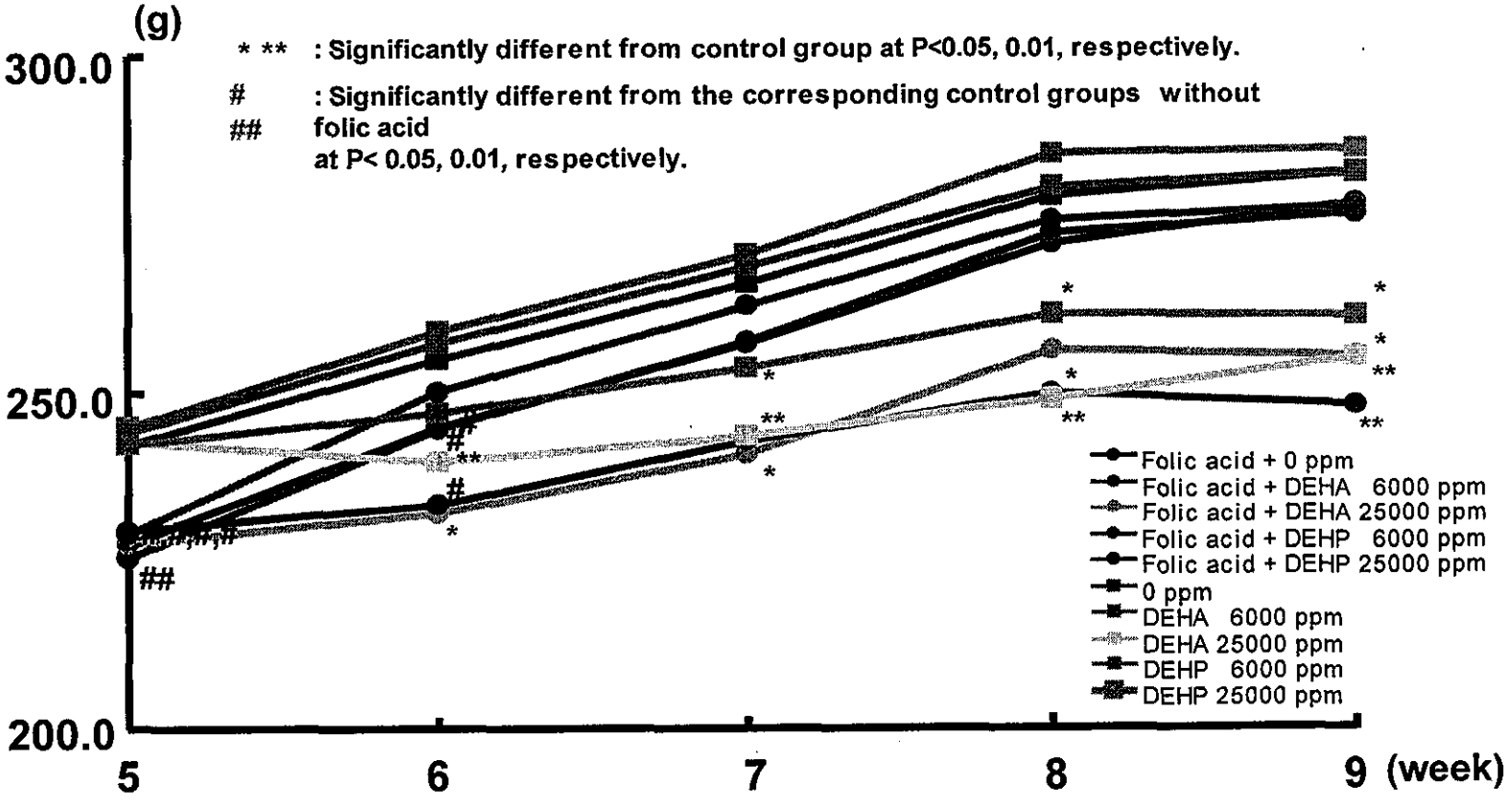
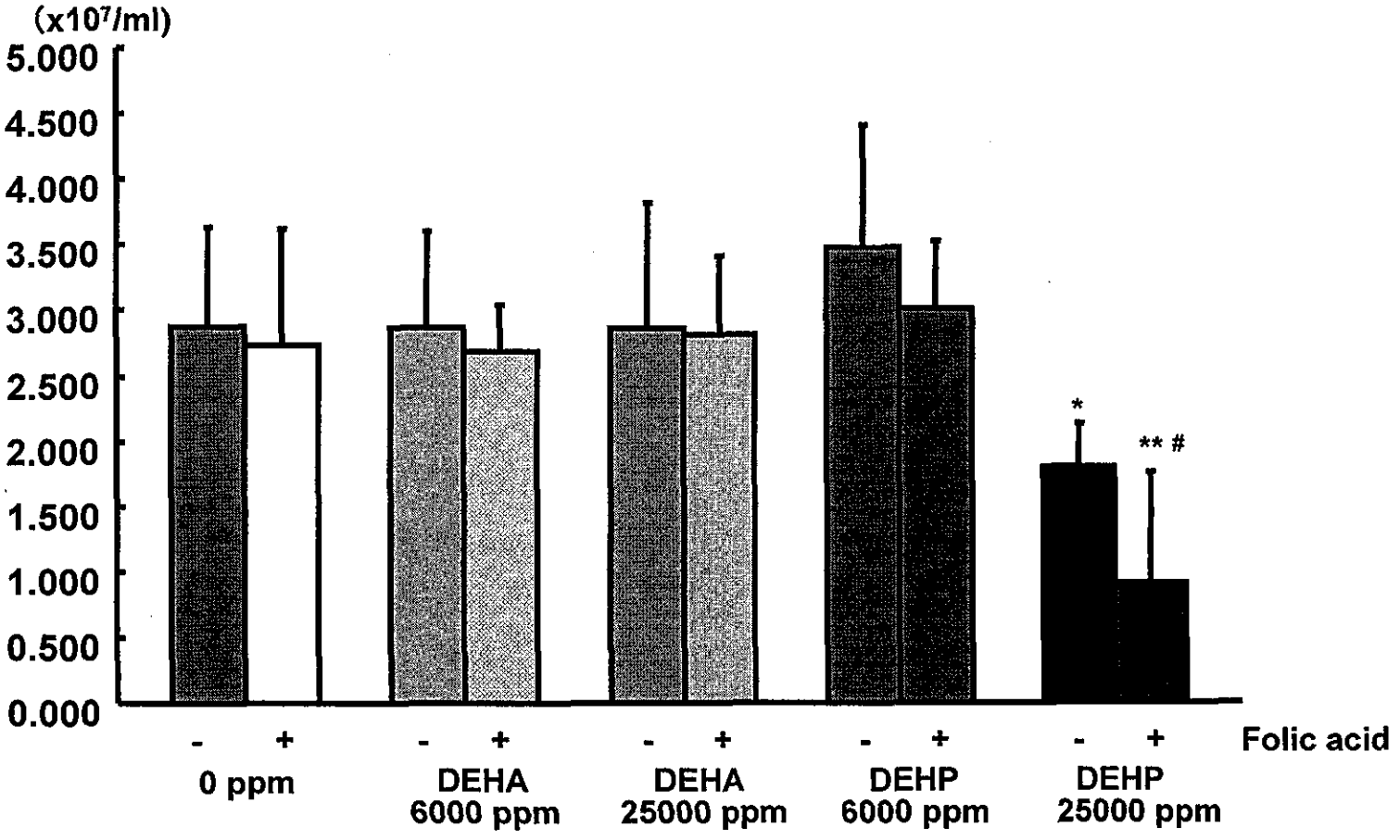
Fig. 2-10


(g)
300.0
250.0
200.0
5
6
7
8
9 (week)
* ** : Significantly different from control group at P<0.05, 0.01, respectively.
# : Significantly different from the corresponding control groups without folic acid
at P< 0.05, 0.01, respectively.
Folic acid + 0 ppm
Folic acid + DEHA 6000 ppm
Folic acid + DEHA 25000 ppm
Folic acid + DEHP 6000 ppm
Folic acid + DEHP 25000 ppm
0 ppm
DEHA 6000 ppm
DEHA 25000 ppm
DEHP 6000 ppm
DEHP 25000 ppm

体重曲線

Fig. 2-11

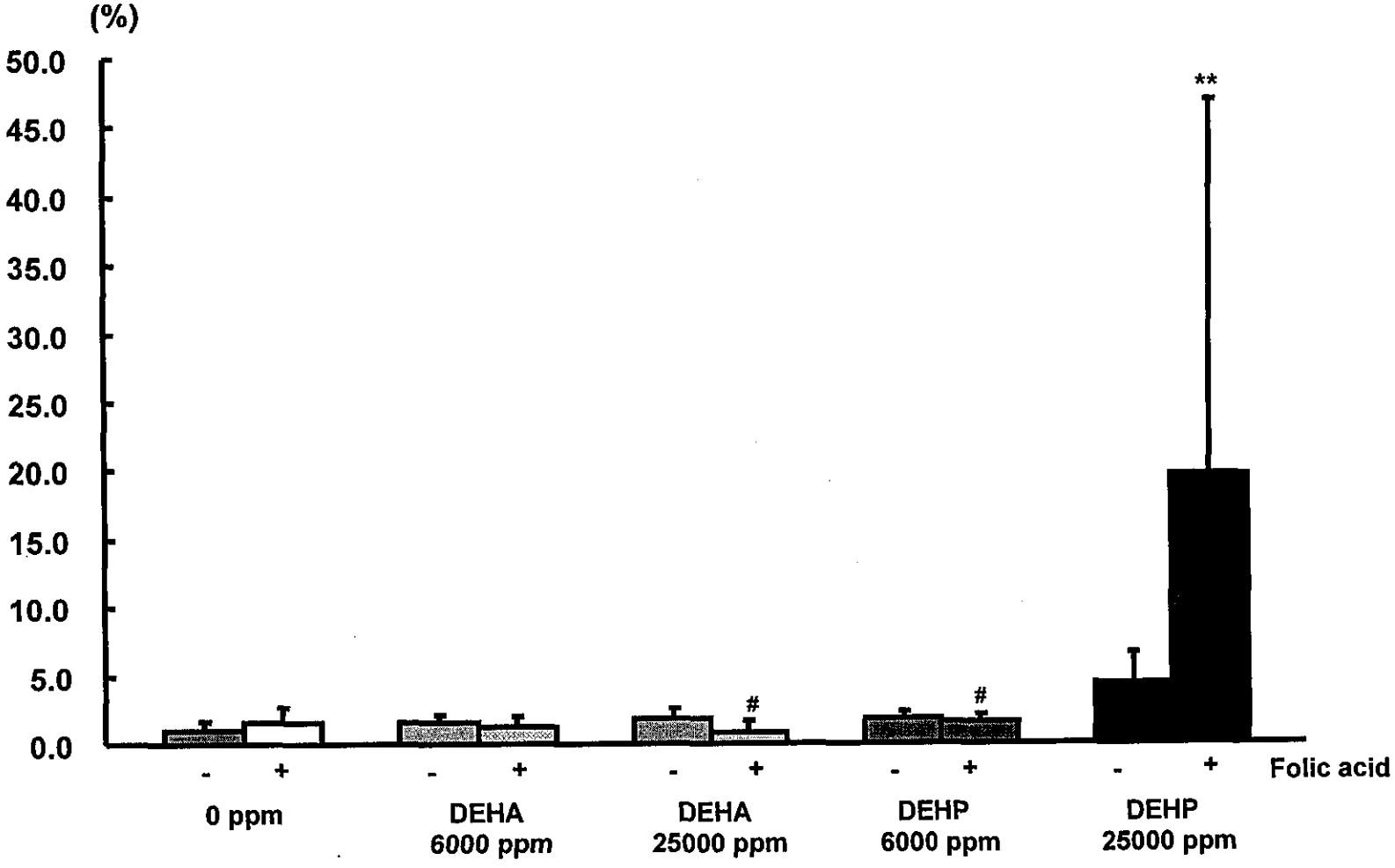
精巣上体内の精子数



* ** : Significantly different from control group at P< 0.05, 0.01, respectively.
' # : Significantly different fromnon-initiation group at P<0.05, respectively.

Fig. 2-12

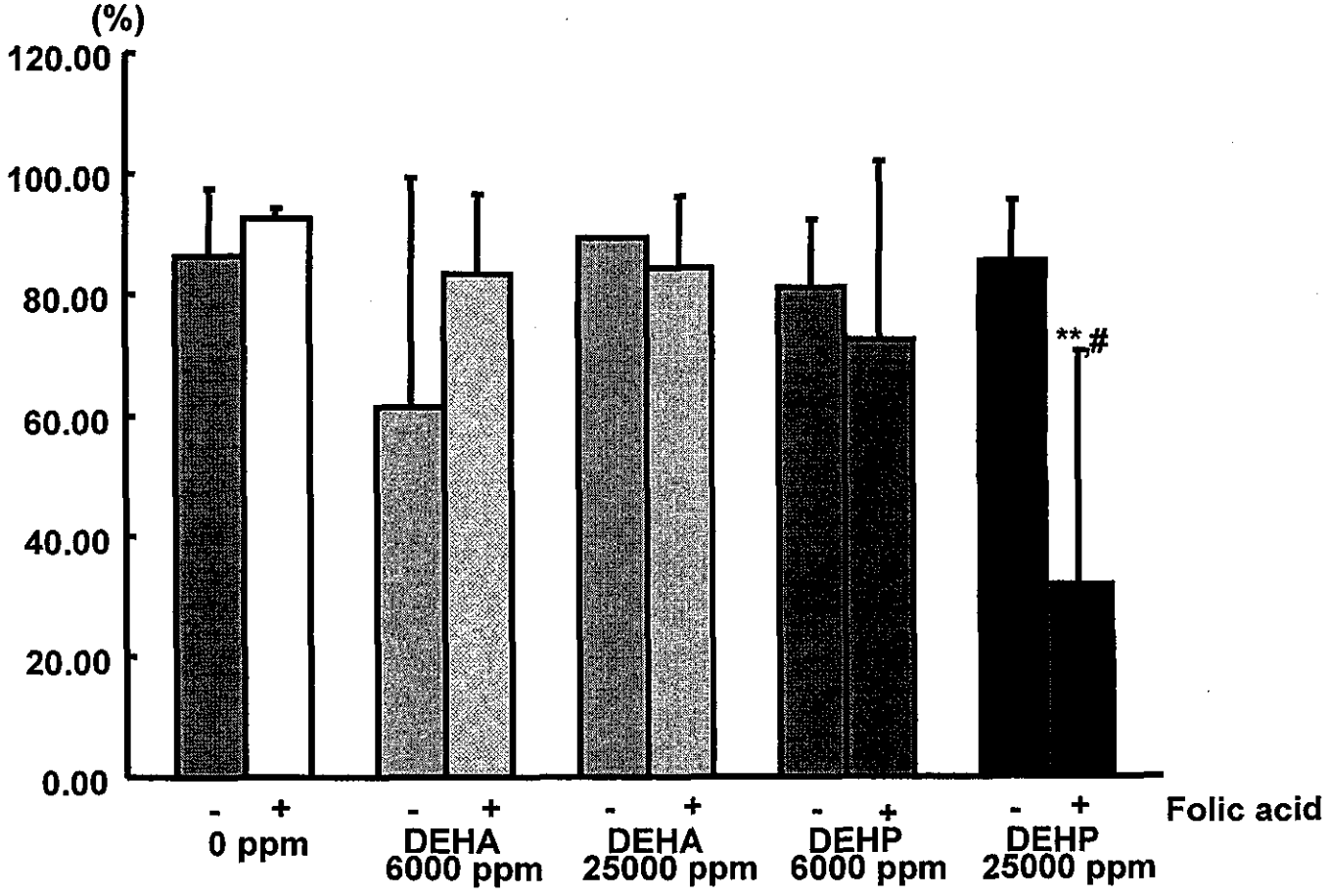
形態異常を示す精子の割合



** : Significantly different from control group at P<0.01, respectively.
# : Significantly different fromnon-initiation group at P<0.05, respectively.

Fig. 2-13

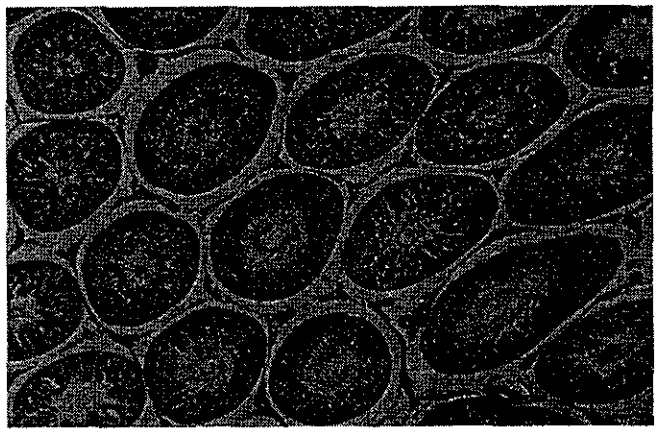
精子運動能(5分間値)



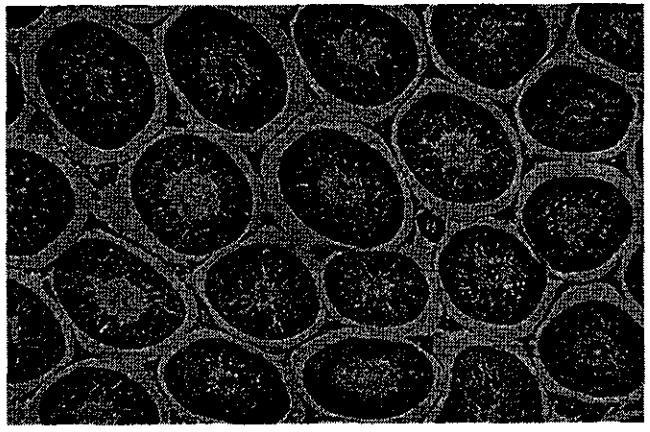
** : Significantly different from control group at P<0.01, respectively.
# : Significantly different from non-initiation group at P<0.05, respectively.

Fig. 2-14

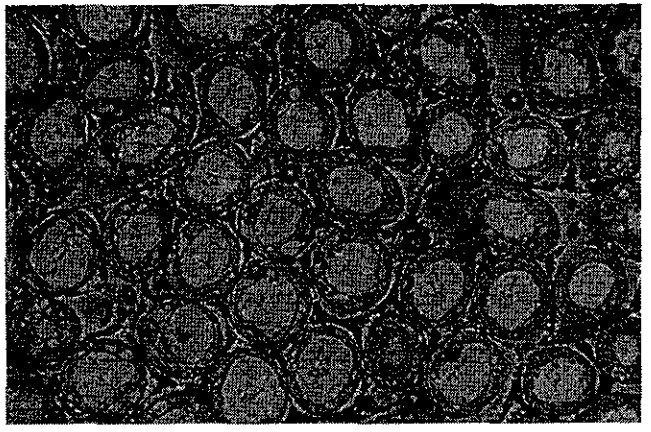
精細管の組織学的変化



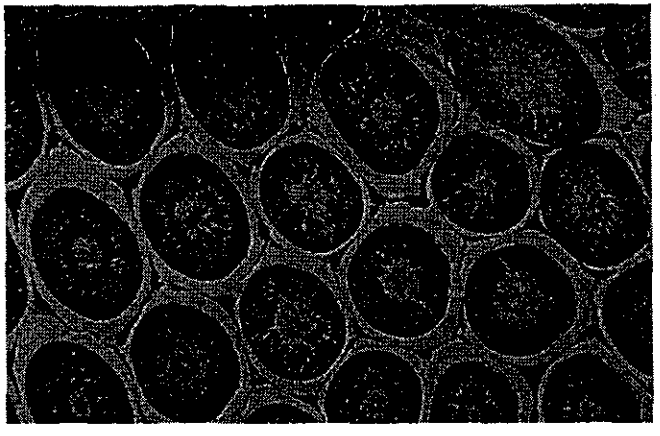
Folic acid + 0 ppm



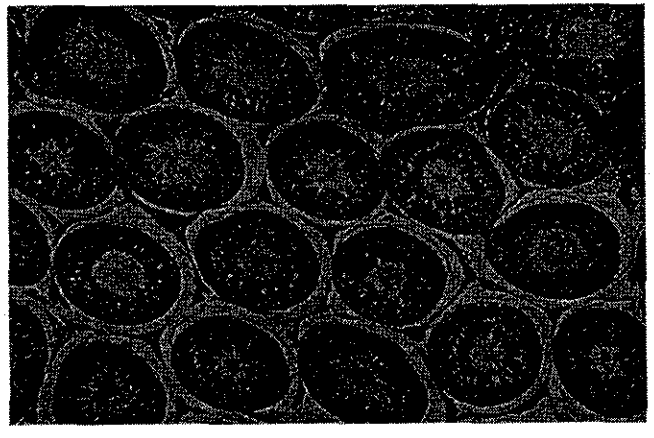
Folic acid + DEHA 25000 ppm



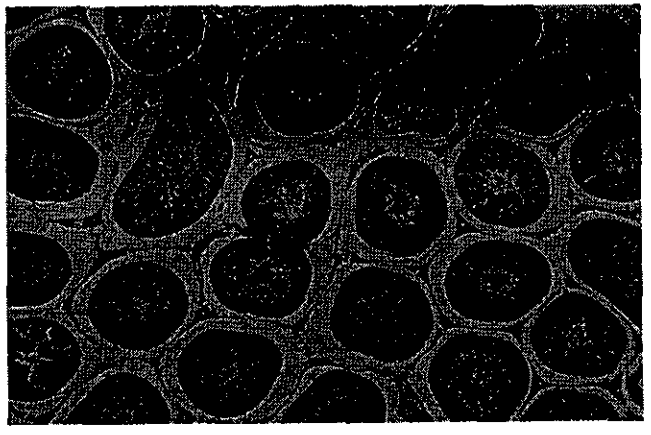
Folic acid + DEHP 25000 ppm



0 ppm

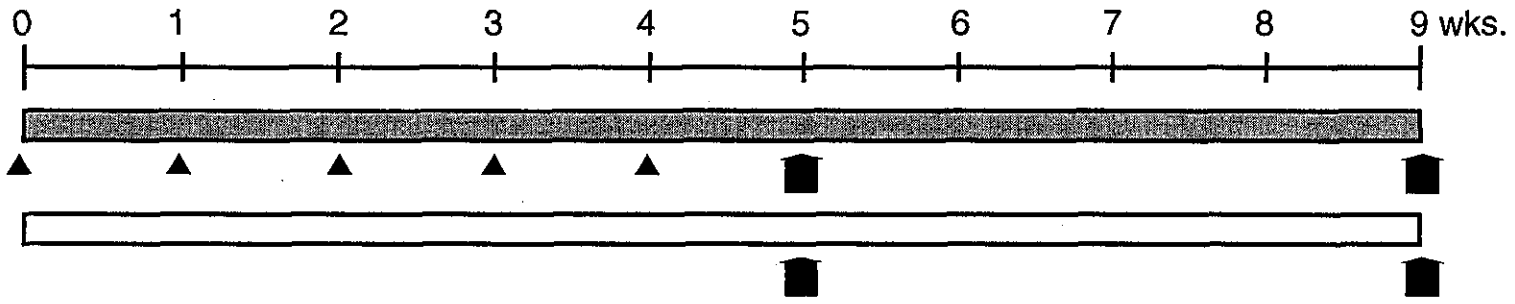


DEHA 25000 ppm



DEHP 25000 ppm

Table 2-1　腎機能低下に伴うDBP精巣毒性増強作用の機構解明



▲ :Folic acid 300 mg/kg s.c. once a week (0.3 M NaHCO3)
▲ :Vehicle (0.3 M NaHCO3)
■ :5週経過時　1000 mg/kg DBP投与→12時間後 採尿・採血 5 rats x 2 groups
■ :9週経過時　1000 mg/kg DBP投与→12時間後 採尿・採血 5 rats x 2 groups

血漿・尿・精巣中のDBP、MBPの濃度[測定施設:名城大学　薬学部衛生化学(小嶋 仲夫教授)]
肝・精巣のβ-glucuronidase活性(Western、m-RNA)

Folic acid	DBP	Exper. week	No. of rats	Final B.W. (g)	Kidneys (%)	Urine specific gravity	Serum BUN (mg/dl)
—	+	5	3	248.0	0.69	1.0487	20.30
—(v)	+	5	6	246.2	0.72	1.0405	21.52
+	+	5	6	236.8	0.79	1.0358	25.80
—(v)	+	9	6	282.2	0.65	1.0487	20.58
+	+	9	6	279.0	0.71	1.0338 *	25.55 *

*: Significantly different from control group (group 4) at $P<0.05$

Table 2-2

Sperm number, movement ability and morphology abnormality in rats treated with DBP

Group	TAA	DBP (mg/kg/day)	Number (?10^4/ml) Epididymis	Movement ability (%) 5 min	Morphology abnormality (%)
1	+	500	45 ± 70 [a,*]	2.9 ± 4.7 [a,*]	23.2 ± 20.9
2	+	125	1083 ± 368 [a]	57.6 ± 30.1	30.0 ± 9.8 [a]
3	+	31.25	1245 ± 373 [a]	75.4 ± 20.3	20.9 ± 10.4 [a]
4	+	0	1452 ± 533 [a]	78.5 ± 23.3	29.1 ± 10.4 [a]
5	-	500	2748 ± 683	82.4 ± 5.8	17.7 ± 7.8 [**]
6	-	125	2067 ± 287 [**]	85.1 ± 9.1	16.4 ± 2.6 [**]
7	-	31.25	3093 ± 713	86.1 ± 6.4	9.3 ± 4.3 [**]
8	-	0	3172 ± 479	74.2 ± 36.6	2.4 ± 1.1

Data are mean ±SD values; ig: intragastric injection.

[a]Significantly different from each corresponding non-TAA treatment groups (group *5 to 8*) at $p<0.01$

[*]Significantly different from group 4 at $p<0.05$

[**]Significantly different from group 8 at $p<0.05$

Table 2-3

Sperm number, movement ability and morphology abnormality in rats treated with DBP

Group	TAA	DBP (ppm)	Number (?10^7/ml) Epididymis	Movement ability (%) 5 min	Morphology abnormality (%)
1	+	20000	1.656 ± 0.189 [a, b]	85.72 ± 5.31 [a]	19.20 ± 5.61 [a, b]
2	+	10000	2.192 ± 0.486	77.32 ± 11.07	10.20 ± 2.40
3	+	2500	2.081 ± 0.119	71.45 ± 30.13	10.22 ± 2.91
4	+	0	2.574 ± 0.622	78.55 ± 7.47	9.80 ± 1.62
5	-	20000	2.588 ± 0.611	71.72 ± 13.42	9.90 ± 2.56
6	-	10000	2.977 ± 0.412	84.58 ± 6.72	9.30 ± 3.13
7	-	2500	3.186 ± 0.597	83.09 ± 6.80	8.50 ± 2.95
8	-	0	3.118 ± 0.567	79.07 ± 16.00	8.80 ± 2.20

Data are mean ±SD values; ig: intragastric injection.

[a]Significantly different from each corresponding non-TAA treatment groups (group 5 to 8) at $p<0.01$

[b]Significantly different from group 4 at $p<0.05$

Table 2-4

Sperm number, movement ability and morphology abnormality in rats treated with DEHP or DEHA

Group	TAA	Chemical and dose (ppm)	Number (?10^7/ml) Epididymis	Movement ability (%) 5 min	Morphology abnormality (%)
1	+	DEHP 25000	0.465 ± 0.574 [a, b]	25.83 ± 40.23 [a, b]	32.83 ± 35.74 [a, b]
2	+	DEHP 6000	2.608 ± 0.457	84.85 ± 5.14	2.83 ± 2.52
3	+	DEHA 25000	2.434 ± 0.358	88.74 ± 8.67	3.00 ± 1.46
4	+	DEHA 6000	2.355 ± 0.312	93.23 ± 1.97	2.17 ± 2.25
5	+	0	2.236 ± 0.717	84.82 ± 8.37	3.75 ± 2.73
6	-	DEHP 25000	1.773 ± 0.929	77.52 ± 14.30	4.17 ± 2.91
7	-	DEHP 6000	2.999 ± 0.414	91.55 ± 5.21	2.92 ± 1.66
8	-	DEHA 25000	2.792 ± 0.752	83.04 ± 17.52	2.50 ± 1.55
9	-	DEHA 6000	2.811 ± 0.800	82.92 ± 13.07	2.00 ± 1.14
10	-	0	2.769 ± 0.584	87.93 ± 5.35	2.00 ± 1.34

Data represent mean±SD

[a]Significantly different from group 5 at $p<0.01$

[b]Significantly different from group 6 at $p<0.01$

Table 2-5

最終体重および肝、腎の相対重量

Group No.	Folic acid	DBP (ppm)	Final B.W. (g)	Liver	Kidneys
1	—	0	275.6 ± 11.8	2.5 ± 0.1	0.65 ± 0.04
2	—	1200	274.0 ± 5.0	2.5 ± 0.1	0.65 ± 0.01
3	—	5000	271.6 ± 10.7	2.8 ± 0.1*	0.68 ± 0.01
4	—	20000	256.6 ± 10.5 *	3.7 ± 0.7 *	0.72 ± 0.02**
5	+	0	263.4 ± 12.5	2.5 ± 0.1	0.71 ± 0.02##
6	+	1200	272.6 ± 6.9	2.5 ± 0.1	0.70 ± 0.02##
7	+	5000	265.4 ± 17.8	2.9 ± 0.1 **	0.75 ± 0.03*, ##
8	+	20000	245.2 ± 12.2*	4.0 ± 0.2**	0.78 ± 0.03**, ##

*,** : Significantly different from control group at P<0.05, 0.01, respectively.

: Significantly different from the corresponding control groups without folic acid at P<0.05, 0.01, respectively.

No. of rat in each group is 5.

Table 2-6

尿の性状

No. of rat in each group is 5.

Group No.	Folic acid	DBP (ppm)	Urine volume (In grams)	pH	Specific gravity	Osmolality (oSm/kg)
1	—	0	3.36 ± 1.13	6.90 ± 0.22	1.0716 ± 0.0059	2.3382 ± 0.1821
2	—	1200	2.94 ± 0.62	7.40 ± 0.42*	1.0704 ± 0.0037	2.2650 ± 0.1325
3	—	5000	2.12 ± 0.49	7.40 ± 0.65	1.0690 ± 0.0060	2.2392 ± 0.1789
4	—	20000	1.92 ± 0.41*	6.40 ± 0.42*	0.0826 ± 0.0019**	2.3892 ± 0.0258
5	+	0	3.29 ± 0.69	7.17 ± 0.39	1.0554 ± 0.0067##	1.8426 ± 0.2469##
6	+	1200	3.34 ± 0.31	7.50 ± 0.30	1.0444 ± 0.0087##	1.4720 ± 0.3048##
7	+	5000	3.64 ± 0.43	7.01 ± 0.32	1.0508 ± 0.0076##	1.6634 ± 0.2573##
8	+	20000	2.90 ± 0.60	6.96 ± 0.35#	1.0644 ± 0.0083##	1.8252 ± 0.2187##

*, **: Significantly different from control group at $P<0.05$, 0.01, respectively

#, ##; $P<0.05$, 0.01 from the corresponding controls without folic acid

Table 2-7 **血中の尿素窒素および血中と尿中のクレアチニン値**

Group No.	Folic acid	DBP Level (ppm)	Serum		Urine
			BUN (mg/dl)	Creatinine (mg/dl)	Creatinine (mg/dl)
1	—	0	21.90 ± 2.29	0.50 ± 0.07	144.08 ± 22.73
2	—	1200	23.06 ± 1.25	0.50 ± 0.00	165.36 ± 14.25
3	—	5000	22.06 ± 1.69	0.52 ± 0.04	150.80 ± 18.88
4	—	20000	21.32 ± 1.19	0.54 ± 0.05	125.68 ± 21.47
5	+	0	29.94 ± 2.61##	0.58 ± 0.04	116.80 ± 23.59
6	+	1200	28.16 ± 4.30	0.56 ± 0.05	98.08 ± 18.83 ##
7	+	5000	28.80 ± 2.43##	0.52 ± 0.04*	100.48 ± 18.11 ##
8	+	20000	26.58 ± 3.13##	0.56 ± 0.05	101.84 ± 20.81

* : Significantly different from control group at P<0.05.

: Significantly different from control group (group 1 vs 5) at P<0.01.

: Significantly different from control group (group 2 vs 6) at P<0.01.

: Significantly different from control group (group 3 vs 7) at P<0.01.

: Significantly different from control group (group 4 vs 8) at P<0.01.

No. of rat in each group is 5.

Table 2-8

精巣と精巣上体の相対重量

Group No.	Folic acid	DBP (ppm)	Testes	Epididymides
1	—	0	1.09 ± 0.08	0.83 ± 0.05
2	—	1200	1.11 ± 0.02	0.85 ± 0.03
3	—	5000	1.12 ± 0.06	0.84 ± 0.03
4	—	20000	0.99 ± 0.11	0.70 ± 0.08 *
5	+	0	1.10 ± 0.12	0.83 ± 0.09
6	+	1200	1.09 ± 0.04	0.83 ± 0.01
7	+	5000	1.14 ± 0.07	0.83 ± 0.03
8	+	20000	0.62 ± 0.27 **, ##	0.54 ± 0.13**, #

*, ** : Significantly different from control group at P<0.05, 0.01, respectively.

: Significantly different from the corresponding control groups without folic acid at P<0.05, 0.01, respectively.

No. of rat in each group is 5.

Table 2-9 **精子数、精子運動能および精子形態異常** No. of rat in each group is 5.

Group No.	Folic acid	DBP (ppm)	Number (x 10^7 / ml) Epididymis	Movement ability (%) 5 minutes	Morphology Abnormality (%)
1	—	0	2.662 ± 0.596	86.58 ± 6.89	0.80 ± 0.84
2	—	1200	2.744 ± 0.517	89.02 ± 5.68	0.40 ± 0.55
3	—	5000	3.022 ± 0.474	81.62 ± 9.46	0.60 ± 0.55
4	—	20000	2.440 ± 1.138	66.06 ± 18.48*	1.60 ± 2.61
5	+	0	2.390 ± 0.604	82.38 ± 13.61	1.00 ± 1.00
6	+	1200	2.768 ± 0.380	90.14 ± 5.89	0.80 ± 0.84
7	+	5000	2.782 ± 0.322	84.74 ± 6.07	0.80 ± 0.84
8	+	20000	0.636 ± 0.755** ##	20.98 ± 39.85*#	4.60 ± 4.16

*, **: Significantly different from control group at P<0.05, 0.01, respectively

#, ##; P<0.05, 0.01 from the corresponding controls without folic acid

Table 2-10

MBP Contents in the Urine of Rats Pretreated with Folic Acid

(Forced Urination 12 hrs After the Administration of DBP)

Group	Folic acid	Wks.	No. of rats	Urine volume (g)	DBP (μg/ml)	MBP (mg/ urine)			
						Untreatment with Enzyme	Treatment with Enzyme	Conjugate	Conjugation (%)
1	—	5	3	0.63 ± 0.15	3.00 ± 1.84 (2)a	9.59 ± 4.73	12.74 ± 1.20	3.16 ± 1.20	26.2 ± 6.85
2	—(v)	5	6	0.27 ± 0.22	6.45 ± 5.74 (4)a	4.99 ± 3.84	9.05 ± 1.78 (4)b	3.02 ± 1.78 (4)b	32.7 ± 6.00 (4)b
3	+	5	6	0.23 ± 0.18	2.37 ± 2.66	3.87 ± 1.77 #	5.62 ± 1.58 #(5)b	1.44 ± 1.58 (5)b	19.9 ± 15.2 (5)b
4	—(v)	9	6	0.10 ± 0.06	4.64 ± 5.54 (5)a	8.90 ± 3.32	11.17 ± 0.63 (4)b	2.64 ± 0.63 (4)b	24.4 ± 5.4 (4)b
5	+	9	6	0.15 ± 0.15	2.86 ± 3.05 (5)a	6.46 ± 2.74	6.53 ± 0.74 *(4)b	1.10 ± 0.74 ** (4)b	16.4 ± 11.1 (4)b

b : Not tested due to insufficient of sample

*, **: Significantly different from control group (group 4) at $P<0.05$, respectively.

: Significantly different from control group (group 1) at $P<0.05$.

Table 2-11

MBP Contents in the Urine of Rats Pretreated with Folic Acid (1) (4-hours collection)

Group	Folic acid	Weeks	No. of rats	Urine Volume (g)	DBP (μg/ml)	MBP (mg/ urine)			
						Untreatment with Enzyme	Treatment with Enzyme	Conjugate	Conjugation (%)
1	—	5	3	1.28 ± 0.68	— (0)a	1.96 ± 2.20	2.80 ± 0.40	0.84 ± 0.27	29.7 ± 5.5
2	—(V)	5	6	1.72 ± 0.80	2.40(1)a	2.21 ± 0.54	2.99 ± 0.71	0.78 ± 0.31	25.6 ± 8.2
3	+	5	6	1.92 ± 0.97	0.20(1)a	1.70 ± 0.65	2.55 ± 0.76	0.85 ± 0.36	34.0 ± 14.6
4	—(V)	9	6	1.00 ± 0.43	— (0)a	3.39 ± 0.91#	4.49 ± 1.00#	1.10 ± 0.27	24.9 ± 6.3
5	+	9	6	1.62 ± 0.96	— (0)a	2.38 ± 1.24	3.48 ± 1.67	1.12 ± 0.73	31.0 ± 17.5

a : Other samples were ND (not detected).

: Significantly different from control group (group 1) at $P<0.05$.

Table 2-12

The Amount of MBP in the Blood of Rats Pretreated with Folic Acid

Group	Folic acid	No. of Rats	DBP (μg/ml)		MBP (μg/ml)	Glucuronidated MBP (μg/ml)
1	—	3	—	(0)a	203 ± 4.0	6.4 ± 1.2 (2)
2	—(v)	6	0.10 —	(0)a	241 ± 39.1	5.9 ± 4.3
3	+	6	0.10 —	(2)a	282 ± 70.3 #	18.6 ± 11.6 *
4	—(v)	6	0.17 ± 0.12	(1)a	226 ± 43.5	14.8 ± 6.5
5	+	6	0.10 ± 0.00	(3)a	249 ± 36.7	9.4 ± 4.0 (5)

a : Other samples were ND (not detected).
: Significantly different from control group (group 1) at $P<0.05$.
* : Significantly different from control group (group 2) at $P<0.05$.

Table 2-13

The Amount of MBP in the Testis of Rats Pretreated with Folic Acid

Group	Folic acid	Week	No. of rats	Testis Weight (g)	DBP (μg/g)	MBP (μg/g)	Content of MBP (μg/ testis)
1	—	5	3	0.77 ± 0.02	— (0)a	46.70 ± 1.60	36.0 ± 2.0
2	—(v)	5	6	0.70 ± 0.11	— (0)a	56.80 ± 6.63 #	40.2 ± 9.6
3	+	5	6	0.70 ± 0.06	0.90 ± 1.13 (2)a	69.72 ± 25.34	49.3 ± 19.8
4	—(v)	9	6	0.74 ± 0.08 #, *	0.20 — (1)a	46.68 ± 13.62	33.7 ± 7.2
5	+	9	6	0.64 ± 0.09	0.23 ± 0.23 (3)a	56.68 ± 15.46	35.5 ± 8.8

a : Other samples were ND (not detected).

* : Significantly different from control group (group 4) at $P<0.05$, respectively.

\# : Significantly different from control group (group 1) at $P<0.05$.

Table 2-14

最終体重および各種腎機能指標値

Folic acid	Test chemical	Level (ppm)	Final B.W. (g)	Weight of kidneys (%)	Urine specific gravity	Urine volume (g)	Serum BUN	Serum Creatinine (mg/dl)
+	-	0	281.6	0.73##	1.060	1.96#	25.64#	0.50
+	DEHA	6000	280.0	0.82**,##	1.056	2.18##	25.90##	0.50 #
+	DEHA	25000	259.8*	0.88#	1.062	2.50##	27.78##	0.56 *
+	DEHP	6000	280.4	0.81**,##	1.050*,#	2.20##	28.18#	0.50
+	DEHP	25000	250.0**	0.96	1.068	2.50#	41.78**,##	0.60 **, #
-	-	0	286.0	0.64	1.069	1.32	20.04	0.50
-	DEHA	6000	289.8	0.68*	1.071	1.28	20.00	0.56 *
-	DEHA	25000	258.6**	0.80**	1.060	1.58	20.02	0.52
-	DEHP	6000	285.6	0.72**	1.078	0.96	22.06	0.52
-	DEHP	25000	263.2*	0.80**	1.070	1.98	29.26 **	0.54

*, ** : Significantly different from control group at P<0.05, 0.01, respectively.

#, ## : Significantly different from the corresponding control groups without folic acid at P< 0.05, 0.01, respectively.

Table 2-15 精巣、精巣上体、精嚢および前立腺重量

Group No.	Folic acid	Test chemical	Level (ppm)	Testes	Epididymides	Seminal vesicles	Prostate
1	+	-	0	1.05±0.06	0.31±0.02	0.31±0.04	0.25±0.03
2	+	DEHA	6000	1.05±0.05	0.29±0.02	0.32±0.05	0.23±0.03
3	+	DEHA	25000	1.16±0.04 **	0.31±0.03	0.30±0.03 ##	0.21±0.04
4	+	DEHP	6000	1.07±0.07	0.29±0.01	0.32±0.03	0.22±0.02
5	+	DEHP	25000	0.74±0.24 *, #	0.27±0.06 *	0.25±0.05 *	0.19±0.03 **
6	-	-	0	1.07±0.07	0.30±0.02	0.30±0.03	0.22±0.02
7	-	DEHA	6000	1.06±0.04	0.29±0.02	0.30±0.02	0.21±0.03
8	-	DEHA	25000	1.17±0.04 *	0.34±0.04 *	0.36±0.02 **	0.23±0.02 *
9	-	DEHP	6000	1.04±0.06	0.29±0.03	0.33±0.05	0.23±0.02
10	-	DEHP	25000	1.01±0.06	0.30±0.02	0.25±0.06	0.19±0.03

The data are shown by g/100g.

*,** : Significantly different from control group at P<0.05, 0.01, respectively.

#, ## : Significantly different from the corresponding control groups without folic acid at P< 0.05, 0.01 respectively.

No. of rat in each group is 5.