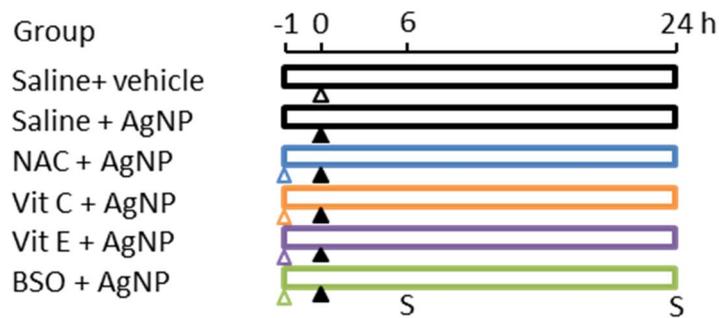


- ▲ Test chemicals exposure (i.p. , 2 ml/mouse)
  - ① Citrate (vehicle)
  - ② 10 nm AgNP (0.2 mg)
  - ③ 60 nm AgNP (1.2 mg)
  - ④ 100 nm AgNP (2 mg)
  
- ▲ Sacrifice

**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】

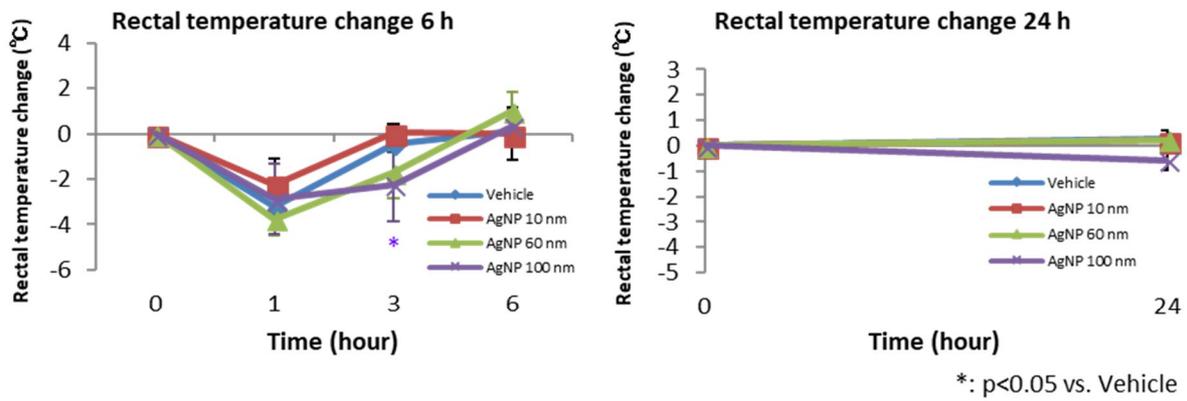


Figure 3. 相対肝重量及び体温変化【実験 A6】

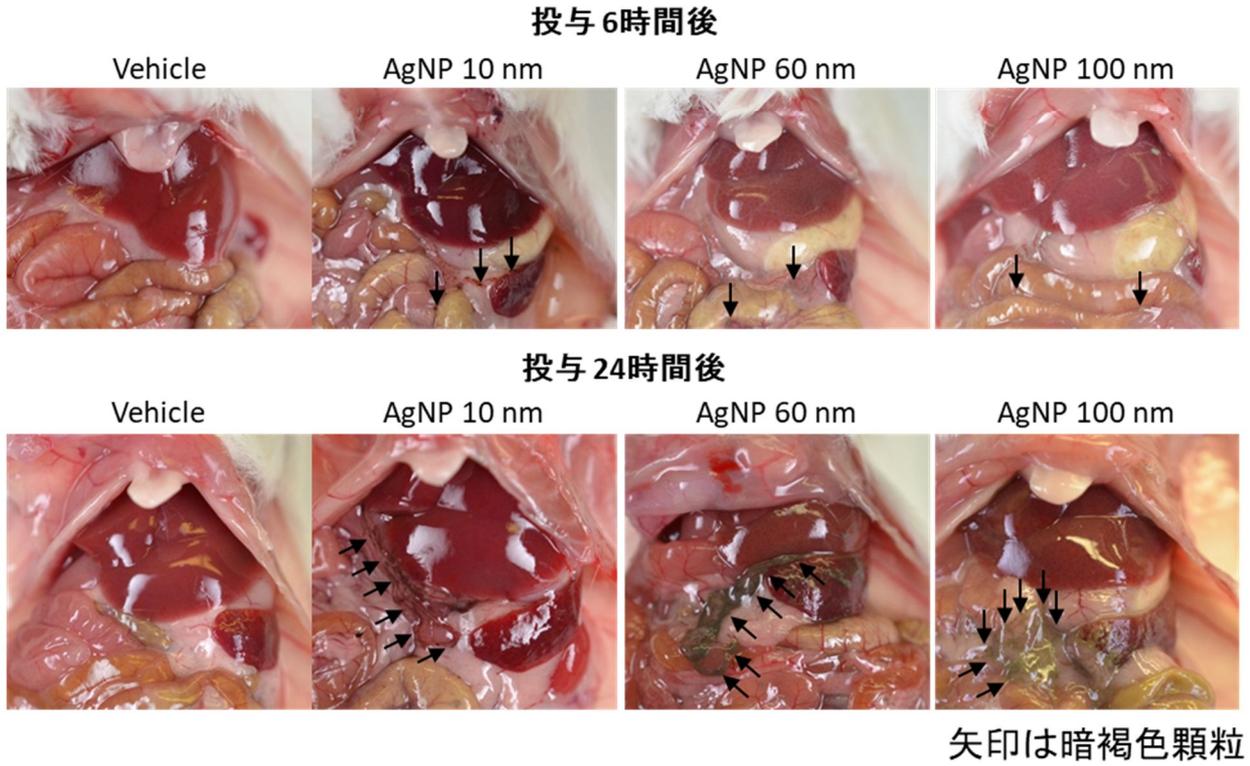


Figure 4. 剖検時の肉眼変化【実験 A6】

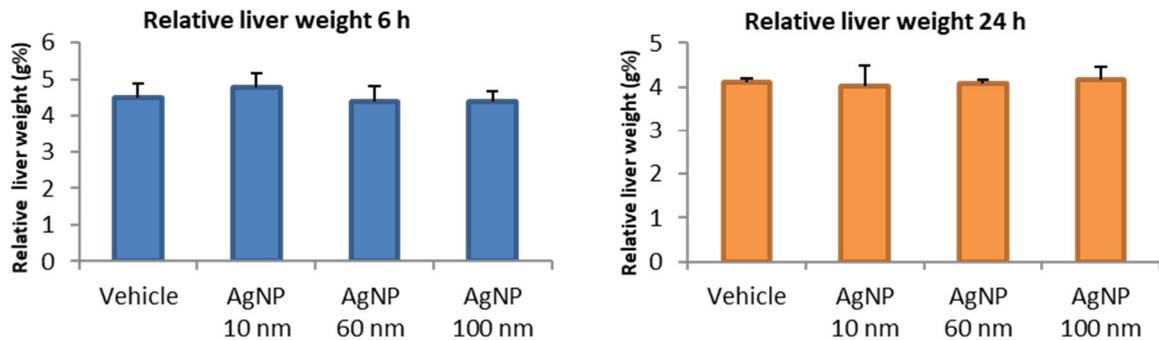


Figure 5. 相対肝重量【実験 A6】

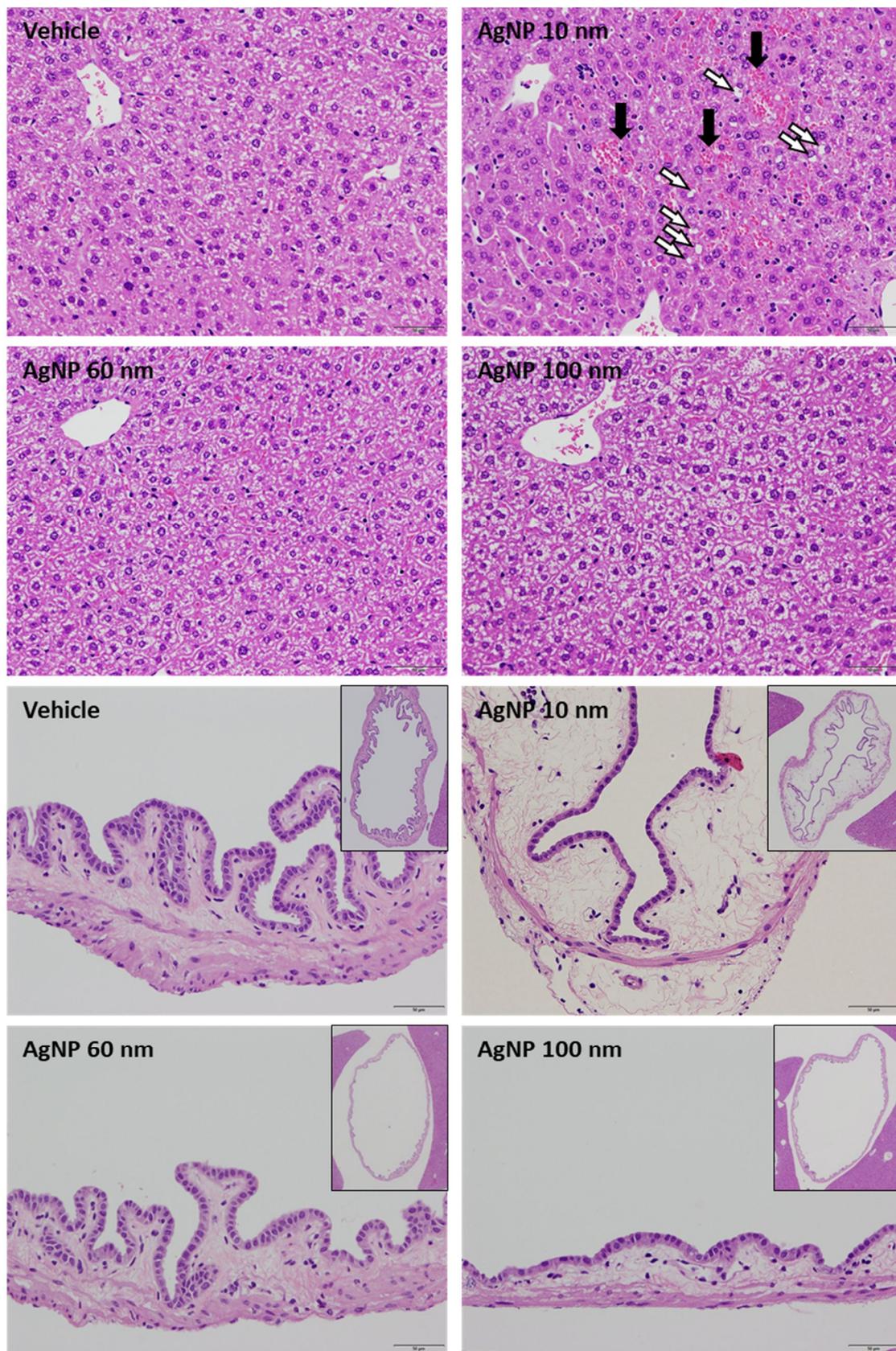
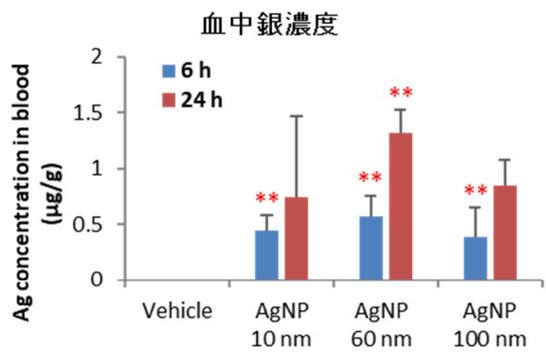
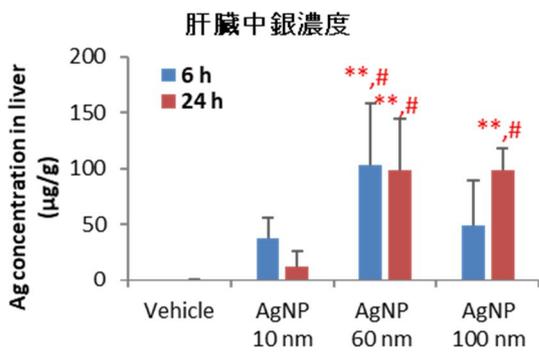
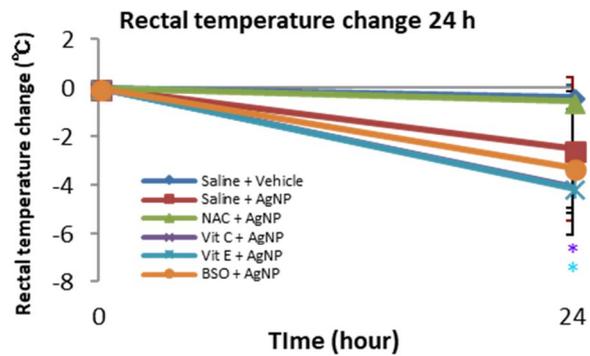
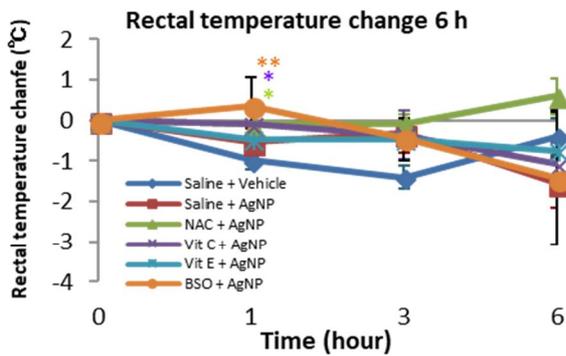


Figure 6. 肝臓及び胆嚢の病理組織写真 **【実験 A6】**  
 AgNP 10 nm 群の肝臓のうっ血（黒矢印）、肝細胞の空胞化（白矢印）及び胆嚢粘膜下並びに漿膜の浮腫が認められた。



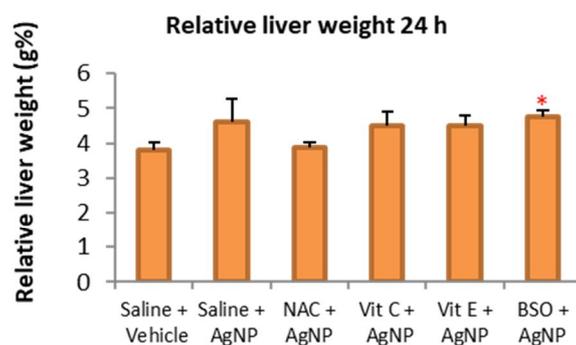
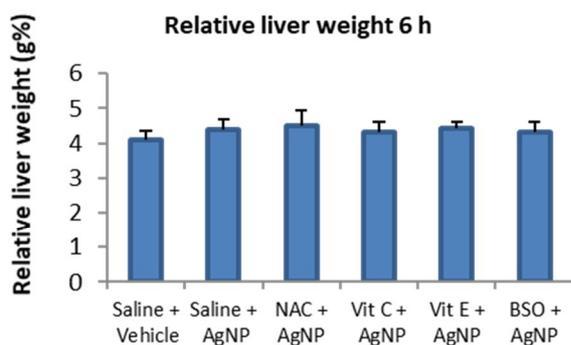
\*\* : p<0.01 vs. Vehicle  
# : p<0.05 vs. AgNP 10 nm

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】**

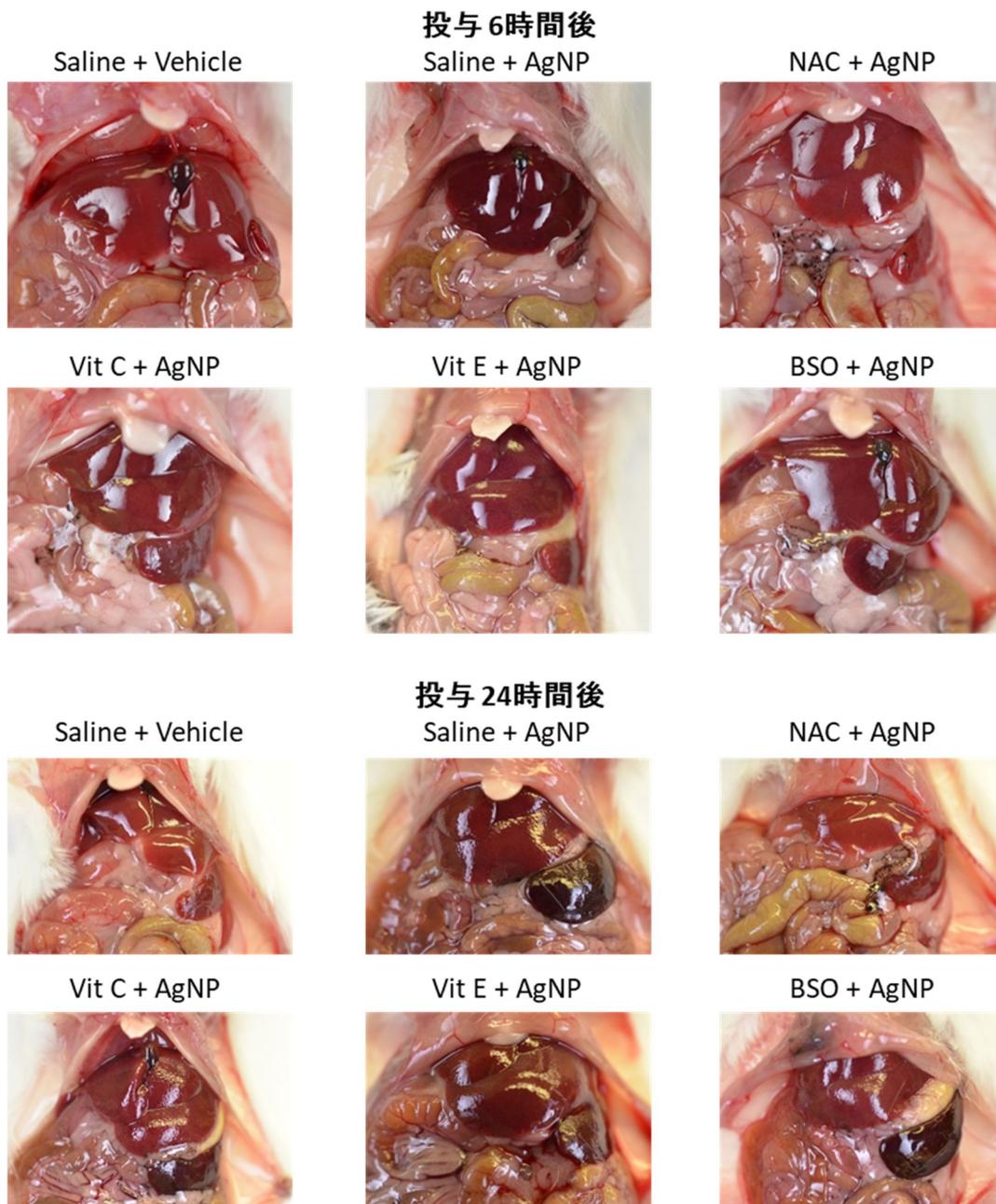


Figure 10. 剖検時の肉眼変化 【実験 A7】

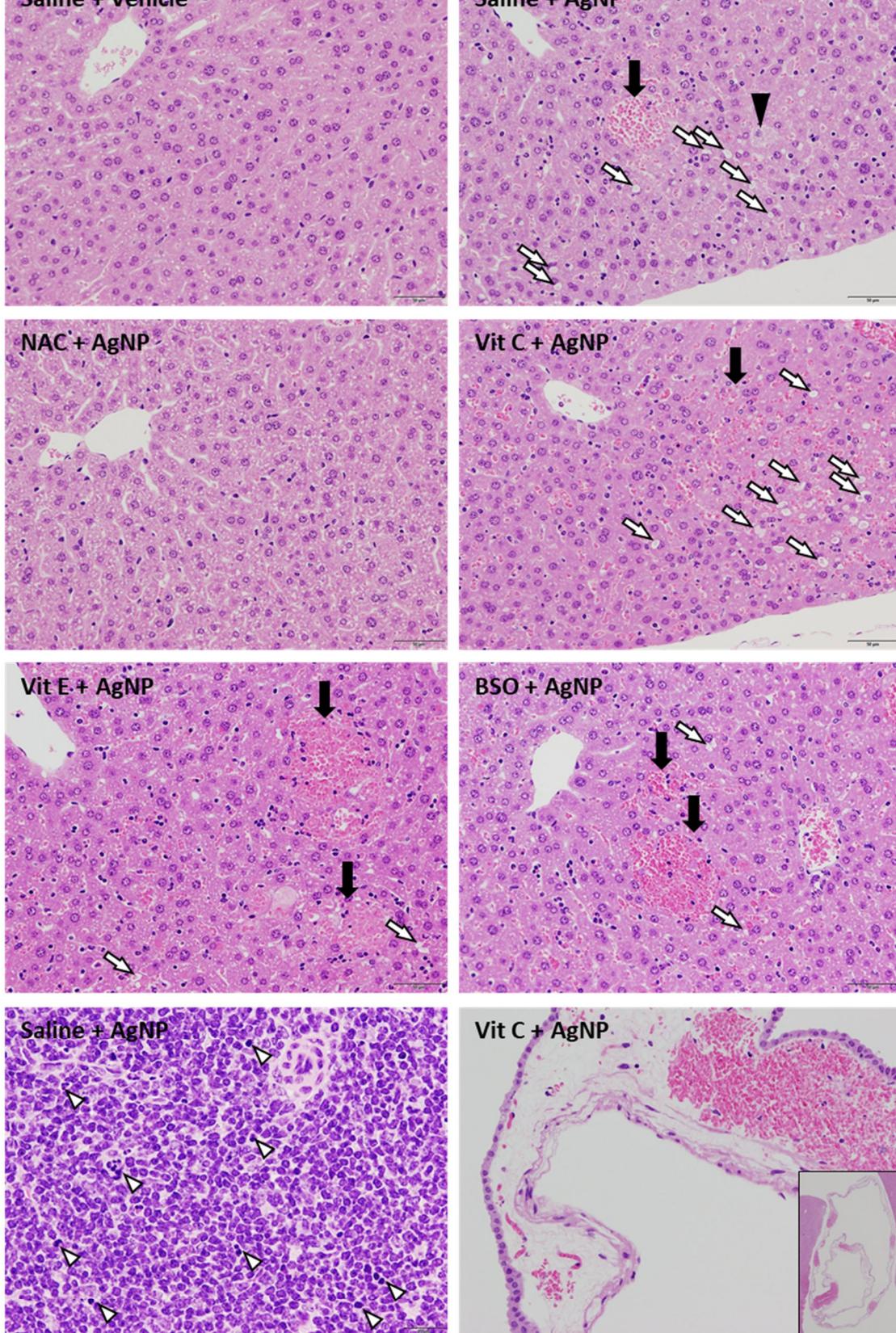


Figure 11. 肝臓、胆嚢、脾臓及び胆嚢の病理組織写真 **【実験 A7】**

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 <sup>-1</sup> )	2.2 x 10 <sup>14</sup>	9.1 x 10 <sup>11</sup>	1.7 x 10 <sup>11</sup>
Surface Area (m <sup>2</sup> /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
		Hemorrhage	1	0	0
Lung	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 <sup>a</sup>	3 <sup>b</sup>	4	4 <sup>b*</sup>	4 <sup>b*</sup>	5*
Mesenterium	Apoptosis	0 <sup>a</sup>	0 <sup>b</sup>	1	1 <sup>b</sup>	0 <sup>b</sup>	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
	++	0	0	0	2	0	0
Thymus	Apoptosis, cortex	0	1	0	2	3	2
	Apoptosis, medulla	0	0	0	2	3	1
	Hemorrhage	0	1	0	0	1	0
Thoracic lymph node	Dark brown pigment deposition, lymphocyte+	0	2	3	2	3	2
	Apoptosis	0	1	0	1	1	2
	Hemorrhage	0	0	0	0	1	0
Mesenterium	Inflammatory cell foci	3	2	3	3	3	2
	+	2	2	3	3	1	1
	++	1	0	0	0	2	1
	Dark brown pigment deposition, lymphocyte	0	2	3	3	3	2
Mesenteric lymph nodes	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