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The Effects of Mushroom Components on the Proliferation of HeLa Cell Line *in Vitro*

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Abstract□In order to find out nutritional effects of Korean edible mushrooms on the multiplication of tissue cells, the alcohol extracts and acid hydrolysates of eleven species of mushrooms were added to Earle's BSS. A comparison of the respective multiplications of HeLa cells in this solution and in control solution of TC-199. Yielded the following results: The alcohol extracts and acid hydrolysates of *Coprinus comatus, Agaricus campestris, Agaricus bisporus, Lentinus edodes, Tricholoma matsutake, Pleurotus ostreatus, Ramaria botrytis* and *Pholiota nameko* influenced favorably the maintenance of the normal form and monolayer of HeLa cells .The growth curves of HeLa cells in the cultures containing, respectively, the alcohol extracts and acid hydrolysates of these eight mushrooms showed that five species, i.e., *Coprinus comatus, Agaricus campestris, Agaricus bisporus, Lentinus edodes* and *Tricholoma matsutake* effected an excellent multiplication and that the other three species were less effective than those five species. As to the effects on the cell multiplication, no marked difference was observed between the alcohol extracts and the acid hydrolysates of the mushrooms tested.

Keyphrases□eleven edible mushrooms—Basidiomycetes—the genera *Coprinus, Agaricus, Lentinus, Tricholoma, Pleurotus, Ramaria* and *Pholiota* —effects of their extracts acid hydrolysates on HeLa cells—tissue culture—

Qualitative analysis of amino acid components contained in 15 species of edible mushrooms were first reported by Kim(1958)[1] and similar studies were successively reported by Huh(1960)[2], Ro and Pyo(1975)[3][4] and Chung(1976)[5]. But nutritional effects of the amino acids of edible mushrooms were not yet investigated. Therefore it is necessary to determine whether the amino acids of mushrooms act as good nutrients for tissue cells.

In this paper an attempt was made to determine the effects of the alcohol extracts[6] and the acid hydrolysate[7] of 11 different edible mushrooms (Table I) on the proliferation of HeLa cell line *in vitro*, considering that there might be certain nutritional effects of the various amino acids and their constituents on the growth and development of the tissue cells.

MATERIALS AND METHODS

HeLa cells[8] were used which were derived from the epithelium of the human carcinoma and subcultured by successive passages for many generations.[9]

Earle's balanced salt solution(Earle's BSS)[10] and TC-199 tissue culture medium[11][12] (Difco, Detroit, Michigan) were used as control cell culture medium. Predetermined

Table I: The edible mushrooms examined

Code	Scientific name	Family name
S-1	*Coprinus comatus* (Fr.) S. F. Gray	Coprinaceae
S-2	*Agaricus bisporus* (Lange) Imbach	Agaricaceae
S-3	*Agaricus campestris* L. ex Fr.	Agaricaceae
S-4	*Lentinus edodes* (Berk.) Sing. = *Cortinellus edodes* (Berk.) S. Ito et Imai	Polyporaceae
S-5	*Tricholoma matsutake* S. Ito et Imai Sing. = *Armillaria matsutake* S. Ito et Imai	Tricholomataceae
S-6	*Pleurotus ostreatus* (Fr.) Quél.	Polyporaceae
S-7	*Remaria botrytis* (Pers.) Ricken = *Clavaria botrytis* Pers.	Clavariaceae
S-8	*Pholiota nameko* (I. Ito) S. Ito et Imai	Strophariaceae
S-9	*Auricularia auricula-judae* (Fr.) Quél.	Auriculariaceae
S-10	*Tremella fuciformis* Berk.	Tremellaceae
S-11	*Gyrophora esculenta*	Gyrophoraceae

Table II: Optimal concentration of mushroom alcoholic extracts added to Earle's BSS for cell culture

Sample number	S-1ex.	S-2ex.	S-3ex.	S-4ex.	S-5ex.	S-6ex.	S-7ex.	S-8ex.	S-9ex.	S-10ex.	S-11ex.
Wt. of fungus equivalent to total AA (g)	1.00	2.00	2.00	4.00	4.00	2.53	16.00	8.78	80.00	180.00	220.00
amino acid (mg/l)											
L-arginine HCl	5.44	2.90	0.44	7.60	11.36	1.19	18.08	5.09	—	21.60	—
L-histidine HCl	1.38	0.84	—	1.72	2.68	1.16	—	3.07	1.60	1.80	2.20
L-lysine HCl	3.21	1.98	1.56	2.84	4.08	0.03	11.36	1.67	—	3.60	2.20
DL-phenylalanine	0.25	1.42	1.00	0.76	2.32	1.16	0.32	0.70	3.20	3.60	2.20
DL-methionine	0.72	0.14	trace	1.08	0.84	0.20	0.32	trace	—	—	—
DL-serine	4.03	1.94	1.66	4.28	3.96	2.30	2.40	4.98	16.80	3.60	4.40
DL-threonine	7.63	3.14	1.34	16.68	7.12	7.84	2.56	0.80	5.60	1.80	2.20
DL-leucine	5.86	2.54	2.70	1.48	3.08	2.56	1.60	3.60	3.20	1.80	2.20
DL-isoleucine	3.79	1.60	1.76	1.12	1.88	1.94	0.96	3.25	2.40	1.80	2.20
DL-valine	4.58	1.82	1.68	0.04	2.76	3.29	1.76	4.48	4.00	3.60	2.20
DL-aspartic acid	2.78	1.78	1.88	11.44	3.96	6.08	4.64	3.60	27.00	1.80	4.40
DL-alpha-alanine	9.77	10.54	9.74	5.96	9.16	10.72	7.68	9.48	4.00	5.40	17.60
L-proline	1.69	25.02	22.32	0.84	4.44	0.99	3.04	4.74	—	3.60	6.60
glycine	1.24	1.10	0.50	3.44	1.12	1.60	2.08	2.20	1.60	1.80	2.20
L-glutamine	2.46	13.96	3.86	9.84	13.40	7.20	7.20	18.09	8.80	3.60	6.60
L-cystine	0.11	1.38	0.20	0.12	0.24	0.56	—	1.32	—	—	2.20
L-tyrosine	6.29	0.36	trace	0.56	1.36	1.34	0.80	1.31	8.00	1.80	2.20
Total	71.42	71.64	50.66	66.72	74.86	54.27	71.68	67.76	71.20	59.40	52.80

optimal concentrations of the alcohol extracts (Table II) and the acid hydrolysate (Table III) of 11 different mushrooms[13)14)] were added to the basal medium which consists of Earle's BSS containing 100 μg/ml streptomycin, 100 units/ml sodium penicillin G and 5% fetal

Table III: Optimal concentration of the acid hydrolysates of mushrooms added to Earle's BSS for cell culture

Sample number	S-1hy.	S-2hy.	S-3hy.	S-4hy.	S-5hy.	S-6hy.	S-7hy.	S-8hy.	S-9hy.	S-10hy.	S-11hy.
Wt. of fungus equivalent to total AA (g)	0.20	0.20	0.30	0.30	05.0	0.70	0.40	0.70	1.10	1.10	1.00
L-arginine HCl	5.46	3.26	5.41	5.70	4.97	5.21	4.84	6.61	7.03	5.89	4.67
L-histidine HCl	1.46	1.18	1.53	4.56	2.93	1.96	4.63	2.21	2.55	1.27	17.10
L-lysine HCl	3.67	2.85	4.68	1.19	2.29	4.40	0.69	5.42	5.03	4.37	4.20
DL-phenylalanine	2.46	1.70	2.54	2.33	1.75	2.44	2.71	2.58	3.18	2.02	2.49
DL-methionine	0.76	0.13	0.21	0.39	0.53	0.18	0.14	—	—	0.23	trace
DL-serine	4.24	3.86	2.53	3.32	3.11	2.82	3.17	3.16	2.48	4.90	3.90
DL-threonine	5.11	4.09	2.80	4.50	2.88	3.29	4.12	3.32	2.87	4.53	4.26
DL-leucine	4.71	3.36	4.80	4.07	3.27	4.87	4.80	5.20	5.21	4.37	4.87
DL-isoleucine	3.36	2.39	3.45	3.05	2.65	3.35	3.37	3.58	4.81	3.22	3.52
DL-valine	4.88	3.02	3.54	3.59	2.49	8.36	5.22	4.13	4.37	4.40	4.61
DL-aspartic acid	12.21	9.03	5.66	8.39	7.48	5.61	7.03	6.54	4.94	8.65	6.81
DL-alpha-alanine	6.47	5.27	4.87	4.33	4.19	5.47	4.14	1.08	0.05	0.03	5.22
L-proline	2.43	4.24	7.10	2.24	2.22	4.03	3.57	3.77	3.87	3.09	3.26
glycine	4.18	3.06	3.43	4.34	3.79	4.94	3.60	3.69	3.49	4.33	.401
L-glutamine	14.55	11.05	11.63	24.64	13.10	13.81	7.50	9.66	6.18	9.40	8.16
L-cystine	0.1	0.19	0.10	0.34	3.085	—	trace	0.15	—	—	trace
L-tyrosine	0.12	trace	0.34	1.24	1.25	0.74	0.52	trace	1.34	0.92	1.40
Total	75.49	58.76	65.50	77.59	61.95	66.75	60.04	64.56	59.62	59.06	64.15

calf serum. Then these mixtures(pH 7.2) were sterilized by filtration through milipore filter. HeLa cells were detached from the surface of culture vessels[15)16)] with trypsin-versene solution[17)] (Difco 1:250) and the concentration was adjusted to 2×10^5 cells/ml in culture medium, and then 1.0ml of cell suspension was placed in each Leighton tube and incubated 37°C for three days.[18)]

Appearance of the proliferating cells was observed[19)] under an inverted microscope twice a day and the density of the cells in the culture media was counted by means of hemocytometer at 24, 48 and 72 hours of incubation by discarding the culture medium, dispersing the cells by one ml of trypsin-versene solution and resuspending the cells into appropriate amounts. To minimize the experimental errors, each procedure was run in triplicate.

RESULTS AND DISCUSSION

The morphology of the cultured cells in the Earle's BSS mixtures was as normal as that of the cells in the TC-199 medium, forming the normal epithelial cell monolayer along the wall of the culture vessels.

The cells in the Earle's BSS degenerated gradually as time passed and the morphology of cells changed into round shapes until all the cells were destroyed. (Figs. 1,2, and 3)

The appearance and density of the cells cultured for 24, 48 and 72 hours in the media containing the alcohol extracts and the acid hydrolysates of 11 different mushrooms are shown in Plate 1. (Fig. 4) The alcohol extracts

of *Coprinus comatus, Agaricus campestris, Agaricus bisporus, Lentinus edodes and Tricholoma matsutake* and the acid hydrolysates of these five mushrooms had favorable effects on the cell proliferation both in their appearance and density as compared with those of the control groups in TC-199 medium. When the alcohol extracts and the acid hydrolysates of *Pleurotus ostreatus, Ramaria botrytis* and *Pholiota nameko* were added to the media, the cell proliferation was well maintained until 48 hours and thereafter cellular degeneration appeared. The alcohol extracts and the acid hydrolysates of *Auricularia auricula-judae, Tremella fuciformis* Berk. and *Gyrophora esculenta* affected the cell proliferation to such degree that they kept on proliferating longer than 24 hours, and all the cells underwent degeneration.

CONCLUSION

In order to find out nutritional effects of edible mushrooms on the multiplication of tissue cells, the alcohol extracts and acid hydrolysates of eleven species of mushrooms were added to solution B (Earle's BSS containing 5% fetal calf serum). A comparison of the respective multiplications of HeLa cells in this solution and in control solution A (TC-199 solution containing 5% fetal calf serum) yielded the following conclusion:

1. The alcohol extracts and acid hydrolysates of *Corinus comatus, Agaricus campestris, Agaricus bisporus, Lentinus edodes, Tricholoma matsutake, Pleurotus ostreatus, Ramaria botrytis* and *Pholiota nameko* influenced favorably the maintenance of the normal form and monolayer of HeLa cells.

2. The growth curves of HeLa cells in the cultures containing, respectively, the alcohol extracts and acid hydrolysates of these eight mushrooms showed that five species, i. e., *Coprinus comatus, Agaricus campestris, Agaricus bisporus, Lentinus edodes* and *Tricholoma matsutake* effected an excellent multiplication and that the other three species were less effective than those five species.

3. As to the effects on the cell multiplication, no marked difference was observed between the alcohol extracts and the acid hydrolysates of the mushrooms tested.

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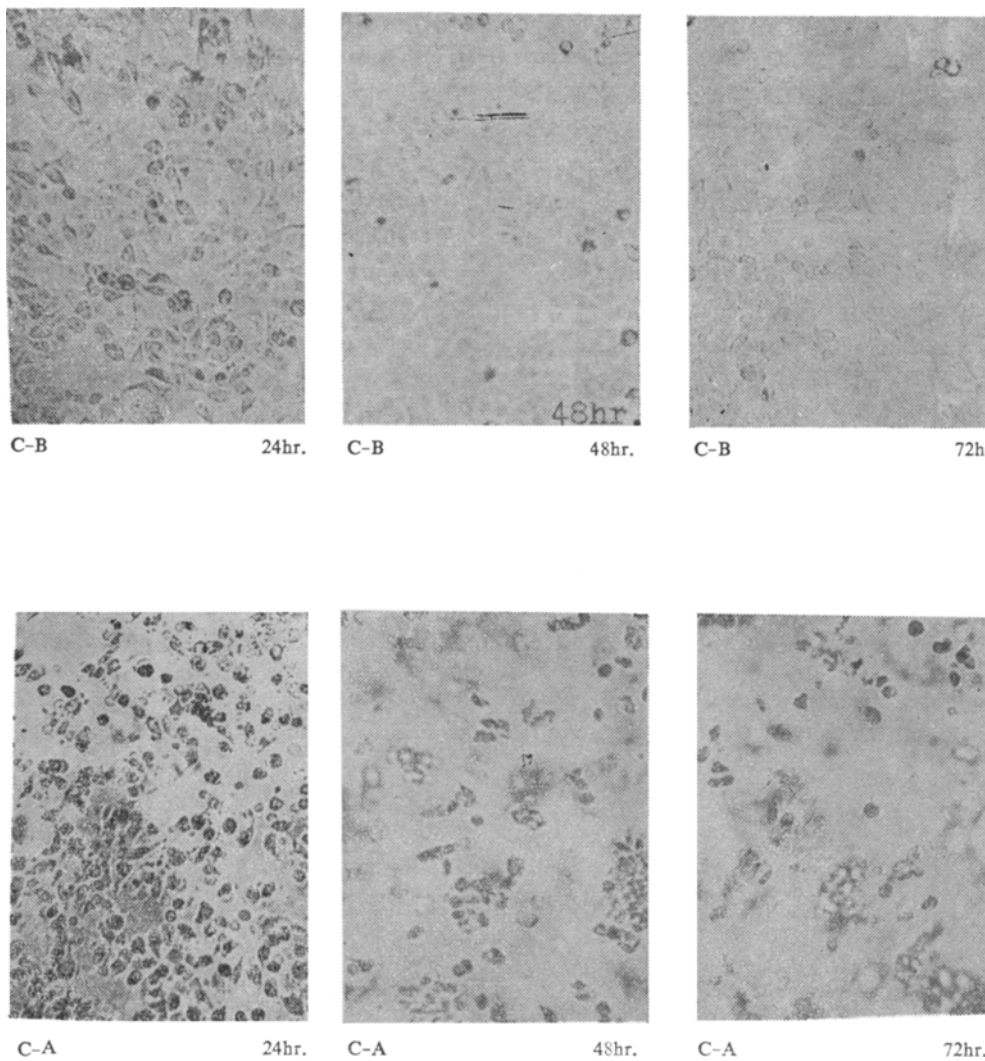

Fig. 1: Photographs showing the morphology and density of the cultured cells in TC-199 medium(C-A) and 5% fetal calf serum contained Earle's BSS (C-B) at different incubation period.

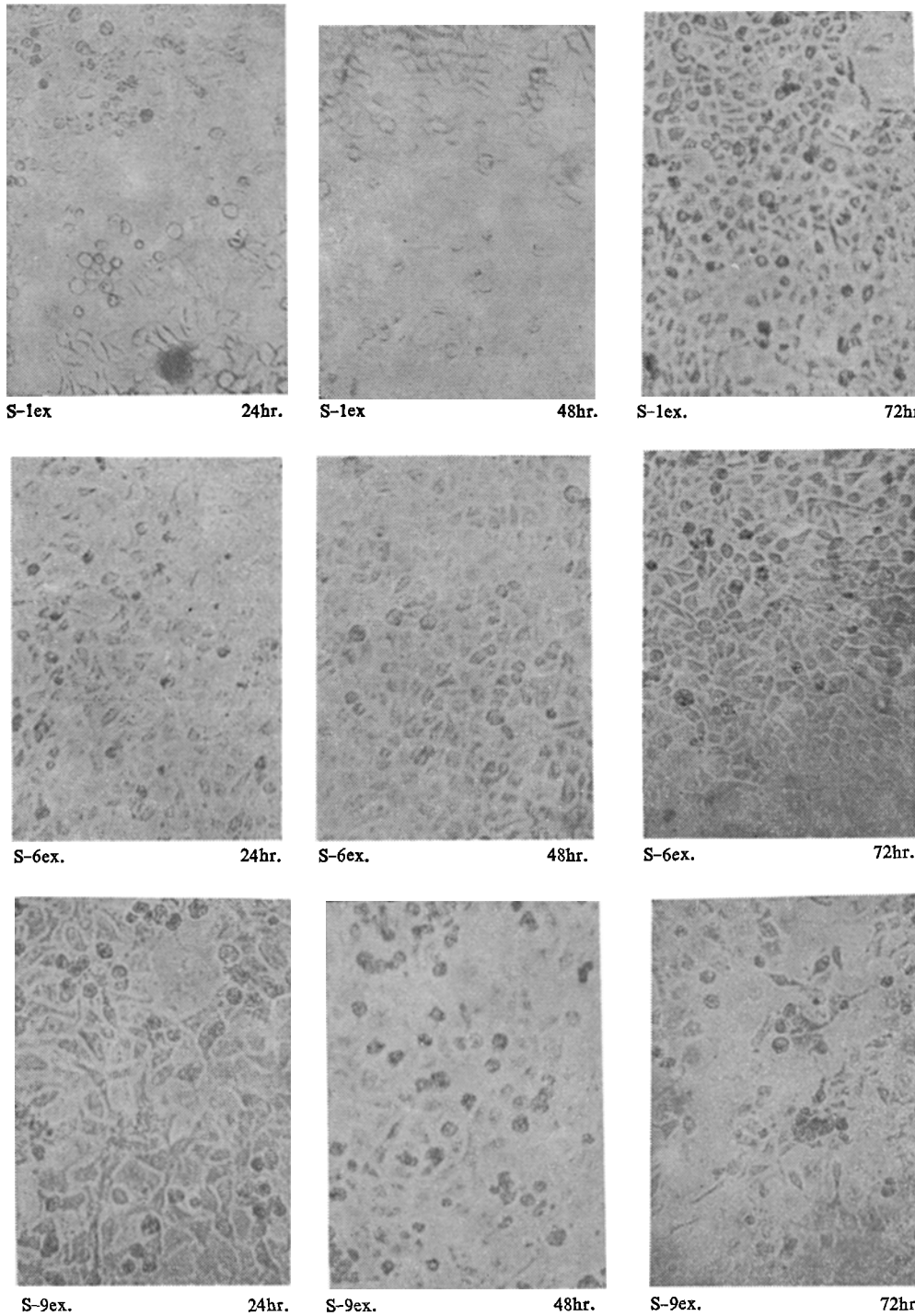

Fig: 2. Photographs showing the morphology and density of the cultured cells in media containing mushroom alcoholic extracts at different incubation period. S-1~11: as shown in Table I.

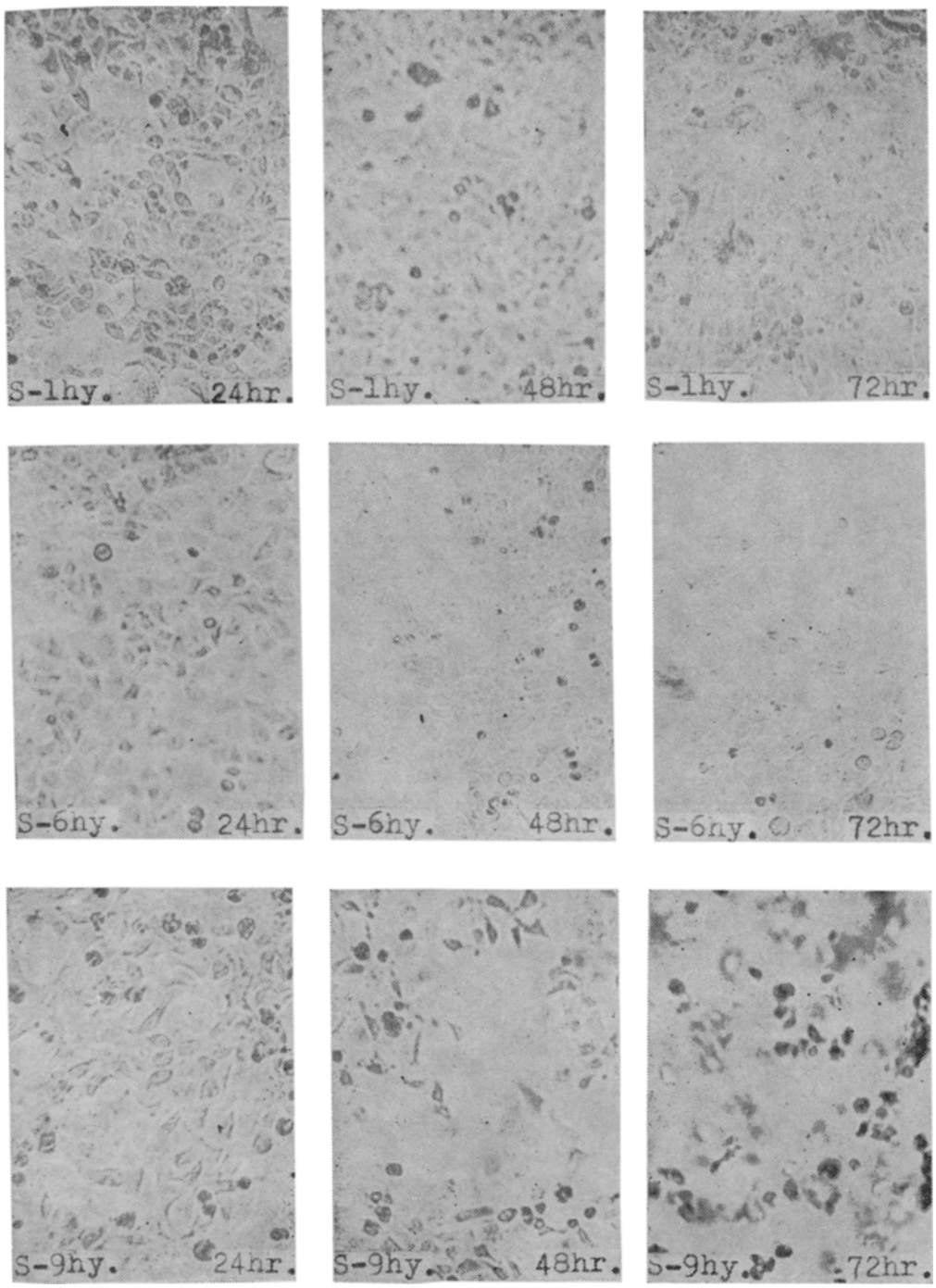

Fig 3: Photographs showing the morphology and density of the cultured cells in media containing mushroom acid hydrolysates at different incubation period. S-1~11: as shown in Table I.

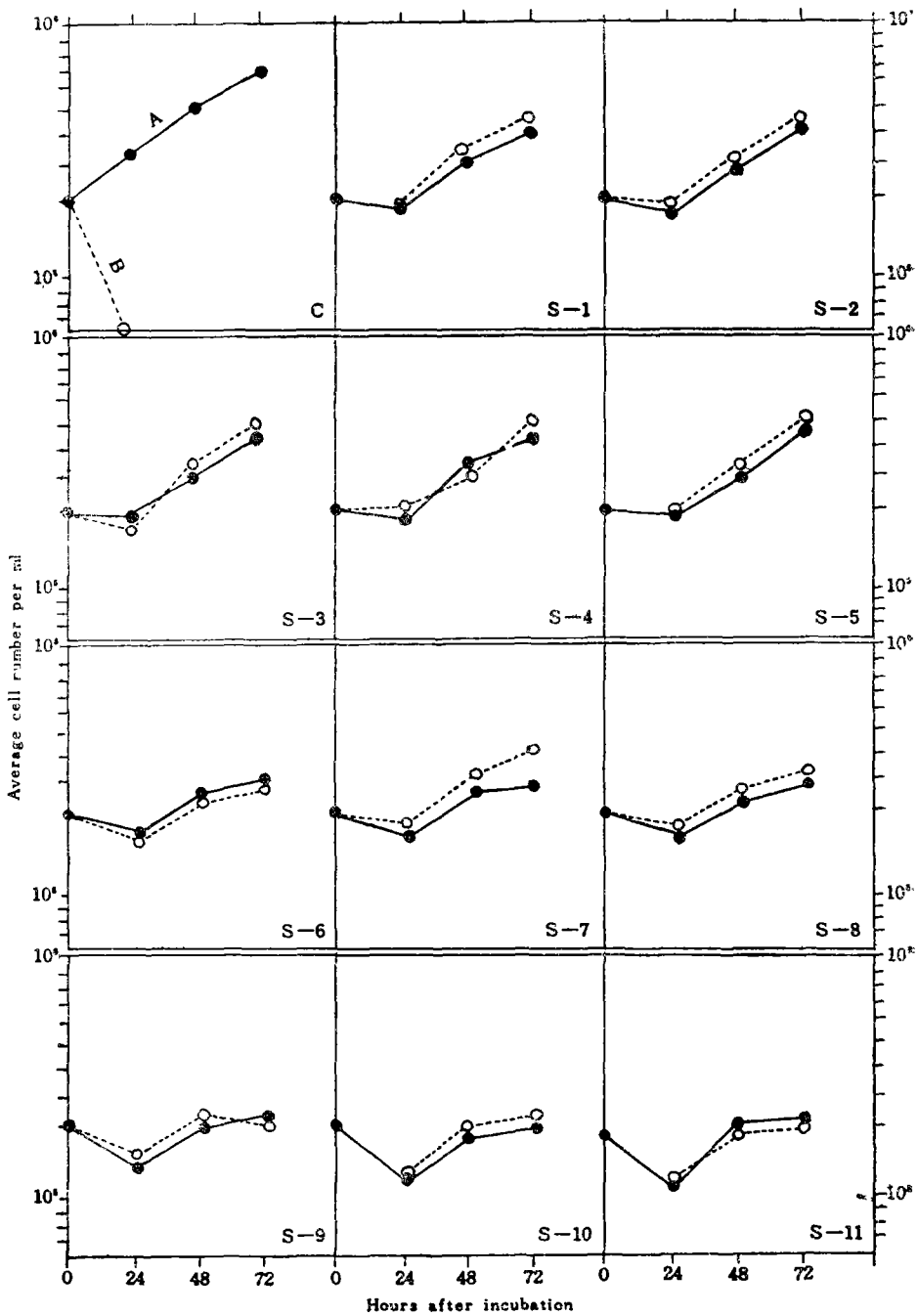

Fig. 4: Average number of cell count in B culture medium (Earle's BSS contained 5% fetal calf serum) containing alcoholic extracts (● — ●) and its acid hydrolysates (○ — ○) of mushrooms at different incubation times. C: control, A: TC-1 9 medium, B; 5% fetal calf serum-Earle's BSS. S-1~11: as shown in Table I.