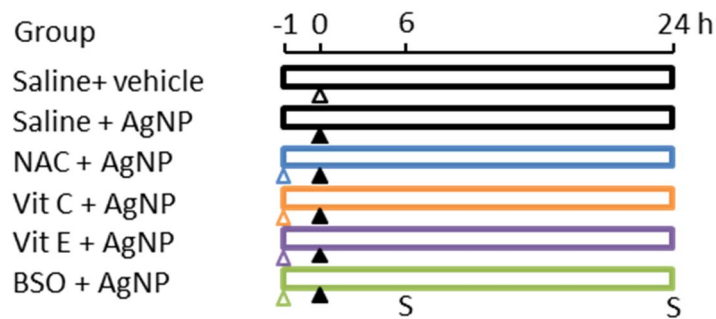


- ▲ Test chemicals exposure (i.p. , 2 ml/mouse)
 - ① Citrate (vehicle)
 - ② 10 nm AgNP (0.2 mg)
 - ③ 60 nm AgNP (1.2 mg)
 - ④ 100 nm AgNP (2 mg)

- ▲ Sacrifice

Figure 1. 実験デザイン 【実験 A6】



Animal 7-week old female BALB/c mouse (n=5/group)

- △ Vehicle (2 mM citrate, i.p., 300 μl/mouse)
- ▲ Silver nanoparticles (10 nm AgNP, i.p., 0.4 mg/mouse)
- △ N-acetyl-l-cysteine (NAC, i.g., 2000 mg/kg bw)
- △ Vitamin C (Vit C, i.g., 200 mg/kg bw)
- △ Vitamin E (Vit E, i.g., 100 mg/kg bw)
- △ L-buthionine-(S,R)-sulfoximine (BSO, i.p., 1.6 g/kg bw)
- S Sacrifice

Figure 2. 実験デザイン 【実験 A7】

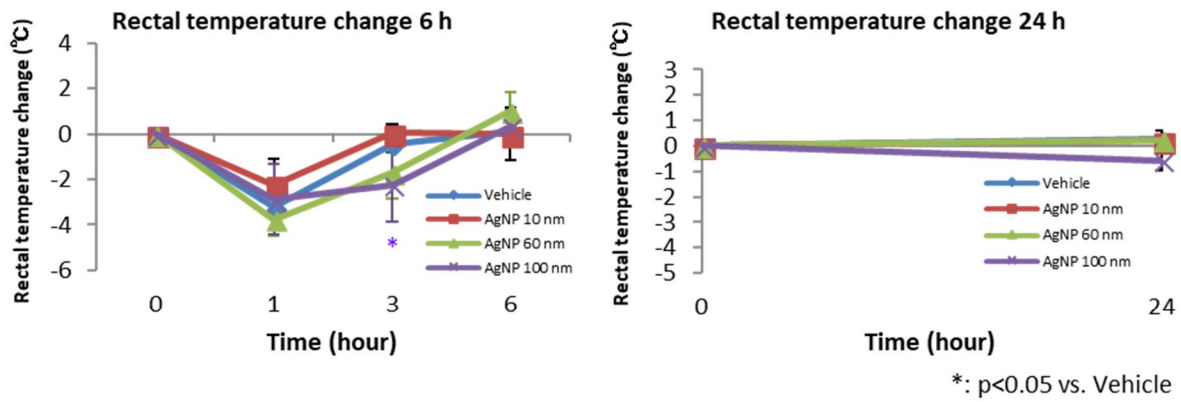


Figure 3. 相対肝重量及び体温変化【実験 A6】

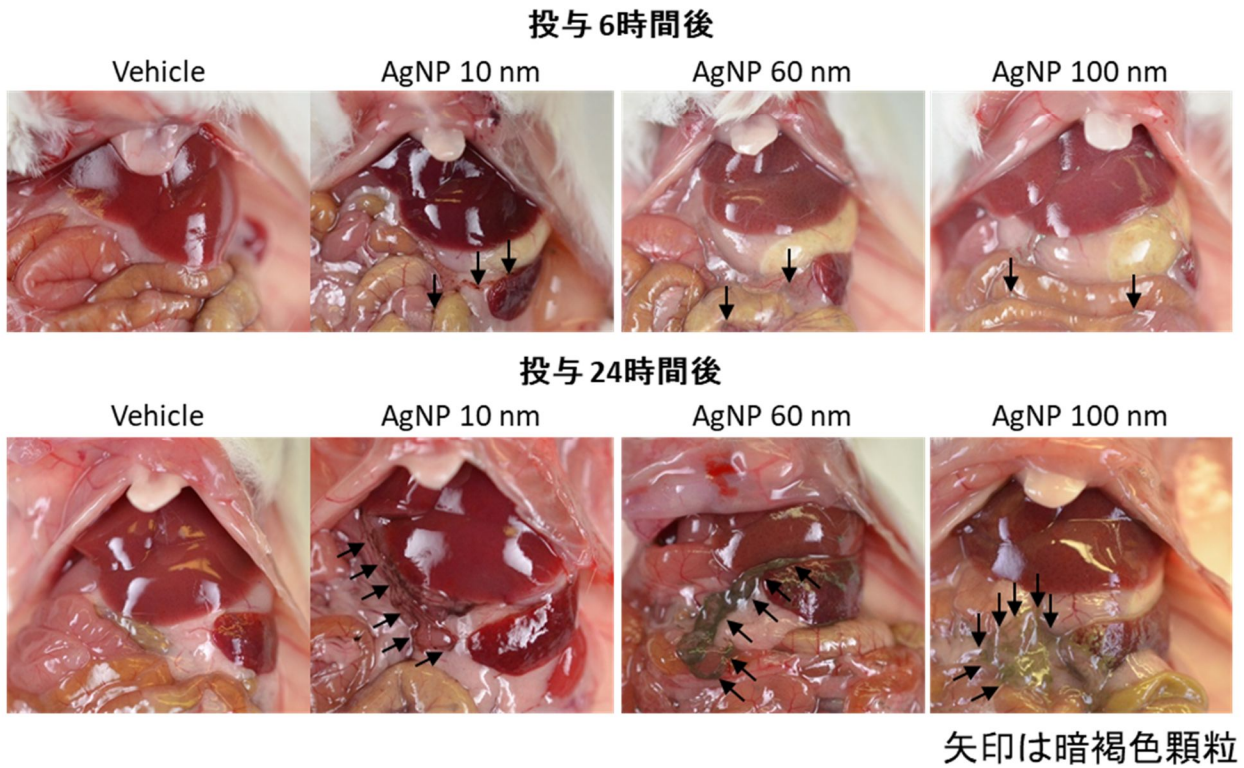


Figure 4. 剖検時の肉眼変化【実験 A6】

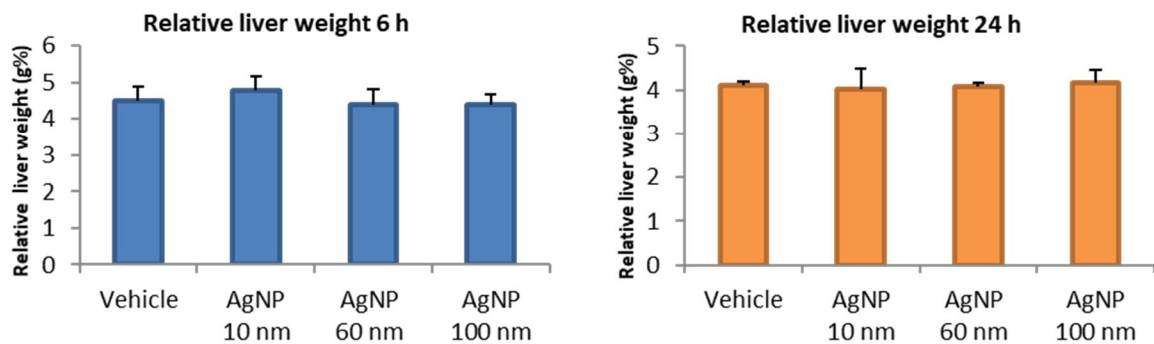


Figure 5. 相対肝重量【実験 A6】

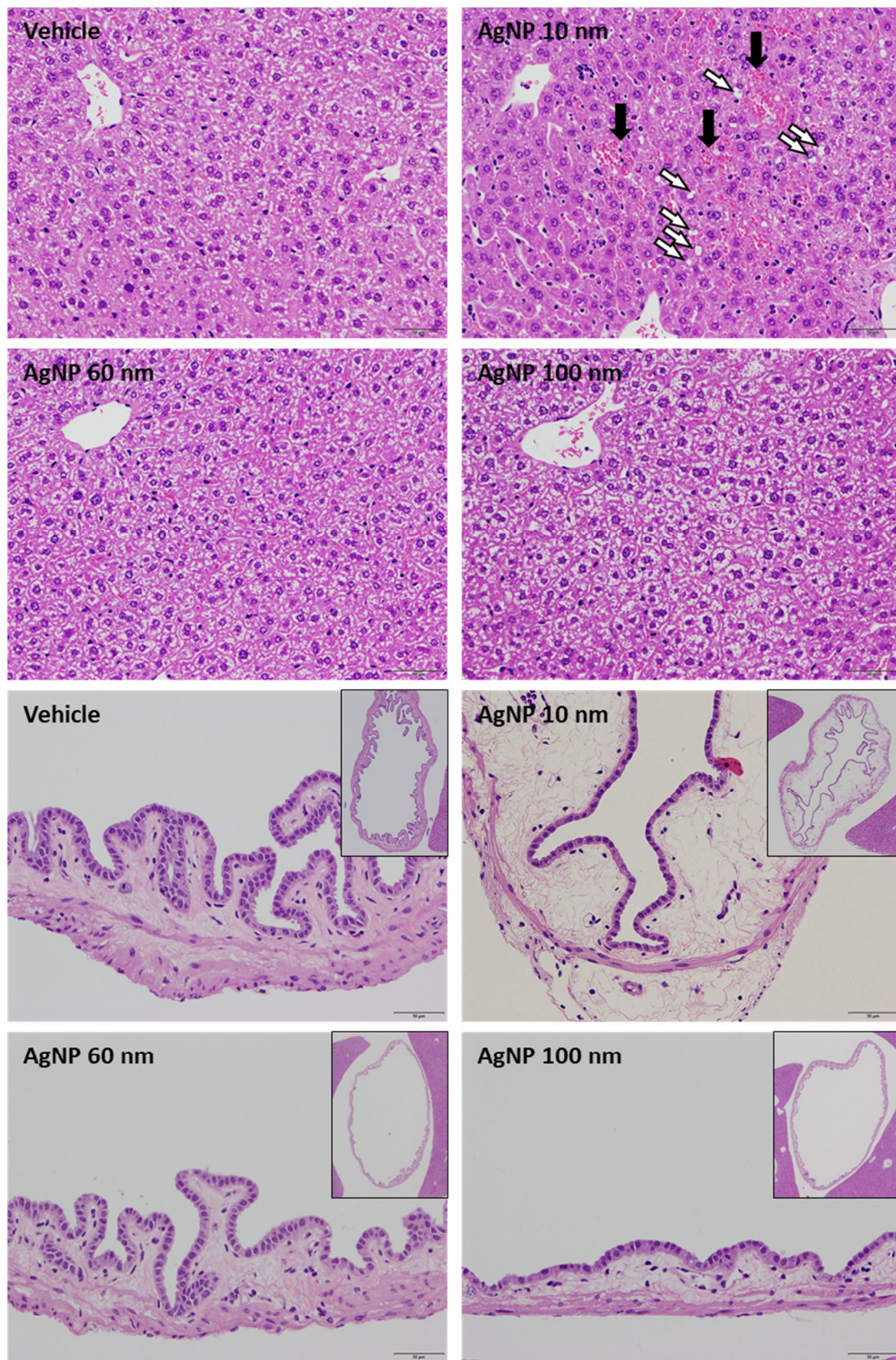
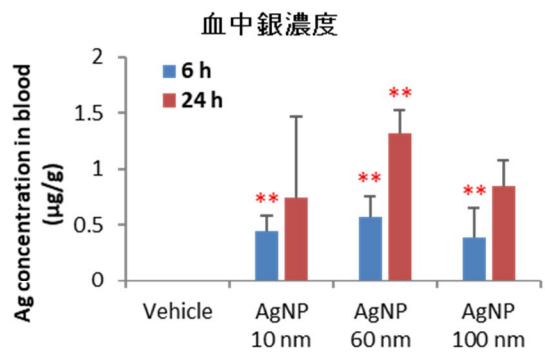
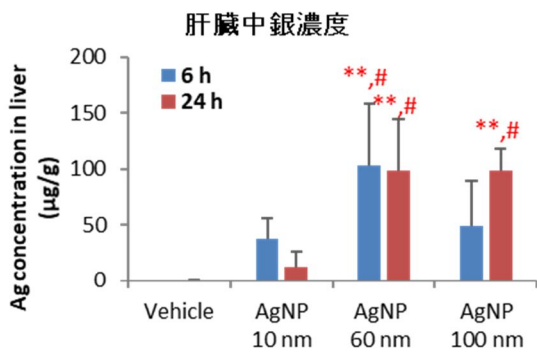
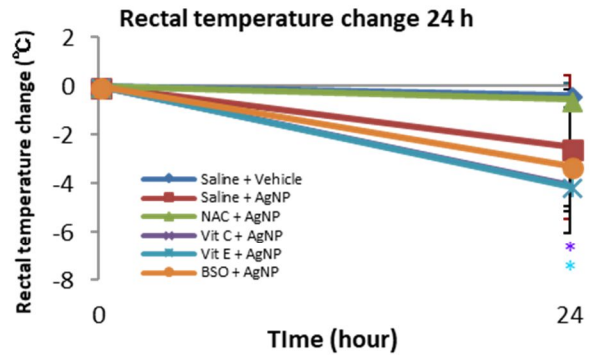
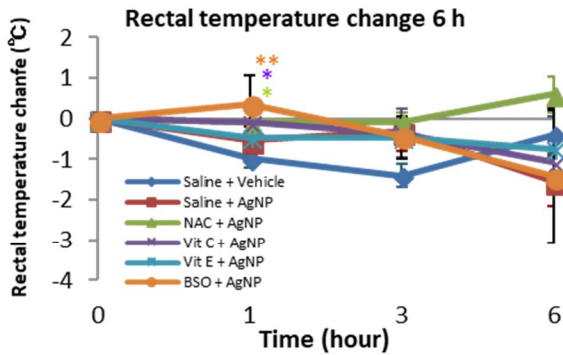


Figure 6. 肝臓及び胆嚢の病理組織写真 **【実験 A6】**
 AgNP 10 nm 群の肝臓のうっ血（黒矢印）、肝細胞の空胞化（白矢印）及び胆嚢粘膜下並びに漿膜の浮腫が認められた。



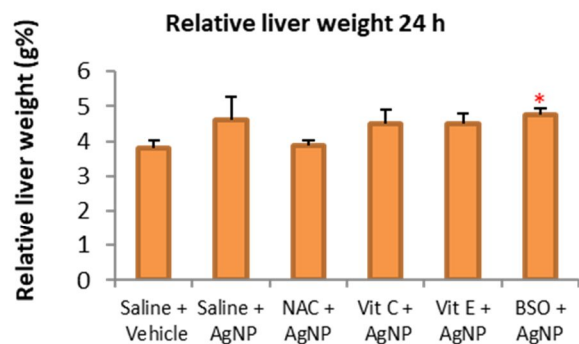
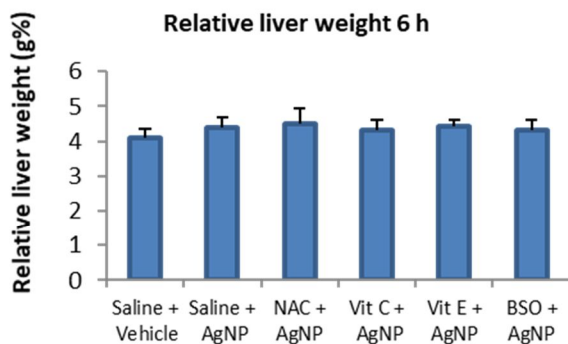
** : p<0.01 vs. Vehicle
: p<0.05 vs. AgNP 10 nm

Figure 7. 肝臓中及び血中銀濃度 **【実験 A6】**



*, **: p<0.05 and p<0.01 vs. Saline + Vehicle

Figure 8. 相对肝重量及び体温変化 **【実験 A7】**



* : p<0.05 vs. Saline + Vehicle

Figure 9. 相对肝重量 **【実験 A7】**

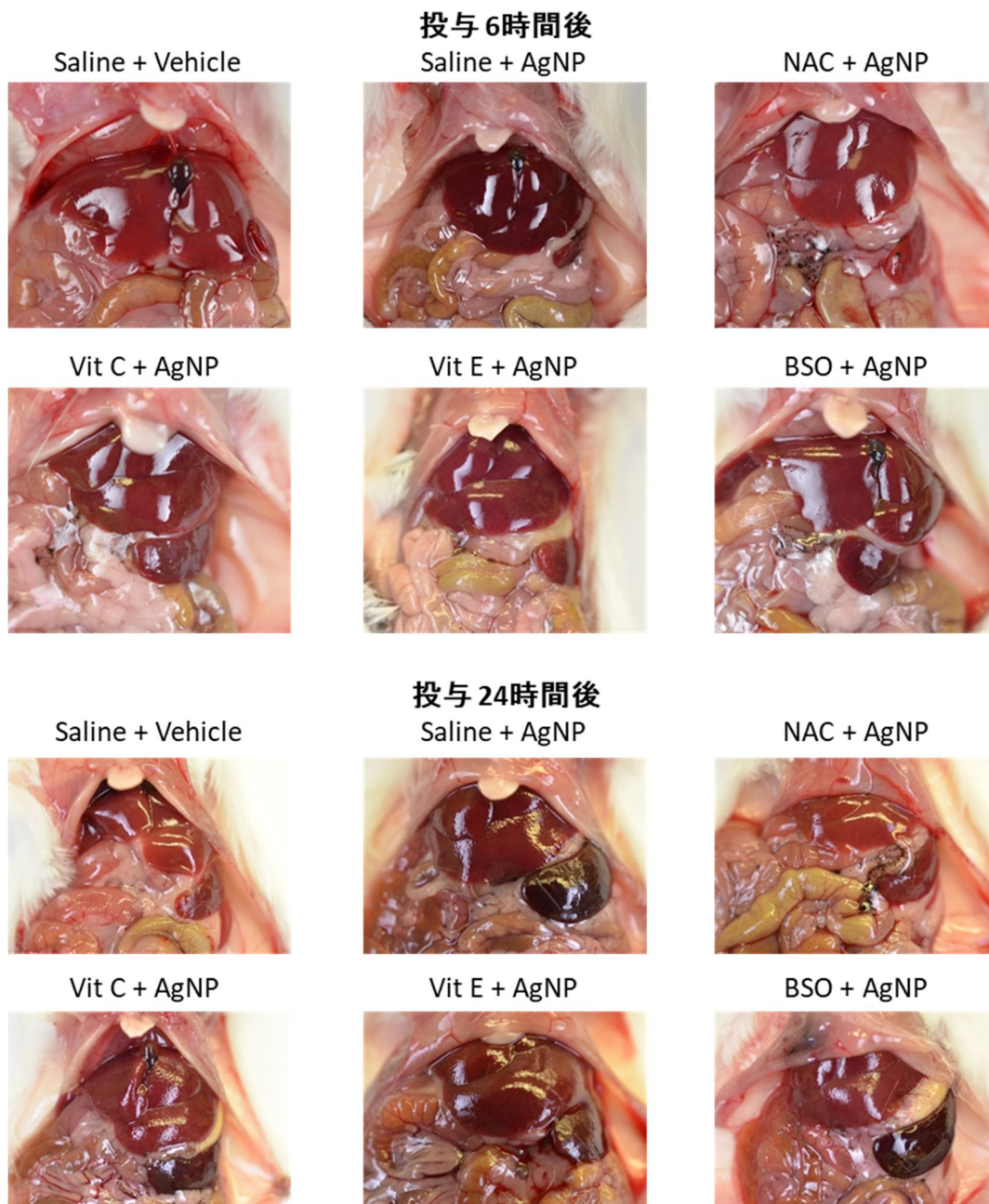


Figure 10. 剖検時の肉眼変化 【実験 A7】

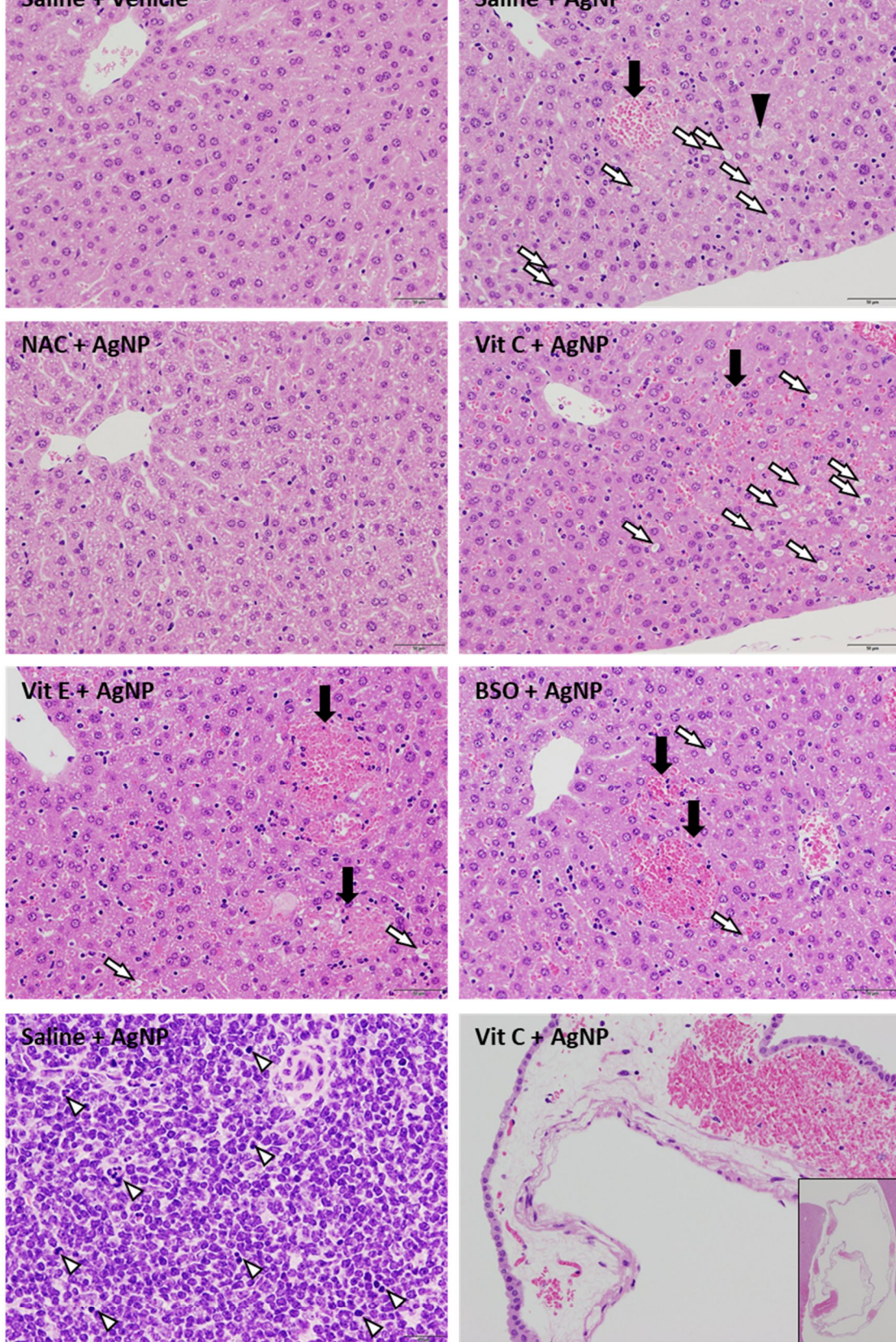


Figure 11. 肝臓、胆嚢、脾臓及び胆嚢の病理組織写真 **【実験 A7】**

Saline + AgNP、Vit C + AgNP、Vit E + AgNP及びBSO + AgNP群の肝臓のうっ血(黒矢印)、肝細胞の空胞化(白矢印)、Saline + AgNP群の肝臓の単細胞壊死(黒矢頭)、脾臓のアポトーシス(白矢頭)及びVit C + AgNP群の胆嚢粘膜下・漿膜の浮腫並びに出血が認められた。

Table 1. Characteristics of tested AgNP reported by the manufacturer in the datasheet 【実験 A6】

	10 nm AgNP	60 nm AgNP	100 nm AgNP
Lot No.	DMW0198	DMW0164	DMW0120
Diameter (nm)	9.6 ± 2	59.8 ± 6.2	104.3 ± 12.6
Coefficient of Variation (%)	20.4	10.3	12.1
Particle Concentration (mL ⁻¹)	2.2 x 10 ¹⁴	9.1 x 10 ¹¹	1.7 x 10 ¹¹
Surface Area (m ² /g)	55.1	9.4	5.3
Mass Concentration (mg/mL)	1.07	1.07	1.05
Endotoxin Concentration (EU/mL)	< 5	< 2.5	< 2.5
Zeta Potential (mV)	-39.4	-46.5	-49.3
pH of Solution	7.7	7.5	7.3
Hydrodynamic Diameter (nm)	13.3	65.8	107
Silver Purity (%)	99.99	99.99	99.99

Table 2. Histopathological findings for Balb/C mice treated with AgNP up to 6 hours 【実験 A6】

Organ and lesions	Treatment	6 h				
		Vehicle	10 nm AgNP	60 nm AgNP	100 nm AgNP	
	No. of animals	5	5	6	5	
Liver	Congestion, intermediate zone	0	4*	0	0	
	Increased cellular component in sinusoid	0	3	0	0	
	Vacuolation, hepatocyte	0	4*	0	0	
	Oval cell hyperplasia	0	1	0	0	
	Multinucleated cell	0	1	0	0	
Gallbladder	Edema, subserosa	0	4*	0	0	
Spleen	Congestion	0	1	0	1	
	Apoptosis, white pulp	0	0	2	2	
Thymus	Apoptosis, cortex	1	0	4	3	
	+	1	0	3	0	
	++	0	0	1	3	
	Apoptosis, medulla	0	0	1	1	
Thoracic lymph node	Dark brown pigment deposition, lymphocyte +	0a	4	5	3b	
Thoracic lymph node	+	0a	0	5	3b	
	++	0a	4	0	0b	
	Apoptosis	0a	0	0	2b	
	Cell infiltration, neutrophil	0a	1	1	0b	
Mesenterium	Inflammatory cell foci	5	5	6	5	
	+	5	4	2	5	
	++	0	1	4	0	
	Dark brown pigment deposition, lymphocyte	0	4*	6**	5**	
Mesenteric lymph nodes	Apoptosis, cortex	1	3	3	2	
Kidney	Regenerative tubules	5	5	5	4	
Heart	Thrombus, right ventricle	1	0	0	0	
	Thrombus, left ventricle	0	0	1	1	
	Thrombus, left atrium	0	0	1	0	
	Thrombus, intra mural	0	0	1	0	
	Mineralization, epicardium	4	4	5	6	
	Single cell necrosis	0	0	0	2	
	Vacuolation	0	0	1	0	
	Megalocyte, cardiomyocyte	0	0	0	2	
	Lung	Hemorrhage	1	0	0	0
		Dark brown pigment deposition, alveolar macrophage +	0	0	2	0
Pancreas	Hemorrhage	0	2	0	1	

*,**; significantly different from the vehicle group at p<0.05 and 0.01, respectively. a; n=2, b; n=5.

Organ and lesions	Treatment	24 hr			
		Vehicle	10 nm AgNP	60 nm AgNP	100 nm AgNP
	No. of animals	3	3	3	3
Liver	Microgranuloma	0	1	1	0
Spleen	Apoptosis, white pulp	0	1	0	0
Thoracic lymph node	Dark brown pigment deposition, lymphocyte +/-	0	1	3	3
	+	0	1	0	0
	++	0	0	2	2
	+++	0	0	1	1
Mesenterium	Inflammatory cell foci	3	3	3	3
	+	0	2	2	0
	++	3	1	1	3
	Dark brown pigment deposition, lymphocyte	0	2	3	3
Mesenteric lymph nodes	Apoptosis, cortex	1	1	0	0
Kidney	Regenerative tubules	3	3	2	3
Heart	Mineralization, epicardium	3	2	2	3
Lung	Dark brown pigment deposition, alveolar macrophage	0	1	0	1
	+	0	1	0	0
	++	0	0	0	1

Table 4. Serum biochemistry for Balb/C mouse treated with AgNP and antioxidants 【実験 A7】

No. of animals		6 h					
		Saline + Vehicle	Saline + AgNP	NAC + AgNP	Vit C + AgNP	Vit E + AgNP	BSO + AgNP
		4	5	5	5	5	4
TP	g/dL	4.5 ± 0.3	4.1 ± 0.3	4.6 ± 0.1	4.1 ± 0.1	4.3 ± 0.2	4.0 ± 0.5*
ALB	g/dL	3.1 ± 0.1	2.8 ± 0.2**	3.1 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.7 ± 0.2**
A/G		2.2 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.3 ± 0.1	2.0 ± 0.1	2.3 ± 0.7
BUN	mg/dL	19.4 ± 3.7	20.7 ± 2.9	23.4 ± 3.4	23.6 ± 2.2	22.4 ± 2.8	24.0 ± 7.7
Cre	mg/dL	0.14 ± 0.02	0.14 ± 0.03	0.12 ± 0.02	0.12 ± 0.02	0.14 ± 0.04	0.12 ± 0.02
Na	mEq/L	152 ± 2	153 ± 3	152 ± 1	151 ± 1	150 ± 2	150 ± 3
K	mEq/L	4.7 ± 0.4	4.7 ± 0.5	3.9 ± 0.4	5.0 ± 0.7	4.9 ± 0.5	5.7 ± 1.4
Cl	mEq/L	110 ± 6	109 ± 8	114 ± 1	110 ± 5	106 ± 8	108 ± 5
Ca	mg/dL	7.5 ± 0.3	7.2 ± 0.7	7.5 ± 0.4	6.7 ± 0.7	7.7 ± 0.6	6.9 ± 0.7
IP	mg/dL	10.1 ± 2.3	10.0 ± 0.9	9.1 ± 0.9	11.8 ± 1.9	10.1 ± 1.5	12.2 ± 3.1
AST	IU/L	113 ± 59	214 ± 85	110 ± 41	210 ± 43	253 ± 68**	134 ± 11
ALT	IU/L	37 ± 12	16 ± 27	50 ± 42	5 ± 2	27 ± 28	19 ± 6
ALP	IU/L	506 ± 21	476 ± 40	443 ± 21	454 ± 54	506 ± 19	411 ± 35**
γGTP	IU/L	<3	<3	<3	<3	<3	<3
T-CHO	mg/dL	68 ± 4	59 ± 2**	64 ± 3	61 ± 5	68 ± 4	57 ± 3**
TG	mg/dL	30 ± 8	14 ± 3*	23 ± 9	21 ± 9	20 ± 7	13 ± 5**
BIL	mg/dL	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.11 ± 0.05	0.11 ± 0.08
Glucose	mg/dL	182 ± 18	98 ± 9**	189 ± 31	117 ± 18**	124 ± 22**	123 ± 32**

Each value represents the mean ± S.D.

*, **; significantly different from the vehicle group at p<0.05 and 0.01, respectively.

Table 5. Serum biochemistry for Balb/C mouse treated with AgNP and antioxidants 【実験 A7】

No. of animals		24 h					
		Saline + Vehicle	Saline + AgNP	NAC + AgNP	Vit C + AgNP	Vit E + AgNP	BSO + AgNP
		3	2	3	3	3	2
TP	g/dL	5.0 ± 0.1	4.4 ± 0.6	4.7 ± 0.1	4.1 ± 0.2**	4.3 ± 0.1*	4.2 ± 0.0*
ALB	g/dL	3.3 ± 0.1	2.9 ± 0.4	3.0 ± 0.2	2.8 ± 0.1*	2.9 ± 0.1	2.7 ± 0.1*
A/G		2.0 ± 0.1	1.9 ± 0.1	1.8 ± 0.2	1.9 ± 0.1	2.1 ± 0.1	1.8 ± 0.3
BUN	mg/dL	24.3 ± 2.7	21.6 ± 8.5	22.5 ± 1.5	29.3 ± 4.2	31.5 ± 3.1	24.8 ± 3.1
Cre	mg/dL	0.15 ± 0.02	0.14 ± 0.04	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.00	0.15 ± 0.02
Na	mEq/L	156 ± 2	154 ± 2	155 ± 0	156 ± 1	159 ± 5	157 ± 1
K	mEq/L	4.2 ± 0.3	3.8 ± 0.8	4.5 ± 0.2	3.9 ± 0.4	3.6 ± 0.4	3.6 ± 0.3
Cl	mEq/L	112 ± 7	109 ± 9	117 ± 1	105 ± 8	101 ± 4	110 ± 11
Ca	mg/dL	8.4 ± 0.3	8.0 ± 0.6	8.7 ± 0.2	7.8 ± 0.6	8.0 ± 0.0	8.1 ± 0.7
IP	mg/dL	10.2 ± 1.0	8.4 ± 2.2	9.9 ± 0.6	8.2 ± 0.7	8.5 ± 0.9	8.2 ± 1.7
AST	IU/L	79 ± 4	304 ± 359	69 ± 10	514 ± 166*	395 ± 46	384 ± 102
ALT	IU/L	34 ± 6	27 ± 16	20 ± 6	91 ± 105	22 ± 12	91 ± 47
ALP	IU/L	429 ± 88	400 ± 1	383 ± 29	389 ± 16	441 ± 9	360 ± 6
γGTP	IU/L	<3	<3	<3	<3	<3	<3
T-CHO	mg/dL	84 ± 6	71 ± 2*	78 ± 2	75 ± 3	97 ± 4*	77 ± 6
TG	mg/dL	36 ± 12	31 ± 5	29 ± 10	24 ± 12	29 ± 2	21 ± 18
BIL	mg/dL	0.07 ± 0.03	0.13 ± 0.07	0.07 ± 0.01	0.18 ± 0.05	0.18 ± 0.09	0.16 ± 0.01
Glucose	mg/dL	174 ± 20	125 ± 77	131 ± 18	97 ± 22	87 ± 28	82 ± 14

Each value represents the mean ± S.D.

*, **; significantly different from the vehicle group at p<0.05 and 0.01, respectively.

Table 6. Histopathological findings for Balb/C mice treated with AgNP up to 6 hours 【実験 A7】

		6 hr					
		Treatment	Vehicle	AgNP	NAC + AgNP	Vit C + AgNP	Vit E + AgNP
Organ and lesions	No. of animals	4	5	5	5	5	5
Liver	Congestion, intermediate zone	0	5**	0	5**	5**	3
	+	0	3	0	5**	4*	2
	++	0	2	0	0	1	1
	Increased cellular component in sinusoid	0	5**	0	4*	4*	1
	Vacuolation, hepatocyte	0	5**	0	3	1	3
	+	0	2	0	2	1	2
	++	0	3	0	1	0	1
	Cytoplasmic inclusions, hepatocyte	0	2	0	1	0	1
	+	0	1	0	1	0	1
	++	0	1	0	0	0	0
	Single cell necrosis, hepatocyte	0	1	0	0	0	0
	Focal necrosis, hepatocyte	0	0	0	1	0	0
	Dark brown pigment deposition, Kupffer cell	0	0	0	1	2	0
	+	0	0	0	1	0	0
++	0	0	0	0	2	0	
Gallbladder	Edema, subserosa	0	4*	3	3	1	4
	+	0	2	3	2	1	2
	++	0	2	0	1	0	1
Spleen	Hemorrhage	0	0	0	1	0	0
	Congestion +	0	1	0	0	1	0
	+	0	1	0	0	0	0
	++	0	0	0	0	1	0
	Apoptosis, white pulp	0	2	0	4*	3	4*
	+	0	2	0	4*	1	3
Thymus	++	0	0	0	0	1	1
	+++	0	0	0	0	1	0
	Apoptosis, cortex	0	0	0	2	3	2
	+	0	0	0	2	3	1
Thoracic lymph node	++	0	0	0	0	0	1
	Hemorrhage	0	0	1	0	0	0
	Dark brown pigment deposition, lymphocyte	0 ^a	3 ^b	4	4 ^{b*}	4 ^{b*}	5*
Mesenterium	Apoptosis	0 ^a	0 ^b	1	1 ^b	0 ^b	0
	Inflammatory cell foci +/-	4	5	5	5	5	5
	+	2	3	2	5	4	4
Mesenteric lymph nodes	++	2	2	3	0	1	1
	Dark brown pigment deposition, lymphocyte +	0	5**	5**	5**	5**	5**
	Apoptosis, paracortex	0	0	0	1	0	0
Kidney	Regenerative tubules	4	3	4	4	5	5
Heart	Mineralization, epicardium	3	3	5	5	4	3
Lung	Hemorrhage +	0	1	3	1	1	0
	Dark brown pigment deposition, alveolar macrophage	0	0	0	0	2	0
Pancreas	Hemorrhage	0	0	1	0	1	0

*, **; significantly different from the vehicle group at p<0.05 and 0.01, respectively. a; n=3, b; n=4.

Table 7. Histopathological findings for Balb/C mice treated with AgNP up to 24 hours 【実験 A7】

Organ and lesions	Treatment No. of animals	24 hr					
		Vehicle	AgNP	NAC + AgNP	Vit C + AgNP	Vit E + AgNP	BSO + AgNP
Liver	Congestion, intermediate zone	0	1	0	3	3	2
	+	0	0	0	1	0	1
	++	0	1	0	2	3	1
	Increased cellular component in sinusoid	0	1	0	3	3	1
	Vacuolation, hepatocyte	0	1	0	3	3	1
	+	0	0	0	2	2	1
	++	0	1	0	1	1	0
	Cytoplasmic inclusions, hepatocyte	0	1	0	3	3	1
	+	0	0	0	1	3	1
	++	0	1	0	2	0	0
	Single cell necrosis, hepatocyte	0	1	0	3	2	0
	+	0	1	0	2	2	0
	++	0	0	0	1	0	2
	Focal necrosis, hepatocyte	0	1	0	2	0	0
Dark brown pigment deposition, Kupffer cell	0	1	0	0	2	0	
Gallbladder	Edema, subserosa	0	0	0	2	1	2
	Hemorrhage	0	1	0	2	0	0
Spleen	Congestion	0	1	0	3	2	2
	Apoptosis, white pulp +	0	1	0	2	1	2
	+	0	1	0	0	1	2
Thymus	++	0	0	0	2	0	0
	Apoptosis, cortex	0	1	0	2	3	2
	Apoptosis, medulla	0	0	0	2	3	1
Thoracic lymph node	Hemorrhage	0	1	0	0	1	0
	Dark brown pigment deposition, lymphocyte+	0	2	3	2	3	2
	Apoptosis	0	1	0	1	1	2
Mesenterium	Hemorrhage	0	0	0	0	1	0
	Inflammatory cell foci	3	2	3	3	3	2
	+	2	2	3	3	1	1
	++	1	0	0	0	2	1
Mesenteric lymph nodes	Dark brown pigment deposition, lymphocyte	0	2	3	3	3	2
	Apoptosis, paracortex	0	0	0	1	0	0
Kidney	Regenerative tubules	3	2	3	2	3	2
Heart	Mineralization, epicardium	3	2	3	2	2	1
Lung	High cellularity, alveolar wall	0	1	0	0	0	0
	Hemorrhage	1	2	1	0	2	0
Pancreas	Hemorrhage	0	0	0	1	1	1