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POLYAMINES AND GLUTATHIONE IN LIVERS OF NORMAL RATS OF DIFFERENT AGES AND IN LIVERS OF PREGNANT RATS

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RECENTLY we studied the spermine (SP), spermidine (SPD) and glutathione (GSH) content of normal adult albino rat liver and noted the effect of growth of transplantable Rd/3 sarcoma on the levels of these substances in the liver.[1] We have now determined the amounts of SP, SPD and GSH in livers of normal male and female albino rats of two younger age groups and have confirmed that variations in polyamine levels with increasing age are similar to those reported by Jänne *et al.*[2] We found that the GSH content of rat liver increased markedly (by about 100 per cent in the case of male rats) from the 5th to 8th week of age. Since the rate of growth of Rd/3 sarcoma is comparable with that of foetal tissue, we examined the effect of pregnancy on the concentrations of SP, SPD and GSH in rat liver.

EXPERIMENTAL

Age experiments. Two groups of young stock albino rats in the age range 33–38 days (mean 36 days) and 51–59 days (mean 54 days) were used. Since weaning, the rats had been maintained on water and Diet 86 (supplied by Oxoid Ltd., London). Rats were killed by chest bleeding under ether anaesthesia during 10.00–11.00 a.m. on the day of the experiment and the livers were perfused with ice-cold normal saline and stored in solid carbon dioxide.

Pregnancy and tumour transplantation experiments. Adult stock female albino rats maintained on water and Diet 86 were mated with stock male albino rats. At various stages of pregnancy rats were killed as described above and the perfused livers were stored in solid carbon dioxide. Uterus and conceptus were removed *en bloc* and the combined weight expressed as a percentage of rat total body weight (P%). The technique for transplantation of Rd/3 sarcoma has already been described.[1] Polyamine and glutathione content of the frozen livers were determined by modifications[1] of the methods described by Raina[2] and Saville.[3]

RESULTS AND DISCUSSION

Effect of Age. In Table 1 are shown the polyamine and GSH contents of livers of male and female rats of the young age groups together with values for normal adult rats (age greater than 100 days) which were obtained in our earlier study.[1]

Student's *t* distribution values[5] for some of the differences of means of polyamines

Table 1. Effect of age and sex on the glutathione and polyamine content (mean ± S.D.) of rat liver

	Male rats			Female rats		
Age (days)	36	54	>100	36	54	>100
No. of rats	9	11	16	9	10	5
Body w. (g)	111 ± 16	207 ± 17	361 ± 61	102 ± 13	143 ± 13	203 ± 18
Liver % of total body wt.	5·0 ± 0·22	5·0 ± 0·35	3·3 ± 0·17	4·9 ± 0·33	4·8 ± 0·22	3·9 ± 0·21
Liver GSH (mg/100 g)	84 ± 11·9	158 ± 25·8	184 ± 15·6	99 ± 25·1	157 ± 14·2	155 ± 11·4
Liver SP (μg/g)	91 ± 9·5	97 ± 4·9	97 ± 5·3	96 ± 2·7	111 ± 5·8	104 ± 5·5
Liver SPD (μg/g)	111 ± 12·3	77 ± 9·4	50 ± 9·8	97 ± 8·2	82 ± 11·9	58 ± 8·9
Liver SPD/SP	1·227 ± 0·168	0·797 ± 0·125	0·519 ± 0·101	1·003 ± 0·080	0·733 ± 0·097	0·560 ± 0·116

TABLE 2. STUDENT'S *t* DISTRIBUTION FOR DIFFERENCES OF MEANS OF RAT LIVER POLYAMINE AND GLUTATHIONE LEVELS

	Males (36 days) compared with: Males (54 days)	Males (>100 days)	Females (36 days) compared with: Females (54 days)	Females (>100 days)
GSH	7·519 (0·1%)*	6·241 (0·1%)	5·964 (0·1%)	4·347 (1%)
SP	1·740 (<5%)	1·803 (<5%)	6·655 (0·1%)	3·270 (1%)
SPD	6·618 (0·1%)	16·141 (0·1%)	3·029 (1%)	6·595 (0·1%)
SPD/SP	9·776 (0·1%)	12·613 (0·1%)	6·224 (0·1%)	6·602 (0·1%)

* Level of significance.

and GSH in various age groups are recorded in Table 2.

Liver SP content was least in the youngest age group and it increased slightly with increasing age in both sexes. On the other hand, liver SPD content was highest in the youngest group and declined to about half this level in the oldest group. Similar trends were observed by Jänne *et al.*[2] as may be seen from the following data for SP and SPD content of male rat liver taken from their work.

Rat age (Months)	SP	SPD	SPD/SP
	μg/g liver wet wt.		
1	134	188	1·403
3	141	129	0·915
9	138	84	0·609

The amounts of liver polyamines found by Jänne *et al.*[2] are greater than those in our rats of comparable age and further the liver SPD/SP ratio is lower in our strain than in that of the Finnish workers. It is interesting that Caldarera, Cozzani and Moruzzi[6] reported SP and SPD levels of 97 and 84 μg/g fresh liver for male rats weighing 160–200 g. These values are in better agreement with our results than with those of Jänne *et al.*[2] Probably the discrepancies are attributable to strain differences. In our experiments, frozen livers were stored overnight before carrying out polyamine determinations but the results were practically the same as those obtained when fresh liver specimens were processed at once for polyamine determination as was done by Jänne *et al.*[2]

In agreement with the Finnish authors[2] we found the SPD content of rat kidney to be less than half that for rat liver. Mean values ± S.D. for SPD and SP in the kidneys of 4 male rats aged 54 days were 28 ± 3·7 and 89 ± 15·6 μg/g frozen tissue (SPD/SP = 0·315) whereas the corresponding values for the livers of these animals were 63 ± 1·3 and 102 ± 3·1 μg/g frozen tissue respectively (SPD/SP = 0·618). Jänne *et al.*[2] give values of 76 and 177 μg/g (SPD/SP = 0·429) for 1-month-old rat kidney SPD and SP. We found that liver GSH content increased markedly from the 5th to 8th week of life in both sexes. In older male rats there was a further increase in liver GSH. In female rats however the adult level of liver GSH had already been attained at the 8th week of age.

Effect of Pregnancy and presence of Tumour. Table 3 shows the mean values and S.D's for GSH, SPD and SP content of livers of normal and pregnant female rats and of livers of female rats bearing Rd/3 sarcoma. For comparison, data from our previous

Table 3. Mean glutathione, spermidine and spermine content of livers of pregnant rats and Rd/3 sarcoma rats

Sex	Female			Male	
Condition	Normal	Pregnant	Rd/3 Tumour	Normal	Rd/3 Tumour
No. of rats	16	16	14	16	16
Total body w. (g)	223 ± 33	285 ± 37	211 ± 20	361 ± 61	339 ± 32
Liver %	3·8 ± 0·36	3·9 ± 0·24	4·4 ± 0·55	3·3 ± 0·17	3·4 ± 0·30
T% or P%	—	9·1 ± 5·1	10·9 ± 4·8	—	4·5 ± 5·0
Liver GSH (mg/100 g)	168 ± 10·2	151 ± 20·9	118 ± 27·9	184 ± 15·6	165 ± 37·1
Liver SPD (μg/g)	63 ± 23·5	129 ± 34·8	151·5 ± 38·1	50 ± 9·8	104 ± 41·4
Liver SP (μg/g)	113 ± 8·1	112 ± 5·7	119·7 ± 16·6	97 ± 5·3	112 ± 12·2
Liver SPD/SP	0·564 ± 0·104	1·150 ± 0·324	1·259 ± 0·222	0·519 ± 0·101	0·918 ± 0·284

report[1] for normal male rats and for male rats with Rd/3 sarcoma are included in this Table.

Correlation coefficients (r) and Student's *t* values were calculated from the values for liver GSH, SPD and SP at various stages of pregnancy (P%) or in the presence of tumours (T%). These results are given in Table 4.

TABLE 4. CORRELATION COEFFICIENTS (*r*) WITH *t* TEST FOR COMPARISON OF TUMOUR PERCENTAGE (*T*%) AND PREGNANCY (*P*%) WITH CONCENTRATIONS OF GSH, SPD AND SP IN LIVER

Comparing T% or P% with:	Male		Female			
	Rd/3 Tumour		Rd/3 Tumour		Pregnant	
	r	*t*	*r*	*t*	*r*	*t*
GSH	−0·938	10·162	−0·541	2·227	−0·567	2·573
SPD	+0·967	14·201	+0·496	1·981	+0·871	6·647
SP	+0·703	3·705	+0·141	0·493	−0·171	0·650
SPD/SP	+0·921	8·860	+0·578	2·454	+0·861	6·329

In the course of pregnancy, as during the growth of Rd/3 tumour in male or female rats, the concentration of GSH in the liver declined and that of SPD increased. The SP concentration remained practically unchanged in both situations. For tumour-bearing female rats correlation coefficients were in general smaller and less significant than for male rats with Rd/3 tumour. In pregnant animals, the correlation coefficient for liver GSH compared with P% was of the same order as that for liver GSH compared with T% in female rats with Rd/3 tumour. A higher *r* value was found for liver SPD in pregnant rats as compared with tumour-bearing female rats. Thus, qualitatively, changes in liver levels of GSH, SPD and SP invoked by rapidly growing foetal tissues or Rd/3 transplantable sarcoma are similar.

McHenry[7] compared the effect of tumour growth and pregnancy on some biochemical changes in rats and found that the growth of foetal tissue did not alter the host metabolism in the same way as did tumour growth. He suggested that these differences were probably related to loss in carcass weight and protein in the tumour animals and to gain in carcass weight and protein in pregnant animals. Our experiments show that the amounts of GSH, SPD and SP in the liver are apparently not influenced by loss or gain of carcass protein. Zeller[8] found that the livers of pregnant rats contained more diamine oxidase than did normal liver, a factor which may be related to the marked increase in liver SPD which we have noted in pregnancy. No data is available for the occurrence of diamine oxidase in liver of tumour rats.

In conclusion it may be noted that in livers of 2 rats 1 day *post partum*, GSH, SPD and SP levels were 152, 150 mg/100 g, 101, 111 μg/g and 188, 154 μg/g respectively as compared with values of 165 mg/100 g, 108 μg/g and 74 μg/g for the liver of a normal female rat. In the case of a pregnant rat (P = 8·3%) which also had a tumour (T = 19%) liver GSH was 106 mg/100 g and SP and SPD values were 129 and 250 μg/g respectively.

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