The Nature of the Sex-Linked Differences in Glutathione Peroxidase Activity and Aerobic Oxidation of Glutathione in Male and Female Rat Liver

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(Received 2 June 1969)

1. Glutathione peroxidase activity in the livers of sham-operated female rats was about 60% higher than in similarly treated male rats. The value in the ovariectomized female was about the same as that in the castrated or sham-operated male. 2. Glutathione peroxidase activity changed during the oestrous cycle. The highest value was in oestrus, and was about 50% higher than the lowest activity, which was found in dioestrus. The activity in proestrus and in metoestrus was respectively about 20 and 30% higher than in dioestrus. 3. In the pregnant female 1 or 2 days before term, glutathione peroxidase activity was about 20% higher than that in the female in oestrus. 4. Subcutaneous implants of both oestradiol and progesterone in the gonadectomized rats increased the glutathione peroxidase activity approximately to the values found in the female at oestrus. 5. The rate of aerobic oxidation of GSH in the female rat liver was about 80% higher than in the male and about 110% higher than in the gonadectomized rats. Treatment of gonadectomized rats with subcutaneous implants of oestradiol and of progesterone increased the rate of oxidation of GSH by about 100%. 6. In the presence of azide the rate of GSH oxidation in the male and in the female was respectively about 3.5- and 2.1-fold that in the absence of azide. In castrated or ovariectomized rats the increase due to the presence of azide was about 2.4-fold. In the gonadectomized rats treated with oestradiol or progesterone the rate of GSH oxidation in the presence of azide was about 2.2-fold that in its absence. 7. The rate of lipid peroxidation in female was 15-30-fold that in male or in gonadectomized rats. Treatment of the gonadectomized rats with oestradiol or with progesterone increased the rate of lipid peroxidation up to values that were even higher than in the female. In the presence of GSH the formation of malonaldehyde from peroxides was virtually eliminated. 8. The results suggest that the sex-linked differences in glutathione peroxidase activity, in the rate of GSH oxidation and in the rate of lipid peroxidation are due to the female sex hormones. 9. It is suggested that both the catalase activity and the rate of hydrogen peroxide formation are higher in the male than in the female. 10. Sex-linked changes in glutathione peroxidase, in the rate of GSH oxidation and in the rate of lipid peroxide formation are discussed in relation to the metabolism of oestrogens in the liver and also to the possible nature of those sex-linked changes.

Glutathione peroxidase activity is higher in mature female than in mature male rat liver (Pinto & Bartley, 1968, 1969a) and there is the same difference in the rate of the aerobic oxidation of GSH (Pinto & Bartley, 1969a). The main purpose of the investigation reported in this paper was to study the factors responsible for those sex differences. Therefore the effect in the rat of castration, ovariectomy and oestradiol and progesterone implants on glutathione peroxidase and glutathione

reductase activities in the liver were studied. The effect of these factors on the rate of aerobic GSH oxidation and on the rate of lipid peroxidation was also studied since lipid peroxides are substrates for glutathione peroxidase. Measurements of glutathione peroxidase activity and of glutathione reductase activity in pregnant rats and during the oestrous cycle were also made. The experiments showed that the female hormones are responsible for the sex differences and probably for the high

values of glutathione peroxidase activity in pregnancy. They also show that the changes in female sex hormones associated with the oestrous cycle are probably the cause of the changes in the activities of glutathione peroxidase during the cycle.

A preliminary communication of some of these results has been published (Pinto & Bartley, 1968).

MATERIAL AND METHODS

Materials. The implants of progesterone and of 17- β -oestradiol (oestradiol/cholesterol ratio 1:9) were obtained from Organon, Crown House, Morden, Surrey. All the other reagents were as described in previous work (Pinto & Bartley, 1969a,b).

Animals, operations and subcutaneous implants. All the animals used were Wistar rats from the Sheffield University animal house. Rats used for the series of experiments in Table 1 were 28–30 days old and in the experiments shown in Table 2 rats were 60–70 days old when operated on. In both cases rats were killed 4–7 weeks after the operations were performed. Oestradiol implants (8.8 ± 0.5 mg.; mean $\pm$ s.d.) were put under the skin in six castrated males and six spayed females. In another six castrated males and six spayed females the implants were of progesterone (9.2 ± 0.5 mg.). Castrated, ovariectomized and sham-operated male and female rats (three of each) were used as controls. Pregnant rats were about 5 months old and were killed 1 or 2 days before delivery.

Vaginal smears. Smears were taken daily at about 10 a.m. for at least 2 weeks before the female rats (4–5 months old) were used for experiments. The activities of glutathione peroxidase and of glutathione reductase were measured only in the livers of animals that showed cyclic behaviour. The stage of the oestrous cycle was based on smears taken after killing the animals that were to be used in the experiments.

Perfusion of the liver, preparation of homogenates and incubations. These were carried out as described by Pinto & Bartley (1969a) except for the preparation of the homogenate for measuring the rate of aerobic oxidation. This was made in 0.155 M-KCl since it was also used for lipid peroxide determination and sucrose interferes with the thiobarbituric acid test (Christophersen, 1968).

Determination of GSSG and rate of aerobic oxidation of GSH. Measurements of GSSG and of the rate of GSH oxidation were done as previously described by Pinto & Bartley (1969a).

Determination of the rate of lipid peroxide formation. Measurements of lipid peroxides were made by the thiobarbituric acid method as described by Hunter, Gebicki, Hoffsten, Weinstein & Scott (1963). For the determination of the rate of lipid peroxidation the homogenates were incubated for the same time (1 hr.) and the incubation mixture was the same as that used for the determination of the rate of GSSG oxidation. When no GSH was added to the incubation mixture 0.3 ml. of 0.155 M-KCl replaced the 32.5 mM-GSH. The rate of lipid peroxidation was expressed in nmoles of malonaldehyde formed/hr./g. of fresh liver. The molar extinction coefficient of the malonaldehyde used for the calculations was 1.54×10^5 at 532 nm.

RESULTS

Influence of castration and of ovariectomy on glutathione peroxidase and glutathione reductase activities in rat liver (Tables 1 and 2). Table 1 shows that there was no significant difference between glutathione peroxidase activity in the liver of the male rat castrated before 30 days old and killed when they were 60–90 days old and that of the

Table 1. *Influence of castration and ovariectomy on the GSH peroxidase activity*

Rats were operated on when they were 28–30 days old and killed 4–7 weeks later. Mean values are given $\pm$ s.d. For further details see the Materials and Methods Section.

	GSH peroxidase activity (μ moles of GSSG formed/ min./g. of fresh liver)	No. of expts.
Sham-operated male	50 ± 4	4
Castrated male	54 ± 5	5
Sham-operated female	75 ± 7	5
Spayed female	56 ± 5	5

Table 2. *Effect of castration, ovariectomy and oestradiol and progesterone subcutaneous implants on glutathione peroxidase activity*

Rats were operated on when they were 60–70 days old and killed 4–7 weeks later. Mean values are given $\pm$ s.d. For further details see the Materials and Methods Section.

Sex	Implants	GSH peroxidase activity (μ moles of GSSG formed/ min./g. of fresh liver)	No. of expts.
Sham-operated male	Nil	53 ± 2	3
Sham-operated female	Nil	88 ± 8	3
Castrated male	Nil	55 ± 2	3
	Oestradiol	85 ± 10	4
	Progesterone	88 ± 5	4
Spayed female	Nil	60 ± 5	3
	Oestradiol	93 ± 9	4
	Progesterone	91 ± 8	4

sham-operated male. The value for the sham-operated female ($75 \pm 7 \mu\text{moles}$ of GSSG formed/min./g. of fresh liver; mean $\pm$ S.D.) was about 35% higher than that of the spayed female. Table 2 shows that the value for the liver of the male castrated at about 70 days old was practically the same as that of the sham-operated male. The activity of the sham-operated female ($88 \pm 8 \mu\text{moles}$ of GSSG formed/min./g. of fresh liver) was about 50% higher than that of the spayed female and about 65% higher than the value for the male liver. In general these experiments show that only the female gonad affects the enzyme activity and that the increased value of glutathione peroxidase in female liver in relation to the male and spayed female appears to be almost entirely of gonadal origin since the value for the ovariectomized female was only about 10% higher than that for the male.

There was no significant difference in glutathione reductase activity between castrated male, ovariectomized female and the sham-operated rats.

Changes in glutathione peroxidase activity related to the oestrous cycle in rat liver (Table 3). Glutathione peroxidase activity was minimal in dioestrus ($66 \pm 6 \mu\text{moles}$ of GSSG formed/min./g. of fresh liver; mean $\pm$ S.D.) and maximal in oestrus, the activity being about 50% higher in oestrus than in dioestrus. Intermediate values were found in proestrus and in metoestrus that were respectively about 20% and 30% higher than glutathione peroxidase activity in dioestrus.

Glutathione reductase activity during oestrous cycle (Table 3). The activity was maximal in dioestrus ($4.1 \pm 0.4 \mu\text{moles}$ of GSSG formed/min./g. of fresh liver; mean $\pm$ S.D.), about 15% higher than in oestrus. Changes in glutathione reductase activity were small and not significant with the possible exception of the difference between dioestrus and oestrus ($P = 0.03$).

Changes in the glutathione peroxidase/glutathione reductase activity ratio in relation to the oestrous cycle (Table 3). This ratio in oestrus (11.5 ± 0.7 ; mean $\pm$ S.D.) was about 75% higher than in dioestrus

and about 45% higher than the ratio in proestrus and metoestrus. The pattern of the ratio is like that of glutathione peroxidase during the oestrous cycle, but the differences are sharper.

Glutathione peroxidase, glutathione reductase and glutathione peroxidase/glutathione reductase activity ratio in the liver of the pregnant female rat. Glutathione peroxidase activity (Table 3) in the liver of the pregnant female ($116 \pm 10 \mu\text{moles}$ of GSSG formed/min./g. of fresh liver; mean $\pm$ S.D.) was about 40% higher than the mean value for the liver of the non-pregnant female (Pinto & Bartley, 1969a) and about 20% higher than that of the female in oestrus (Table 3).

The activity of glutathione reductase in the liver of pregnant female rats was approximately the same as that in the liver of non-pregnant females. The glutathione peroxidase/glutathione reductase activity ratio was about 45% higher than the mean of non-pregnant females and about 10% higher than the value of the ratio for the female in oestrus.

Influence of oestradiol and progesterone implants in the castrated male and ovariectomized female rat on glutathione peroxidase and on reductase activities in the liver (Table 2). The glutathione peroxidase activity in the liver of the castrated rat that received oestradiol implants and that with progesterone implanted were about the same. They were also approximately the same as the activities of glutathione peroxidase in the spayed female treated with oestradiol and that treated with progesterone. The treatment of the castrated male and ovariectomized female with either of the female sex hormones increased the activity of glutathione peroxidase about 60% and reproduced the values in female rat liver.

In general it appears that glutathione reductase activity was not affected by the implants of oestradiol or of progesterone.

Influence of sex, castration and ovariectomy on the rate of aerobic oxidation of GSH in rat liver (Table 4). The rate of aerobic oxidation of GSH in the female rat liver ($5.2 \pm 0.2 \mu\text{moles}$ of GSSG formed/hr./g. of

Table 3. *Changes in glutathione peroxidase, glutathione reductase and their activity ratio in pregnancy and during the oestrous cycle in rat liver*

Rats were 4–5 months old when killed. Mean values are given $\pm$ S.D. For further details see the Materials and Methods section.

State of the rat	GSH peroxidase activity (μmoles of GSSG formed/ min./g. of fresh liver)	GSSG reductase activity (μmoles of GSSG reduced/ min./g. of fresh liver)	GSH peroxidase/ GSH reductase activity ratio	No. of expts.
Pregnant	116 ± 10	3.6 ± 0.3	5.0 ± 0.4	5
Dioestrus	66 ± 6	4.1 ± 0.4	2.6 ± 0.2	12
Proestrus	80 ± 6	3.4 ± 0.3	3.8 ± 0.2	6
Oestrus	98 ± 7	3.4 ± 0.3	4.6 ± 0.3	8
Metoestrus	87 ± 6	3.6 ± 0.3	3.8 ± 0.3	9

Table 4. *Influence of sex, castration, ovariectomy and oestradiol and progesterone implants on the rate of GSH oxidation in the absence and in the presence of azide*Mean values are given $\pm$ s.d. Other details are given in Table 2.

Sex	Implant	GSH aerobic oxidation (μ moles of GSSG formed/ hr./g. of fresh liver)	Aerobic GSH oxidation in the presence of 10 mM-azide (μ moles of GSSG formed/hr./g. of fresh liver)	No. of expts.
Male	Nil	2.9 ± 0.3	10.2 ± 0.4	4
Female	Nil	5.2 ± 0.2	11.1 ± 0.5	4
Castrated male	Nil	2.5 ± 0.3	5.8 ± 0.3	2
	Oestradiol	5.0 ± 0.2	10.3 ± 0.2	2
	Progesterone	4.8	10.0	1
Spayed female	Nil	2.4 ± 0.2	6.2 ± 0.2	3
	Oestradiol	4.6 ± 0.2	10.9 ± 0.3	2
	Progesterone	4.9 ± 0.1	10.8 ± 0.2	2

fresh liver) was about 80% higher than in the male liver. The rate in the liver of the castrated male was about the same as the rate in the liver of the spayed female. Both values were about 55% lower than that of female and about 15% lower than in male liver. The higher rate of GSH oxidation in untreated males than in the rats without gonads suggests an effect of the male sex hormones in enhancing GSH oxidation in male liver.

Effect of oestradiol and progesterone implants in castrated and spayed rats on the rate of GSH oxidation in liver. Table 4 shows that the rate of GSH oxidation in the liver of castrated male and ovariectomized female rats increased by about 100% when treated with oestradiol or with progesterone, and reproduced the rate of GSH oxidation in female liver.

Effect of azide on the rate of oxidation of GSH in rat liver (Table 4). In the presence of azide (an inhibitor of catalase but not of glutathione peroxidase) a higher rate of GSH oxidation was expected to occur due to the disappearance of competition for hydrogen peroxide and lipid hydroperoxides between glutathione peroxidase and catalase. With 10 mM-azide the rate of GSH oxidation was increased 3.5-fold in the male rat liver and 2.2-fold in the female rat liver. The rate of oxidation in the female ($11.1 \pm 0.5 \mu$ moles of GSSG formed/hr./g. of fresh liver) was about 10% higher than in the male. Thus when azide was present the sex-linked difference in the rate of GSH oxidation was much lower than in its absence (10% compared with 80%) and much lower than expected from the difference in peroxidase activity between male and female. When azide was present GSH oxidation in the castrated male ($5.8 \pm 0.3 \mu$ moles of GSSG formed/hr./g. of fresh liver) was about 2.3-fold higher than in the absence of azide. In the presence of azide, GSH oxidation in liver from rats treated with oestradiol and progesterone was about 2.1-fold that in the absence of azide. In the

presence of azide the rate of GSH oxidation in the liver of the spayed female was about 2.6-fold that when no azide was present. In the presence of azide, GSH oxidation in the liver of the spayed rat treated with oestradiol and treated with progesterone was respectively 2.4- and 2.2-fold that in the absence of azide. Thus in the male rat the presence of azide increased the rate of GSH oxidation more than in the castrated rat, in the female or in the castrated rat treated with female sex hormones.

Sex-linked difference in the rate of formation of lipid peroxide in rat liver: effect of castration and ovariectomy on the rate of lipid peroxidation (Table 5). In the female rat liver the rate of lipid peroxidation was about 20-fold that in the male. The rate of formation of lipid peroxides in the liver of the castrated male was of the same order as that in the normal male. The value for the spayed female was about 7% of that in the liver of the normal female. These experiments show a very striking sex difference due to the female gonads.

Effect of oestradiol and progesterone on the rate of lipid peroxidation in the liver of the castrated and of the spayed rats (Table 5). Treatment of the castrated or spayed rats with oestradiol or progesterone increased the rate of lipid peroxidation in the liver 25–100-fold. In general these experiments show that treatment with oestradiol or progesterone of the rats with no gonads increases the rate of lipid peroxidation to the value in the female or even more.

Effect of GSH on the rate of malonaldehyde formation from lipid peroxides in rat liver (Table 5). In the presence of 2.5 mM-GSH the rate of malonaldehyde formed in the male and in the castrated male was about 25% of that in the absence of GSH. In the spayed female the value in the presence of GSH decreased by about 85% as compared with that when GSH was absent, and in the female was less than 4% of the value when no GSH was present.

In the castrated male treated with oestradiol or progesterone the effect of 2.5 mM-GSH on malon-

Table 5. *Influence of sex, castration, ovariectomy and oestradiol and progesterone implants on the rate of lipid peroxide formation in the absence and in the presence of GSH*Mean values are given $\pm$ S.D. Other details are given in Table 2.

Sex	Implants	Lipid peroxide formation (nmoles of malonaldehyde formed/hr./g. of fresh liver)	No. of expts.	Lipid peroxide formation in the presence of 2.4 mM-GSH (nmoles of malonaldehyde formed/hr./g. of fresh liver)	No. of expts.
Male	Nil	4.9 $\pm$ 1.8	5	1.2 $\pm$ 0.7	3
Female	Nil	95 $\pm$ 23	4	3.3 $\pm$ 0.8	2
Castrated male	Nil	3.2 $\pm$ 0.2	2	0.8 $\pm$ 0.1	2
	Oestradiol	240 $\pm$ 15	2	2.1	1
	Progesterone	326 $\pm$ 46	2	2.2	1
Spayed female	Nil	6.5 $\pm$ 1.9	3	1.0	1
	Oestradiol	195 $\pm$ 45	2	2.0	1
	Progesterone	160	1	1.3	1

aldehyde formation was to decrease the amount formed to less than 1% of that found in the absence of added GSH. A similar effect was found in the spayed female treated with oestradiol. In general the presence of 2.5 mM-GSH almost completely suppresses the accumulation of malonaldehyde.

DISCUSSION

Nature of the effect of female sex hormones. The experiments with oestradiol and progesterone implants show that the female hormones are responsible for the differences in the glutathione peroxidase activity of the liver and the rates at which rat liver oxidizes GSH or peroxidizes lipids. Changes in production of oestrogens and progesterone occur during the oestrous cycle (Turner, 1966). Also, production of oestrogens and progesterone is increased in pregnancy and the concentration is highest near term (Eik-Nes & Hall, 1965). Therefore it is probable that changes in glutathione peroxidase activities during the oestrous cycle and in pregnancy are associated with oestradiol and progesterone changes. The effects of the two hormones might be mediated through a common factor possibly involving the adrenals. Oestradiol appears to have a larger effect than progesterone since the oestradiol implants contained only about one-tenth the amount of hormone as the progesterone implants. Also, the weights of the pellets that were taken after the animals were killed showed that only 10% of the oestradiol had been absorbed compared with 90% of the progesterone. A direct effect of oestradiol and progesterone on glutathione peroxidase does not appear to be probable since these hormones (10 μ M) did not increase the enzyme activity in experiments *in vitro* (R. E. Pinto & W. Bartley, unpublished work). Thus sex-linked differences and changes in glutathione peroxidase activities re-

ported in this paper may be due to changes in the enzyme population.

Sex-linked difference of the rate of lipid peroxide in female and male rat liver. The thiobarbituric acid method used as a measure of lipid peroxidation appears to measure malonaldehyde produced as a degradation product of lipid peroxides (Dahle, Hill & Holman, 1962; Niehaus & Samuelsson, 1968), which are formed from unsaturated fatty acids with three, or more, double bonds (Dahle *et al.* 1962). Therefore differences in tissue lipid composition will influence the determination of lipid peroxidation. Thus the 20-fold higher rate of lipid peroxidation found in the female liver than in the male liver was partly due to the higher content of unsaturated lipids (Pudelkewicz, Seufert & Holman, 1968; Aftergood & Alfin-Slater, 1965; Lyman, Hopkins, Sheehan & Tinoco, 1968), but, as the mathematical analysis given in the Appendix shows, there is also a hormonal component.

The suppression of the accumulation of malonaldehyde in the presence of GSH agrees with the findings of Christophersen (1966, 1968) and suggests that peroxides in the presence of GSH (probably also *in vivo*) are metabolized by a route other than malonaldehyde formation, probably the reduction of hydroperoxides by GSH through glutathione peroxidase (see Scheme 1).

Interrelationship of GSH and oestrogen metabolism in rat liver. The metabolism of oestrogens in rat liver involves both protein-bound and water-soluble metabolites (Riegel & Mueller, 1954; Jellinck, Lazier & Copp, 1965; Marks & Hecker, 1968). Rat liver microsomes are able to form oestrogen glucuronides, but in the presence of cell sap, or of GSH, water-soluble oestrogen metabolites are formed that are not glucuronides nor sulphates (Jellinck & Lazier, 1965; Jellinck *et al.* 1965). These metabolites of oestradiol are conjugates of GSH;

Scheme 1. Relationships between GSH, GSH oxidation, lipid peroxidation and oestrogen metabolism in rat liver. ROOH and ROH represent respectively lipid, or fatty acid, unsaturated hydroperoxides and its product of reduction through glutathione peroxidase. (1) is glutathione reductase, (2) is glutathione peroxidase and (3) represents lipid peroxidation. The supply of NADPH is assumed to come from oxidation of substrate by any NADPH-linked dehydrogenase.

specifically, when the oestrogen is oestradiol the water-soluble products are *S*-(2,3,17 β -trihydroxy-1,3,5-oestratrien-1-yl)glutathione and the similar compound in which GSH is linked to 2-hydroxy-oestradiol in position 4 instead of in position 1 (Kuss, 1969). Lipid peroxide formation and the binding of metabolites of oestrogens to proteins appear to be competing processes (Marks & Hecker, 1968). Lipid peroxidation and metabolism of 2-hydroxyoestrone appear to utilize, in part, the same enzymes (Marks & Hecker, 1969). GSH is able to reduce hydroperoxides through glutathione peroxidase (Little & O'Brien, 1968; Christophersen, 1968; Pinto & Bartley, 1969a). Thus GSH, its oxidation, lipid peroxidation and the oestrogen metabolism appear to be related in rat liver. Scheme 1 summarizes these apparent relationships. The rate of formation of water-soluble oestrogen metabolites from hydroxylated oestrone is faster than that from oestrone (Lehmann & Breuer, 1969). Thus hydroxylation of oestrogens, which requires NADPH, might be a rate-limiting step of oestrogen metabolism. GSSG and unsaturated fatty acids

probably compete with oestrogens for NADPH more in the female than in the male because glutathione peroxidase activity and the rates of GSH oxidation and lipid peroxidation are higher in the female than in the male. This might contribute to the lower rate of the metabolism of the oestrogens in the female than in the male (Jellinek & Lucieer, 1965; Lehmann & Breuer, 1969).

Factors controlling the rate of GSH oxidation in rat liver. The rate of aerobic GSH oxidation (Pinto, 1961; Pinto & Bartley, 1969b) is probably dependent on the rates of formation of hydrogen peroxide and of lipid hydroperoxide and on the glutathione peroxidase activity (Pinto & Bartley, 1969a). Since hydrogen peroxide is involved in GSH oxidation (Jocelyn, 1964) catalase is expected to compete with glutathione peroxidase (Mills, 1960) for the hydrogen peroxide. Therefore all these factors may be involved in the sex-linked difference of the rate of GSH oxidation in rat liver. The mean ratio of glutathione peroxidase activity in female to that in the male (about 1.6) and the ratio of the rate of GSH oxidation in female to that in the male (about

1.7) are approximately the same. This suggests that glutathione peroxidase activity could be the determining factor. However, the rate of lipid peroxidation is higher in the female than in the male. This ought to produce a ratio of GSH oxidation in the female to that in the male higher than that of enzyme activity in the female if there are no sex differences in the rate of hydrogen peroxide formation or catalase activity or both. Schultz & Kuiken (1941) reported that catalase activity is higher in male than in female rat liver. This explains the higher increase in the rate of oxidation of GSH in male than female when azide is present. The higher catalase activity in male than in female ought to increase the sex-linked difference in the rate of GSH oxidation. Therefore it must be accepted that the rate of formation of hydrogen peroxide in the male is higher than in the female to explain the approximately equal ratios of enzyme activity and the ratios of the rate of GSH oxidation. This agrees with the findings that activities of some enzymes catalysing the formation of hydrogen peroxide are higher in the male than in the female (Richardson, 1964; Wurtman & Axelrod, 1964).

We thank Dr D. Bellamy for valuable discussions and for providing the facilities for the operations, Mr A. Stevenson for carrying out most of the operations on the rats and Mr G. Loy for making most of the vaginal smears. We also thank Dr S. Ainsworth for advice on mathematical aspects of the paper. R. E. P. thanks the Instituto de Alta Cultura (Portugal) for a fellowship.

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APPENDIX

Calculation of the ratio of the rates of malonaldehyde formation in female and male rat liver due to sex-linked differences in lipid unsaturation

Let us call:

C , number of moles of unsaturated fatty acids/g. of fresh liver;

C' , number of moles of total fatty acids/g. of fresh liver;

X , number of moles of unsaturated fatty acids that do not form malonaldehyde/g. of fresh liver;

Y , number of moles of fatty acids able to form malonaldehyde/g. of fresh liver;

B and A , mean of the number of double bonds of fatty acids that form and do not form malonaldehyde respectively.

The subscripts F and M denote the female and the male respectively. It is obvious that:

$$C_F = X_F + Y_F \quad (1)$$

$$C_M = X_M + Y_M \quad (2)$$

The ratio:

$$R = \frac{\text{rate of malonaldehyde formation in female}}{\text{rate of malonaldehyde formation in male}}$$

is expected to be proportional to Y_F/Y_M and thus:

$$R = R_1 Y_F/Y_M \quad (3)$$

where R_1 is a proportionality constant. The number of double bonds per fatty acid is $(AX + BY)/C'$. Thus:

$$R_2 = \frac{(A_F X_F + B_F Y_F)/C'_F}{(A_M X_M + B_M Y_M)/C'_M}$$

and

$$R_3 = C'_M/C'_F$$

Thus we can write:

$$\frac{A_F X_F + B_F Y_F}{A_M X_M + B_M Y_M} = R_2 R_3 \quad (4)$$

From eqns. (1), (2), (3) and (4) the ratio R was calculated as:

$R =$

$$\frac{R_1 [R_2 R_3 (B_M - A_M) Y_M / C_M + R_2 R_3 A_M - A_F C_F / C_M]}{(B_F - A_F) Y_M / C_M} \quad (5)$$

The experimental values used to calculate R were taken, or calculated, from papers containing data on lipid composition of rat liver. A large error, in some cases up to 40%, has always been assumed when the errors of the data were not given in the papers. From the ratio of the amount of the lipids per gram of liver in male and female rat, $R_3 = 0.8 \pm 0.3$ (Aftergood & Alfin-Slater, 1965),

$$C_F/C_M = 1 \pm 0.3,$$

$$A_F = A_M = 1.5 \pm 0.4,$$

$$Y_M/C_M = 0.25 \pm 0.1$$

(Getz & Bartley, 1961; Pudalkewicz, Seufert & Holman, 1968) and $R_2 = 1.6 \pm 0.3$ (Pudalkewicz *et al.* 1968). Also, $B_M = 4.2 \pm 0.5$ and $B_F = 5.0 \pm 0.5$

(Pudalkewicz *et al.* 1968). From Dahle, Hill & Holman (1962) it may be calculated that

$$R_1 = (B_F - 2)/(B_M - 2)$$

and therefore

$$R_1 = 1.4 \pm 0.5.$$

R was calculated by substitution of these values on eqn. (5). The standard deviation of R was calculated (see Margenau & Murphy, 1956) by using the errors of the above values as standard deviations. The calculated value of R was 2.2 ± 4.0 and the value of the ratio of the lipid malonaldehyde formation in female and male rat liver calculated from Table 5 of the main paper was 19.5 ± 8.5 . Thus the sex-linked difference in the lipid unsaturation can be only partly responsible for the sex-linked difference in the rate of lipid peroxidation.

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