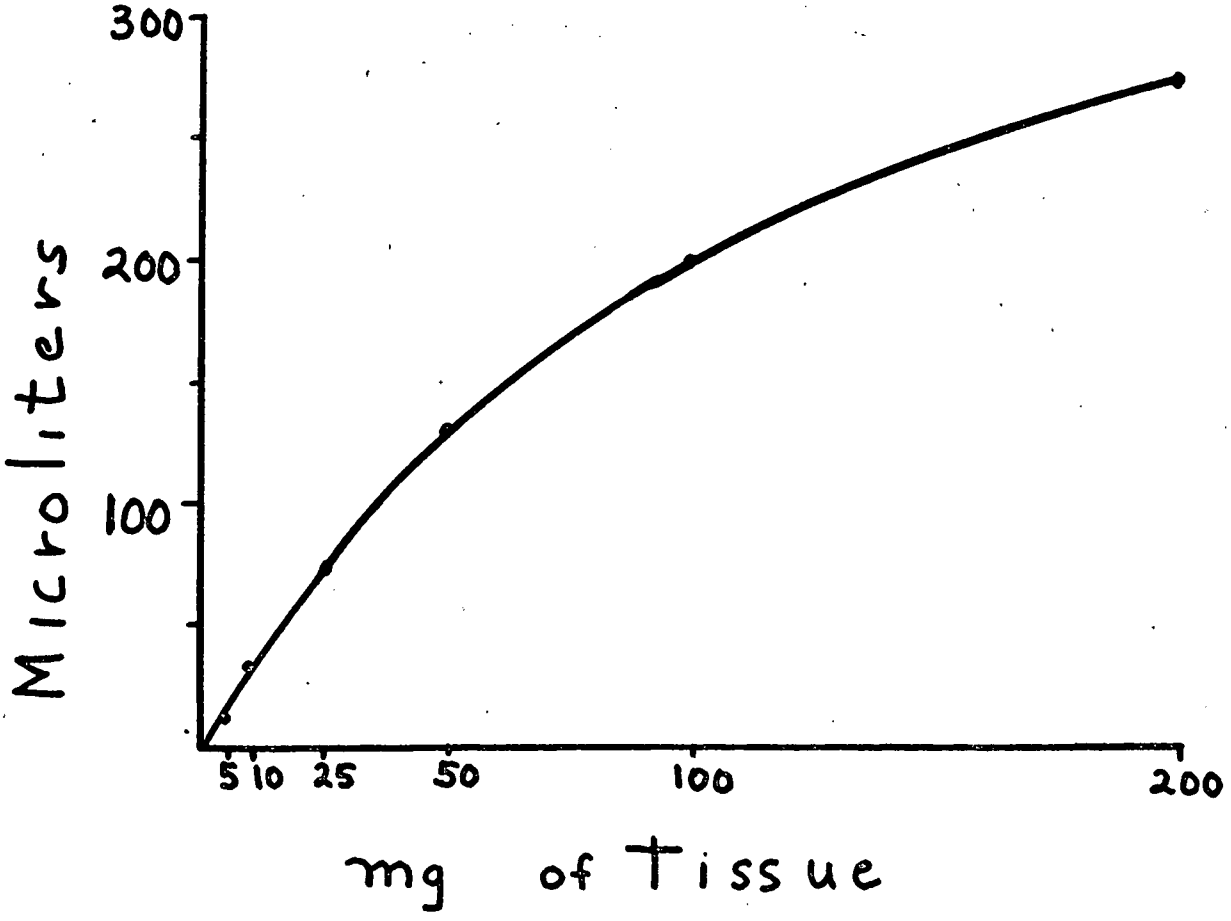

Figure 3

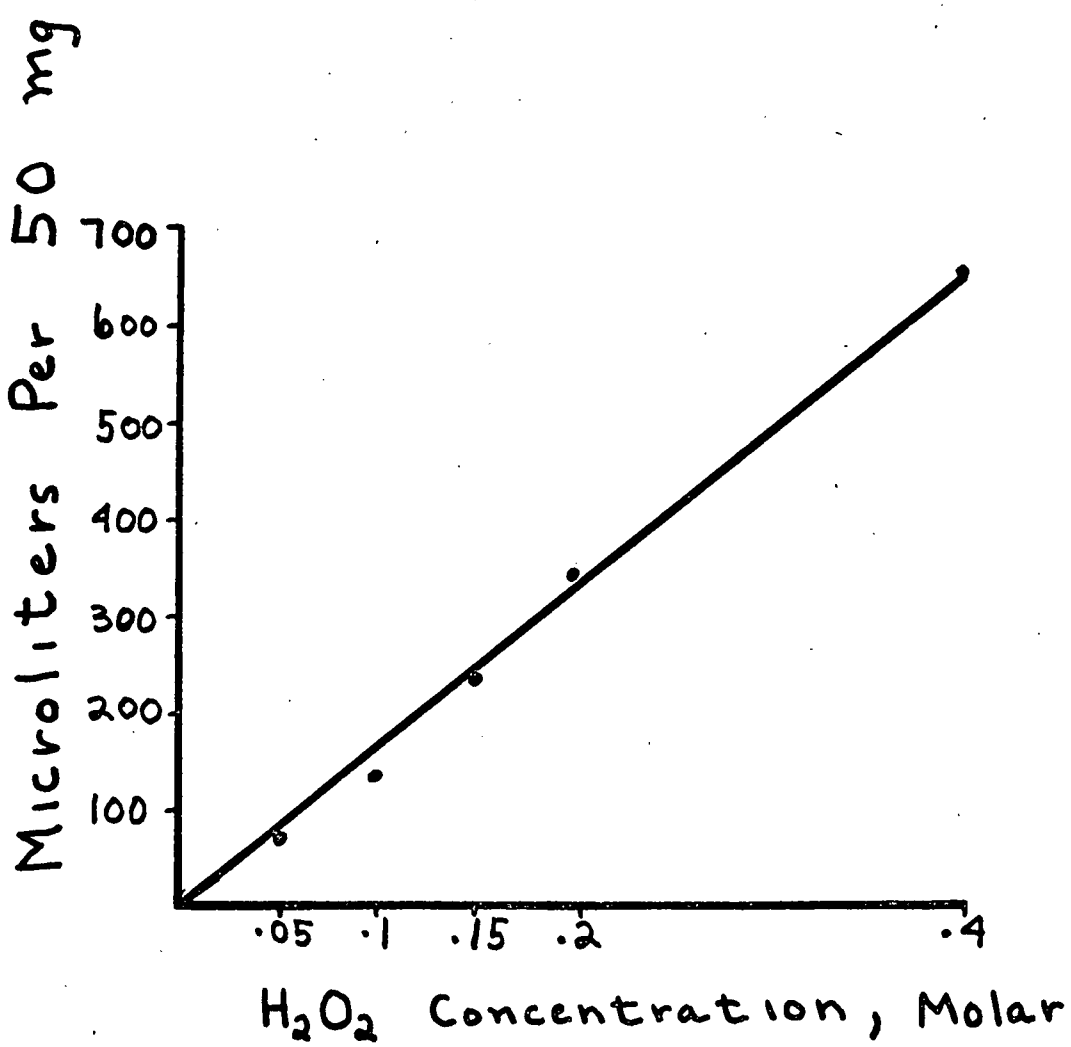
Microliters Per 50 mg
700
600
500
400
300
200
100
.05
.1
.15
.2
.4
H_2O_2 Concentration, Molar

Figure 4

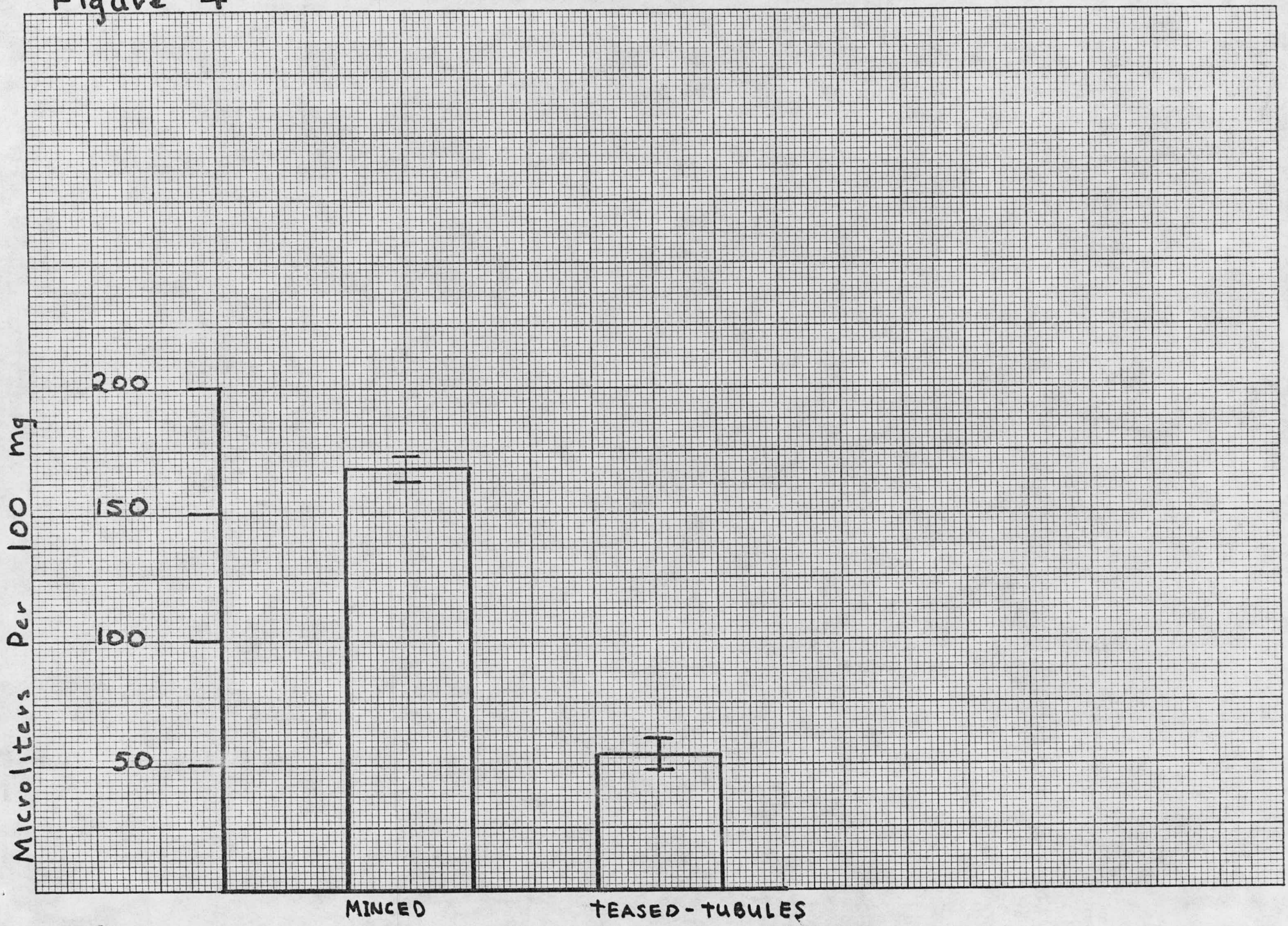

Figure 5

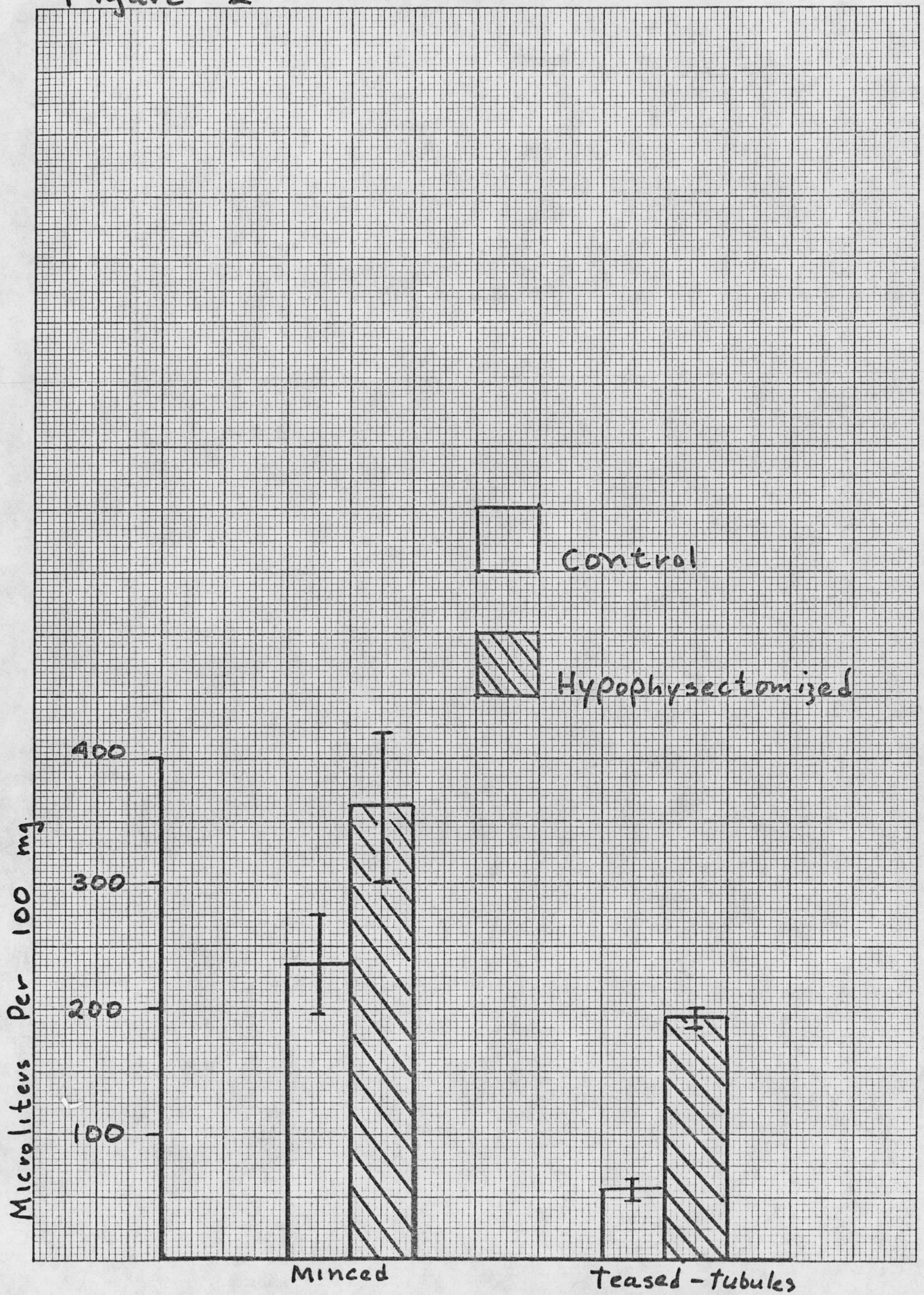

Figure 6

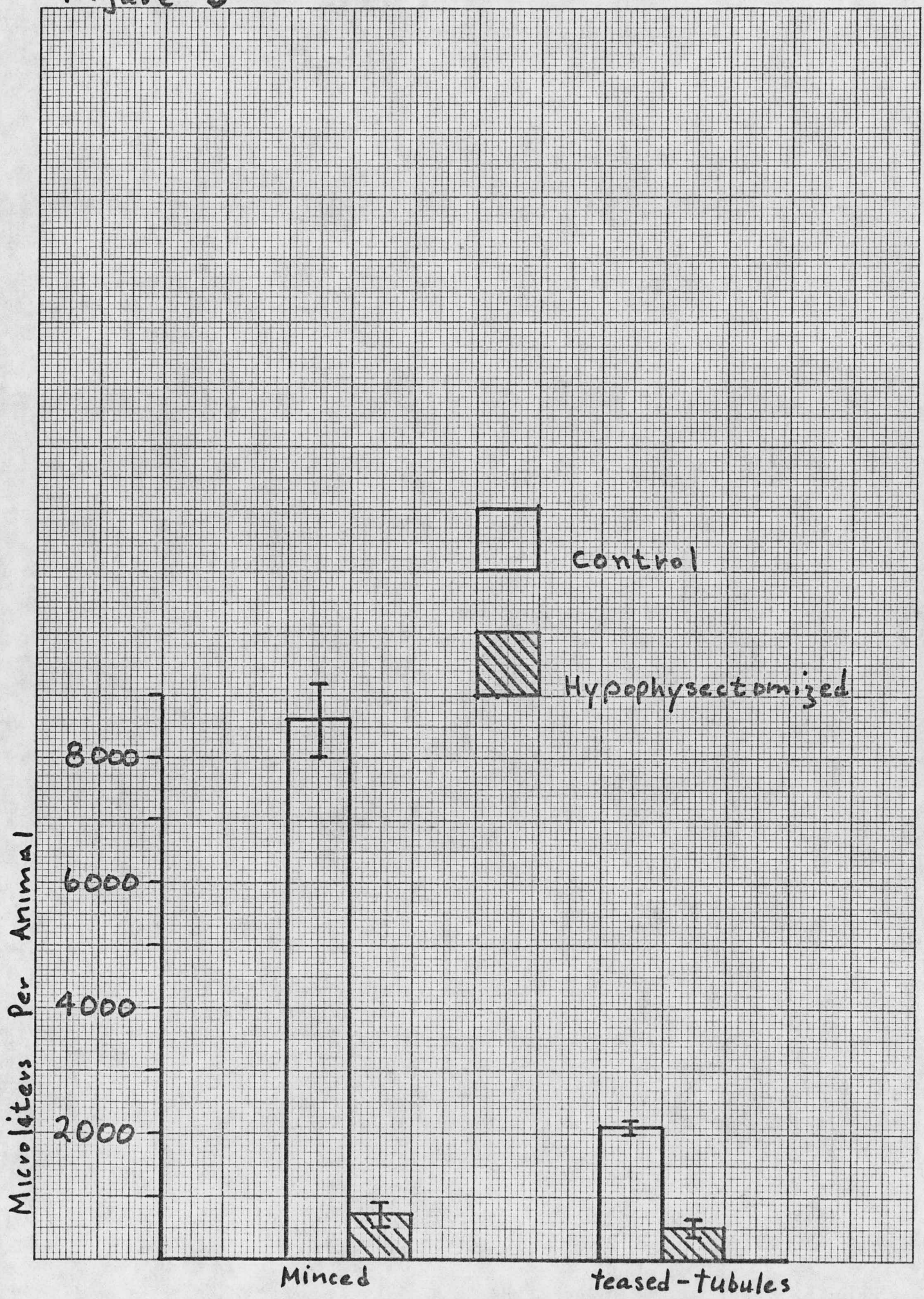

Figure 7