ABSORPTION, DISTRIBUTION AND EXCRETION OF NEOCARZINOSTATIN (NCS) IN MICE AFTER ORAL ADMINISTRATION

KAZUYOSHI TORIYAMA, HIROSHI FUJITA* and NAKAO ISHIDA

Department of Bacteriology, Tohoku University School of Medicine, Sendai, and
Department of Bacteriology, Tsurumi University School of
Dental Medicine*, Yokohama, Japan

(Received for publication February 21, 1974)

Distribution, excretion and toxicity of an antitumor protein, neocarzinostatin (NCS) were examined in mice after oral administration. The oral LD_{50} was 1 g/kg compared to 1 mg/kg after intravenous injection. After oral administration of 200 mg/kg of NCS, the tissue level was low but detectable in lung, skin and pancreas in addition to the tissues of the gastrointestinal tract. The NCS level in lung and skin remained constant through 6 hours. In gastrointestinal tissues after oral administration the level was higher in the stomach than the large intestine or small intestine. The total recovery of orally administered NCS in feces of mice was 26.5% of the given dose during the first 12 hours. Inactivation of NCS by homogenates of small and large intestines (about 50%) was found in *in vitro* experiments.

The antitumor antibiotic, neocarzinostatin, was isolated by ISHIDA *et al.*[1,2] from the culture filtrate of *Streptomyces carzinostaticus* var. F-41. Its was recently shown by MEIENHOFER *et al.*[3] to be an acidic single-chain polypeptide with a molecular weight of 10,700, consisting of 109 L-amino acids. The N- and C-terminal amino acids are alanine and asparagine, respectively. Two cysteinyl disulfide bonds are present in its molecule, and an intact stereochemical structure including cross linkage by disulfide bonds is necessary for biological activity.

The antibiotic is highly active against various ascitic tumors in mice and rats when given intraperitoneally.[1,4,5,6,7] Among various microorganisms, *Sarcina lutea* and a mutant of *Shigella* are known to be most sensitive to the inhibitory action of NCS.[1,8]

After intravenous injection, NCS is well-absorbed and excreted quickly in urine, as reported by FUJITA *et al.*[9,10,16] In mice injected intravenously with a dose of 100 mg/kg, the tissue levels of NCS were reported to be highest in kidney, with decreasing amounts in blood, tumor tissue, skin, stomach, lung, pancreas, thymus and muscle. In some respects, the distribution of NCS resembles that of bleomycin, but is different in that a high NCS concentration is found in stomach and pancreas.[9,16]

Since NCS is resistant to the action of proteolytic enzymes such as trypsin, chymotrypsin, papain and pronase[2,3,11] and is stable at acidic pH,[1,2] a distribution study of NCS after oral administration was conducted in mice with the results reported here. The marked chemotherapeutic effect of NCS on some limited experimental tumors in rats after oral administration will be reported elsewhere by SATOH of Sasaki Institute.[12]

Materials and Methods

Neocarzinostatin: Neocarzinostatin (NCS) was prepared at Kayaku Antibiotics Research

Co., Ltd., Tokyo. The NCS sample was finally purified by chromatography on DEAE cellulose and proved to be a single protein with an activity of 1,560 mcg u/mg.

Toxicity test of NCS: The acute toxicity of NCS was examined not only in mice but also in rats and rabbits. Male mice of *ddN* strain weighing an average of 20 g, male rats of Wistar strain weighing an average of 150 g and male rabbits weighing an average of 2.2 kg were purchased from Saitama Experimental Animal Farm Co. The LD_{50} after single intravenous injection was calculated and compared to that after oral administration. Two-fold dilutions of NCS were made in saline and groups of 10 mice, 10 rats and 2 rabbits were used for each concentration. The observation period was 10 days. The weight change of each animal and gross lesions of moribund animals were examined.

Method of assay for NCS: The concentration of NCS was determined by the cylinder method on thin agar plates[9,18], using *Sarcina lutea* PCI 1001 as the test organism. For testing NCS activity against *Sarcina lutea*, Heart Infusion agar medium (Nissui Seiyaku Co., Ltd.) was used for the maintenance of *Sarcina lutea* and modified MUELLER-HINTON medium (Nissui Seiyaku Co., Ltd.) for the assay. The lowest detectable concentration of NCS with *Sarcina lutea* was 0.02~0.05 mcg/ml.

Distribution of NCS in mice after oral administration: The blood level and distribution of NCS in mouse organs after oral administration of 200 mg/kg were examined, in comparison with those after intravenous injection of the same dose. For these tests, male mice of *ddN* strain weighing an average of 21 g were used. For the distribution study, each organ was removed after exanguination by cardiac puncture and the organs taken from 3 mice were pooled, washed 3 times with 40 ml of ice-cold saline and homogenized in cold distilled water. These homogenates were diluted 5 and 20 times with distilled water before assay. Serum, urine and bile were appropriately diluted with distilled water before assay. From the bioassay results, NCS (mcg) in one gram of tissue or one milliliter of fluid was calculated.

Recovery of NCS in feces of mice: Six male mice, each weighing 20 g, in individual metabolic cages received 0.2 ml of the NCS saline solution (20 mg/ml) orally (200 mg/kg). The feces of each mouse were collected every 6 hours during the first 24 hours, weighed and homogenized in distilled water. The homogenate was diluted 5~40 times with distilled water for assay.

In vitro inactivation of NCS by gastrointestinal tissue homogenate: Five male mice were used. Similar tissues from stomach, small intestine and large intestine were combined, washed three times with 40 ml of saline after the removal of the contents and 20 % tissue homogenates were prepared in saline. To 100 mcg/ml of NCS solution in saline, an equal volume of each tissue homogenate was added, making 50 mcg/ml NCS in 10 % tissue homogenate. All procedures were carried out at 4°C. Aliquots were held at 4°C and 37°C for one hour. The residual activity of NCS in each tissue homogenate after incubation at 37°C was compared with that of the control kept at 4°C.

Results

Acute Toxicity of NCS

As shown in Table 1, the LD_{50} value of NCS in mice intravenously and orally was 0.96 mg/kg and 1,050 mg/kg, respectively. Such a big difference in toxicity between intravenous and oral administrations was also found in rats and rabbits (Table 1). The acute toxic dose of NCS after a single intravenous injection was found to be almost the same in mice, rats and rabbits.

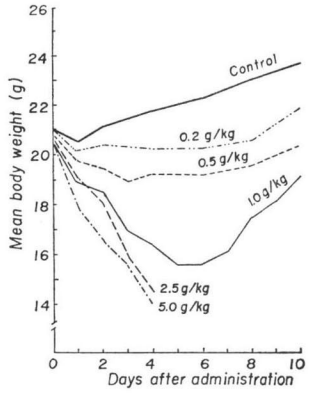
Toxic signs were carefully examined in mice. When the groups of mice were injected intravenously with more than 2.5 mg/kg of NCS, the mice showed a marked loss of body

Table 1. Acute toxicity of neocarzinostatin

Animal	Route*	LD_{50} (10 days) mg/kg
Mouse (ddN, male)	I V	0.96
	P O	1,050
Rat (Wistar, male)	I V	0.83
	P O	>300
Rabbit (male)	I V	0.9~1.0
	P O	>600

* IV: intravenous. PO: per oral.

Fig. 1. Effect of single oral administration of NCS on mouse body weight.



weight and died between 3 and 6 days after injection. The gross lesion of moribund mice was characterized by the presence of hyperemia and hemorrhage in intestine and lung, and also by atrophy of spleen and thymus. When the amount of NCS given was decreased to 0.5 mg/kg, a slight loss of body weight was found during the first 2~3 days and then a gain in weight occurred. These mice showed ruffled fur for the first few days.

The change in body weight after oral administration is illustrated in Fig. 1. When the dose of NCS was more than 2.5 g/kg, body weight of mice rapidly decreased from 20.6 g to 14.3 g within 4 days and all 10 mice died between 4 and 5 days. When the oral dose was 1.0 g/kg, the decrease of body weight was from 20.5 g to 15.6 g within 5 days and then a rapid gain of body weight occurred. However, the initial body weight was not recovered after 10 days and 3 out of 10 mice died on the 4th day and one on the 7th day. With 0.5 g/kg oral administration, a slight decrease of body weight occurred for 3 days. Although a tendency to recover was noticed after 8 days, the gain of body weight was not the same as controls. In the case of a dose of 0.2 g/kg orally, the body weight did not show any decrease or increase during the first 8 days, but thereafter, the recovery of growth rate was appreciable. All 10 mice receiving 0.5 g/kg or 0.2 g/kg orally survived.

Mice receiving one LD_{50} oral dose of NCS (1.0 g/kg) were inactive with ruffled fur and anorexia. Half died between 5 and 8 days. The symptoms were almost the same with mice receiving one LD_{50} intravenous dose (1 mg/kg), although death occurred between 3 and 5 days after intravenous injection.

When the gross lesions of moribund mice were examined after oral administration, hemorrhage in mucosa of pylorus to duodenum or in whole gastrointestinal tissues, mottled hypere-

Table 2. Blood level (mcg/ml) of neocarzinostatin in mouse after intravenous and oral administration

Minutes	3	5	10	15	30	60	90	120
I V	1550	642.5	230	186.3	18.8	3.1	nd	—
P O	nd**	nd	—*	nd	—	0.5	0.2	—

Male ddN mice (20~26 g) were given 200 mg/kg dose of NCS.
* <0.024 mcg/ml. ** not done

emia of lung and atrophy of spleen and thymus were apparent. After oral admintration, the longer the survival period, the more extensive the intestinal hemorrhage.

Distribution of NCS in Mice after Oral Administration

Symptoms and autopsy findings of mice receiving orally one LD_{50} dose of NCS were almost the same as those receiving intravenously one LD_{50} dose. As the absolute amount of NCS introduced to mice was almost 1,000 times greater in the former case, either the low absorption of NCS from the intestinal tract or inactivation of NCS in the intestinal tract might be a possible explanation. Therefore, the absorption and distribution of NCS in mice after oral administration were examined.

When the blood levels of NCS in mice after intravenous and oral administration were compared, the results shown in Table 2 were obtained. After intravenous injection of 200 mg/kg, the blood level of NCS reached a maximum (about 1,550 mcg/ml) within 3 minutes and decreased to half the maximum at 5 minutes. NCS was not detectable in blood 2 hours after injection.

With oral administration of 200 mg/kg, 0.5 mcg/ml blood level was found at 60 minutes and 0.2 mcg/ml at 90 minutes. These results indicate that maximum absorption of NCS in mice occurrs 60~90 minutes after oral administration, and the amount absorbed is quite limited.

Almost the same blood level (0.5 mcg/ml) was obtained within 3 minutes, when 0.5 mg/kg of NCS was given intravenously in another experiment. On this basis, when the ratio of

Table 3. Distribution of neocarzinostatin in mice after 200 mg/kg oral administration

Tissues	mcg/g or mcg/ml			
	10 min.	30 min.	60 min.	90 min.
Stomach	65.7	30.4	15.1	17.1
Small intestine	18.8	12.1	1.6	2.6
Large intestine	0.6	—	—	—
Lung	0.2	0.31	0.4	0.2
Skin	0.2	0.18	0.16	0.5
Pancreas	—*	0.15	0.13	—
Heart	—	—	—	—
Thymus	—	—	—	—
Liver	—	—	—	—
Spleen	—	—	—	—
Kidney	—	—	—	—
Testis	—	—	—	—
Muscle	—	—	—	—
Serum	—	—	0.5	0.2
Blood sediment	—	—	—	—
Urine	—	—	0.2	—
Bile	—	—	—	—
Gastric content	617.5	217.5	312.5	373.5
Small intestine contente	590	378	370	258
Large intestine contente	1.3	0.17	5.2	—

*: <0.025 mcg/g or ml

oral dose to intravenous dose to obtain the same blood level is calculated, the value of 400 is obtained. This ratio is fairly close to the ratio of 1,000 for the LD_{50} of NCS by oral or intravenous administration.

The distribution of NCS in organs of mice after oral administration of 200 mg/kg is shown in Table 3. High concentrations of NCS were detectable at 90 minutes both in the stomach and small intestine after oral admistration, though NCS in these organs might be largely accounted for by residual NCS. The presence of NCS in the large intestine was detectable only at 10 minutes.

The highest concentration of NCS (65.7 mcg/g) found in the stomach 10 minutes after oral administration did not decrease rapidly and the concentration of NCS 90 minutes after administration was comparable to that after 60 minutes. The concentration of NCS in the stomach after oral administration of 200 mg/kg was 4~16 times that found after intravenous injection of 100 mg/kg.[16] After oral administration of 200 mg/kg NCS as much as 18.8 mcg/g was found in the small intestine.

When the concentrations of NCS in the contents of stomach and small intestine were examined, a high concentration of NCS, about 600 mcg/g, was detected at 10 minutes and half that amount 30 minutes after oral administration. Such a high concentration of NCS remained without inactivation when examined 90 minutes after the administration. On the contrary, only a small amount of NCS was detectable in the large intestine content at 10 and 30 minutes and the maximum concentration of 5.2 mcg/g was attained at 60 minutes. When examined at 90 minutes, no significant amount of NCS was detected. It was concluded that a large amount of NCS did not reach the large intestinal tract within the first 90 minutes, although examination was not conducted beyond 90 minutes in this experiment.

Except for gastrointestinal tract tissues and contents, NCS was detectable in lung, skin and pancreas after oral administration. In lung, a 0.2~0.4 mcg/g concentration was maintained for 90 minutes. In skin, 0.16~0.5 mcg/g of NCS was found up to 90 minutes and in pancreas, around 0.15 mcg/g of NCS was detectable 30 and 60 minutes after oral administration.

Different from the results obtained after intravenous injection[16], the concentration of NCS in urine was quite low reflecting the low serum level.

Table 4. Distribution of neocarzinostatin along gastrointestinal tract of mice after 200 mg/kg oral administration

Tissues	mcg/g or mcg/ml			
	2 hrs.	4 hrs.	6 hrs.	48 hrs.
Stomach	2.1	4.1	—	—
Small intestine	1.6	—	—	—
Large intestine	21.8	16.0	9.7	—
Lung	0.42	0.44	0.39	—
Skin	0.34	0.53	0.43	—
Pancreas	trace	trace	trace	—
Serum	—	—	—	—

Fig. 2. Level of NCS in gastrointestinal tract tissues of mice after oral administration of 200 mg/kg of NCS.

Tissues removed from exanguinated mouse were washed three times with saline to remove the contents as much as possible.

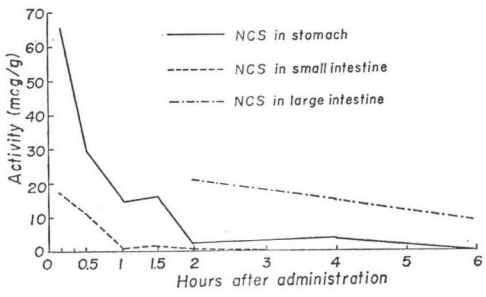


Table 5. Fecal excretion of neocarzinostatin in mice (ddN, male) after oral administration
Dose: 200 mg/kg P.O.

Hours after administration	Mice*	Fecal vol. (g)	NCS activity		Recovery rate (%)	
			Concentration (mcg/g)	Total NCS (mcg)	Individual	Average
0~6	A*	0.156	3,560	555.4	12.51	16.47 ±7.94
	B	0.260	2,332	606.3	15.16	
	C	0.081	3,200	259.2	5.31	
	D	0.448	1,930	864.6	18.24	
	E	0.171	7,280	1,245.0	31.44	
	F	0.240	2,614	627.5	16.17	
6~12	A	0.325	1,340	435.5	9.81	10.03 ±7.18
	B	1.053	16	16.8	0.42	
	C	0.590	1,880	1,109.2	22.73	
	D	0.240	1,724	413.8	8.73	
	E	0.120	2,888	334.6	8.45	
12~18	A	0.230	—			
	B	0.984	—			
	C	0.665	—			
	D	1.880	—			
18~24	A	0.048	—			
	B	0.447	—			
	C	0.126	—			
	D	0.480	—			

* weight of mice; A: 22.2g, B: 20.0g, C: 24.4g, D: 23.7g, E: 19.8g, F: 19.4g.

As NCS tended to remain in various tissues after oral administration (Table 3), the distribution of NCS in mouse organs was reexamined at 2, 4, 6 and 48 hours after oral administration of 200 mg/kg. The results shown in Table 4 and Fig. 2 indicate that 2.1 and 4.1 mcg/g of NCS were found in stomach tissues 2 and 4 hours after administration. In small intestine a small amount of NCS, 1.6 mcg/g, was detectable only at 2 hours. In large intestine, no significant level of NCS was detectable at 90 minutes. However, as shown in Table 4 and Fig. 2, a very high level of NCS, 21.8 mcg/g, was detected in large intestine 2 hours after oral administration and a relatively high level was found even at 6 hours.

The results illustrated in Fig. 2 indicate the early and high distribution of NCS in stomach and the late and continued distribution of NCS in large intestine. However, the concentration of NCS in small intestine was relatively low.

Total Recovery of NCS in Feces

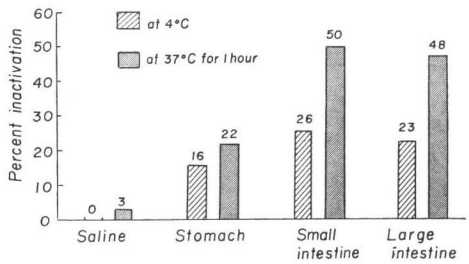
As the blood level and concentrations in tissues other than intestinal tract were low after the oral administration, the absorption of NCS to body fluids by oral route was assumed to be very low. NCS is known to be resistant to the action of known proteases.[2,8,11] Thus a high recovery of NCS in feces was anticipated. However, when the amount of NCS in feces was examined in 6 mice after oral administration of 200 mg/kg, the results shown in Table 5 were obtained. The recovery of NCS in feces was 16.5% (8.5~24.4%) of the given dose

in the first 6 hours and 10.0% (2.9~17.2%) in the next 6 hours. NCS could not be found thereafter. In average, only 26.5% (11.4~41.6%) of the given dose was recovered within 12 hours.

In vitro Inactivation of NCS by Intestinal Tissue Homogenate

As the fecal excretion of NCS after oral administration was found to be relatively low contrary to expectation, the possible inactivation of NCS was examined *in vitro* by incubating a NCS solution with mouse intestinal tissue homogenates. The results obtained are shown in Fig. 3. The decrease of NCS titer at 4°C was 16% with stomach homogenate and 25% with homogenates of small and large intestines. Such a decrease at 4°C might be explained by an adsorption of NCS to these homogenates. When the same mixture was incubated at 37°C for one hour, the inactivation of NCS was found to be less than 3% in saline and 22% in stomach homogenate, but it was as high as 50% in homogenates of small and large intestines. Thus the inactivation of NCS by stomach homogenate was almost negligible, but the inactivation by intestinal homogenates was fairly high *in vitro*. Further studies on the nature of enzymes detectable in small and large intestines or in their contents are under way.

Fig. 3. *In vitro* inactivation of NCS in the presence of 10% gastrointestinal tissue homogenates of normal mice.



* Initial 50 mcg/ml concentration of NCS was used. For other details, see the text.

Discussion

As a part of the preclinical studies, the absorption, distribution and excretion of NCS after oral administration were examined in mice. A big difference in 50% lethal dose (1,000: 1) was found between oral and intravenous administrations and further analysis was conducted to explain this difference.

Symptoms of mice receiving orally 1 g/kg dose (≒1 LD_{50}) and intravenously 1 mg/kg dose (≒1 LD_{50}) were almost identical and the gross lesions of moribund mice were similar between these two groups of mice, except for a stronger hemorrhagic tendency of small and large intestines in mice receiving oral administration. On the basis of such observations, one may anticipate extremely limited absorption of orally given NCS through the intestinal tract into the blood stream. Generally speaking, the absorption of protein from the gastrointestinal wall is known to be extremely low[14] and NCS is a protein of 10,700 molecular weight. In fact, when the amount of NCS to be given to mice to obtain similar blood levels was compared for oral and intravenous administrations, the former procedure required 400-times as much NCS. This ratio is close to the LD_{50} ratio of 1,000, described above. Thus the low toxicity of NCS in mice after oral administration can be explained by low absorption through intestinal wall.

NCS distribution was evident in lung, skin and pancreas in addition to the gastrointestinal tissues after oral administration. Furthermore, atrophy of spleen and thymus was conspicuous after autopsy of the moribund mouse. These results indicate the distribution of a limited but consistent amount of NCS to various organs after oral administration as was found after intravenous injection.[9,10,16]

After intravenous injection of 200 mg/kg dose, the maximum blood level was attained

within 3 minutes and NCS rapidly disappeared from the blood stream. This finding was concordant to the accelerated excretion of NCS into urine through kidney.[16] After oral administration, on the other hand, low and slow absorption of NCS was noticed. The maximum blood level was attained 1 hour after administration, and an appreciable amount of NCS was not detectable in serum from 2 to 6 hours after oral administration (Table 4). However, the maintenance of NCS titer in lung and skin for the period from 2 to 6 hours (Table 4) may suggest the continued absorption through intestinal tract on one hand, and possible absence of inactivation process of NCS in these tissues, on the other. In fact, high concentrations of NCS can be detected for such a period in the tissues of stomach and small intestine (Fig. 2). Other studies (to be reported by KIKUCHI) indicate the absence of NCS-inactivating enzymes in lung and skin.

NCS is quickly excreted in urine when given intravenously, and almost 78% of the initially given dose was recovered.[9,16] NCS given to mice by the oral route was mainly excreted in feces. The all-over recovery in fecal excretion was calculated to be 26.5% (11.4~41.6%) of the given dose. As described above, the acute toxicity of NCS in mice after oral administration should be 400~500 mg/kg on the basis of blood level comparison, but it was actually 1 g/kg. Such a difference might be explained if 50% inactivation of NCS could occur in intestine. Such an assumption was supported by two experimental results, one by the 26.5% recovery of given NCS in feces and another by inactivation which actually occurred *in vitro* by incubating NCS with intestinal homogenates.

The level of NCS concentration in gastrointestinal tissues after a single oral dose was 2.0~66.0 mcg/g in stomach, 1.6~18.8 mcg/g in small intestine, and 9.7~21.8 mcg/g in large intestine. NCS could be found in the stomach for 4 hours, for more than 4 hours in large intestine and for 2 hours in small intestine (Fig. 2). The total recovery of NCS in gastrointestinal tissues of mice after oral administration (200 mg/kg) was almost one percent of the given dose at maximum. When the time course study shown in Table 3 and Fig. 2 is taken into consideration, it appears that a part of NCS found in the gastrointestinal tissues is residual, reflecting the large amount found in the intestine contents. Further detailed studies on the fate of NCS along the gastrointestinal tissues seem to be necessary to determine whether oral administration of this antibiotic is preferable to intravenous administration to attain a high concentration in gastrointestinal tissues.

Meanwhile, clinical trials conducted by ANEHA *et al.*[15] revealed the absorption of NCS to stomach tissues after oral administration. When patients with stomach cancer received 6 mg orally 0.16~1.5 mcg/g was detectable in gastric mucosa and 0.24~0.5 mcg/g in gastric serosa in the initial 3~5 hours. This result can be explained as the consequence of NCS distribution through the blood stream rather than direct contact. However, the possible distribution through lymph duct has not been examined yet.

In animal experiments, oral administration of NCS was found to be effective against AH 66, AH44 and AH130 among 10 tested ascites hepatomas in rats inoculated with 10^7 cells intravenously.[12] In this experiment a 24 mg/kg/day oral dose of NCS was given for consecutive 10 days, starting 72 hours after tumor implantation. AH66 and AH130 are known to cause metastasis into the lung of rats. The data indicate the distribution of NCS into various organs including lung after oral administration.

Acknowledgements

Authors are grateful to Dr. H. SATOH of Sasaki Institute, Tokyo, and Dr. M. KIKUCHI of Department of Bacteriology, Tohoku University School of Medicine, for their helpful discussions and encouragements.

Authors are also indebted to Dr. Y. KOYAMA, Dr. T. MATSUMOTO, Mr. M. SHOJI and all other members of NCS research of Kayaku Antibiotics Research Co., Ltd. for their generous supply of neocarzinostatin and advices on toxicology.

References

1) ISHIDA, N.; K. MIYAZAKI, K. KUMAGAI & M. RIKIMARU: Neocarzinostatin, an antitumor antibiotic of high molecular weight: Isolation, physicochemical properties and biological activities. J. Antibiotics, Ser. A 18: 68~76, 1965
2) MAEDA, H.; K. KUMAGAI & N. ISHIDA: Characterization of neocarzinostatin. J. Antibiotics, Ser. A 19: 235~259, 1966
3) MEIENHOFER, J.; H. MAEDA, C. B. GLASER, J. CZOMBOS & K. KUROMIZU: Primary structure of neocarzinostatin, an antitumor protein. Science 178: 875~876, 1972
4) KUMAGAI, K.: Antitumor activity of carzinostatin. J. Antibiotics, Ser. A 15: 53~59, 1962
5) BRADNER, W.T. & D.J. HUTCHISON: Neocarzinostatin (NCS-69856): an antitumor antibiotic effective against ascitic leukemia L 1210 in mice. Cancer Chemother. Rep. 50: 79~84, 1966
6) SATOH, H. & H. ICHIMURA: Antitumor spectrum of neocarzinostatin on ascites hepatomas in rats. Igaku no Ayumi 74: 499~500, 1970 (in Japanese)
7) SATOH, H. & H. ICHIMURA: Antitumor spectra of various antitumor agents on rat ascites hepatomas. Jap. J. Clin. Med. 29: 170~178, 1971 (in Japanese)
8) KIKUCHI, M.; Y. SASAKI & N. ISHIDA: Variants of *Shigella flexneri* sensitive to neocarzinostatin and actinomycin D. J. Antibiotics 22: 390~391, 1969
9) FUJITA, H.; N. NAKAYAMA, T. SAWABE & K. KIMURA: *In vivo* distribution and inactivation of neocarzinostatin. Jap. J. Antibiotics 23: 471~478, 1970 (in Japanese)
10) FUJITA, H.: Characteristic pattern of *in vivo* distribution, excretion and inactivation of several anticancer agents. Clinic All-Round 20: 1350~1359, 1971 (in Japanese)
11) KITAME, S.; H. MAEDA, K. KUMAGAI & N. ISHIDA: Tryptic digestion of an antitumor protein: Neocarzinostatin. J. Antibiotics 21: 460~462, 1968
12) SATOH, H.: Personal communication
13) MIYAMURA, S. & H. KANAZAWA: An assay of penicillin and streptomycin in body fluids by the cup method using thin agar plate. J. Antibiotics 3: 411~416, 1950 (in Japanese)
14) MORIYA, H.; K. YAMAGUCHI, S. KURIYAMA, M. IWADARE & C. MORIWAKI: Some problems on the studies of the absorption of enzyme preparation after oral administration. Igaku no Ayumi 64: 833~840, 1968 (in Japanese)
15) ANEHA, Y.; K. KIKUCHI, M. KIKUCHI & H. MOTOKI: Study on the oral administration of neocarzinostatin. Proc. Jap. Cancer Assoc.; 31st Ann. Meet. p. 116, 1972 (in Japanese)
16) TORIYAMA, K.; H. FUJITA, T. SAWABE, N. NAKAYAMA & K. KIMURA: Distribution, excretion and inactivation of pure neocarzinostatin. Proceeding of the 20th Annual Meeting of Japanese Chemotherapy, p. 33, 1972 (in Japanese)