$H^+ + Lactate^- \rightleftarrows$ Lactic acid,

as the $[H^+]$ is increased, the equilibrium is shifted to the right favoring the unionized form. At physiologic pH (6–7) the ionized species diffuses more slowly. The poor absorption of lactic acid at the pH of intestinal content is consistent with the conclusion that the osmotic activity of the short chain fatty acids contribute to the watery diarrhea in patients with sugar malabsorption.

It has been suggested that acid solutions damage the mucosa of the small intestine. Several investigators have described villus atrophy, inflammation of the lamina propria, and damaged surface epithelial cells in patients with low intraluminal pH due to Zollinger-Ellison syndrome, gastrojejunostomy, or pancreatic insufficiency (15–17). There were no morphologic changes in the intestine of rats after acid perfusion for 2 hours.

Summary. Lactic acid absorption was studied in anesthetized rats by perfusing isolated segments of small intestine or colon with sodium lactate-1-^{14}C for 2 hours. The concentration and pH of the perfusate were varied. Absorption was maximum at pH 2.8, near the p*K* of lactate, and minimum at physiologic pH. Absorption was directly related to concentration of the lactate perfused and it was not affected by a metabolic inhibitor. The jejunum was the site of maximum absorption. Absorbed lactate was metabolized to $^{14}CO_2$. Poor lactate absorption at physiologic pH and in the distal intestine might lead to an osmotic diarrhea in certain patients with malabsorption.

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Comparative Studies on Pathogenesis of Two Strains of Transplantable Carcinomas of Rabbits, Vx2 and Vx7* (32883)

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Cutaneous papillomas of rabbits induced by Shope papilloma virus (SPV)(1) frequently progress to carcinomas(2). From these malignancies, Rous and his associates have been able to establish several transplantable strains of carcinomas, the so-called Vx series, and among them Vx2(3) and Vx7(4) have been serially transferred for over 20 years to date.

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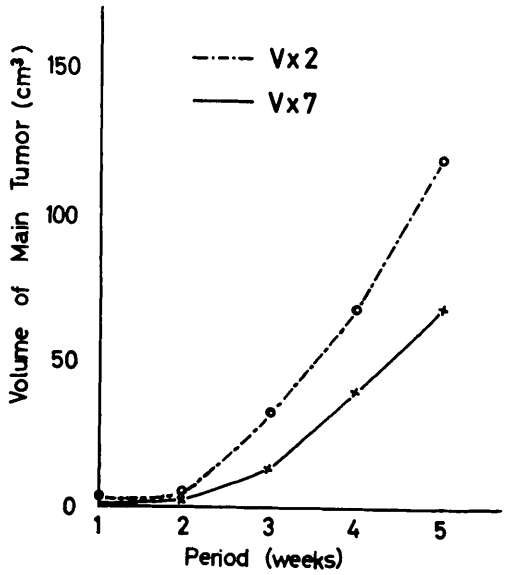


FIG. 1. Growth curve of Vx2 and Vx7 carcinoma transplanted in thigh muscle of domestic rabbits.

The major difference between these two neoplasia is documented as: (a) Vx7 still harbor viral antigen of SPV while Vx2 has lost its possession sometime in the past(3), however, evidence has recently been presented that not only the Vx7 but also Vx2 still retains the SPV antigen(5). (b) Histologically, Vx7 still retains characteristics of squamous cell carcinoma suggesting its epithelial origin whereas Vx2 exhibits features of a more anaplastic type carcinoma, and (c) From the viewpoint of pathogenicity, it has been believed more or less by experience or impression of the investigator that Vx2 is more malignant than Vx7. However, few comparative studies

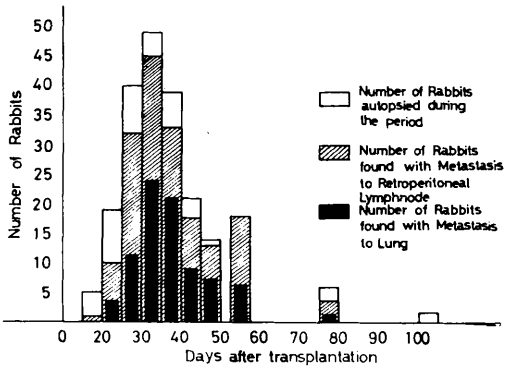


FIG. 2. Incidence of metastases to retroperitoneal lymph nodes and lungs in Vx2-bearing rabbits.

on this aspect of the two carcinomas have been carried out to date.

The present paper describes "quantitative" studies dealing with the comparison of pathogenicity of the two neoplasia with particular reference to their growth pattern and their capacity to induce metastases in remote organs. The data have been collected from 214 Vx2-bearing and 123 Vx7-bearing rabbits studied in our laboratory during the past 6 years.

Materials and Methods. Animals. White domestic rabbits (Nagano strain) of mixed sexes, purchased from a commercial farm,

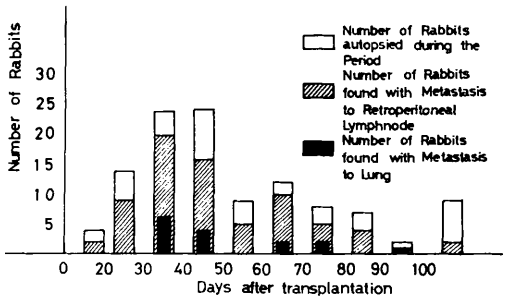


FIG. 3. Incidence of metastases to retroperitoneal lymph nodes and lungs in Vx7-bearing rabbits.

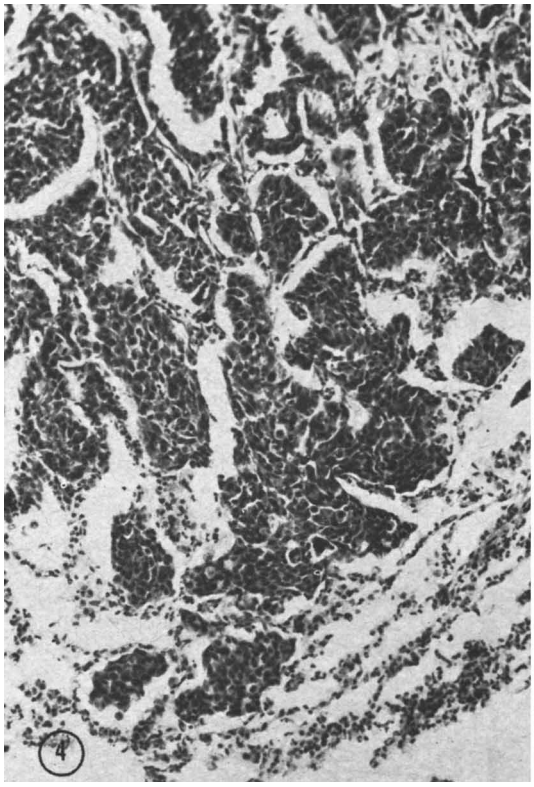
were used. They weighed 2.0–2.5 kg at the beginning of the experiment. The animals were housed individually in metal cages and were maintained on a diet of rabbit pellet (Funahashi Farm Co.) and tap water.

Tumors. Two strains of transplantable carcinomas, Vx2 and Vx7, both of viral etiology were used throughout. They were kindly provided by Dr. Peyton Rous of the Rockefeller Institute. The Vx2 carcinoma was in its eightieth transfer generation and Vx7 carcinoma in its one hundred sixty-six generation when they arrived at our laboratory about 5 years ago. Since then, Vx2 has been carried on for 47 generations and Vx7 for 40 generations to date (November 9, 1967). During this period of time, 213 Vx2-bearing and 114 Vx7-bearing rabbits were sacrificed at various time intervals after the transplantation of the tumors and autopsied to study the mode of development of the tumors and also the frequency of metastases to retroperitoneal lymph nodes, lungs, and other remote organs.

Transplantation. Both Vx2 and Vx7 car-

cinomas were transplanted into the thigh muscle of animals according to our standard procedure previously reported(6).

Observation. The tumors developing in the thigh muscle were measured once a week. The measurement of each radius in three dimensions, i.e., length(a), width(b) and thickness(c), were taken and the tumor volume



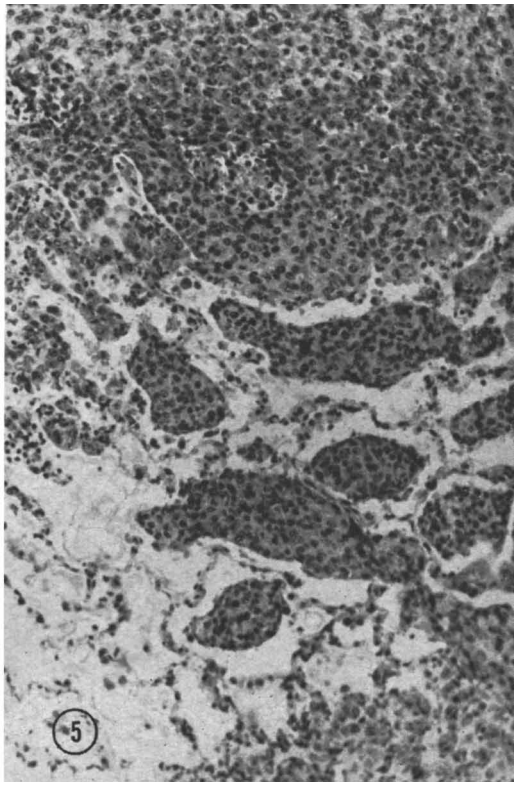


FIG. 4. Histological section of pulmonary metastasis of Vx2 carcinoma in rabbit. H and E stain; × 160.

FIG. 5. Histological section of pulmonary metastasis of Vx7 carcinoma in rabbit. H and E stain; × 160.

TABLE I. Number of Metastatic Nodules in Lungs of Rabbits Transplanted with Vx2 Carcinoma in Thigh Muscle.

Days after transplantation	Number of rabbits autopsied	Number of metastatic nodules in lungs[a]							
		0	1	2–10	11–30	31–60	61–100	100–150	151–
–20	5	5	—	—	—	—	—	—	—
21–25	19	17	—	—	2	—	—	—	—
26–30	40	29	3	—	4	—	—	1	3
31–35	49	25	2	2	9	—	3	—	8
36–40	39	18	2	6	3	4	3	1	2
41–50	35	20	2	2	3	1	2	2	3
51–60	18	12	—	—	3	2	—	—	1
61–	8	6	—	—	1	—	1	—	—
Total	213	132	9	10	25	7	9	4	17

[a] Metastatic nodules grossly countable on the surface of lungs of each animal.

TABLE II. Number of Metastatic Nodules in Lungs of Rabbits Transplanted with Vx7 Carcinoma in Thigh Muscle.

Days after transplantation	Number of rabbits autopsied	Number of metastatic nodules in lungs[a]				
		0	1	2–10	11–30	31–60
–20	4	4	—	—	—	—
21–30	15	15	—	—	—	—
31–40	24	14	1	6	1	2
41–50	24	20	1	2	1	—
51–60	9	9	—	—	—	—
61–70	12	10	—	1	1	—
71–	26	23	1	1	1	—
Total	114	95	3	10	4	2

[a] Metastatic nodules grossly countable on the surface of lungs of each animal.

(cm^3) was calculated, as a closest approximation, by the formula $3/4\ \pi\ abc$ in both cases of Vx2 and Vx7.

When the life of the tumor-bearing animal was to be terminated, the tumor was dissected out carefully. The representative portion was biopsied and fixed in 10% formalin or in Tellyesniczky's fixative for histopathological observation. Histological specimens were also taken from the apparent and suspicious metastatic lesions. These histological sections were carefully examined to determine their neoplastic nature.

Results. Comparison of histological features of Vx2 and Vx7. It has been observed by Rous(7) that Vx7 still retains characteristics of squamous cell carcinoma whereas the Vx2 exhibits a more anaplastic nature. We have also confirmed that such histopathological difference among these two malignant tumors could be consistently observed throughout the course of the present study.

Growth pattern of Vx2 and Vx7 in rabbits. The volume of the tumors was calculated from the measurements taken once every week up to the fifth week on 214 Vx2-bearing rabbits and 123 Vx7-bearing rabbits. The average value of tumor volume (cm^3) in both cases is plotted as growth curves in Fig. 1. It is shown that the growth of Vx2 is more rapid than Vx7.

Metastases in Vx2- and Vx7-bearing rabbits. As a parameter to assess the malignancy of these two carcinomas, their tendency to form metastatic lesions in their primary lymph node, i.e., retroperitoneal lymph nodes, lungs, and other remote organs was considered. The sacrificed animals were examined thoroughly by autopsy to check for all gross metastatic lesions and their frequencies were tabulated.

a. Metastasis to retroperitoneal lymph node and lungs. In rabbits transplanted with Vx2 carcinomas, the metastases to the retroperitoneal lymph node were seen in 82% (174/213) of the animals autopsied on 26–60 days after transplantation. Metastases to lungs were observed in 38%(81/213)(Fig. 2).

In Vx7-bearing rabbits, the frequency of the metastases was significantly lower when compared with that of the Vx2. The ratio of animals found with metastases in the primary lymph nodes and in lungs was 65%(74/114) and 13%(15/114)(Fig. 3).

The histological section of lung metastases in Vx2- and Vx7-bearing rabbits is illustrated in Figs. 4 and 5, respectively.

b. Quantitative aspect of lung metastases. To obtain somewhat quantitative difference in the capacity of both tumors to form metastases in lungs, number of metastatic nodules grossly countable on the surface of the organ was examined. Such data are tabulated in Tables I and II. It is clearly shown that more metastatic lesions were found in Vx2-bearing rabbits than in the Vx7-bearing animals.

c. Metastases to other organs. The findings on metastatic lesions observed in organs other than retroperitoneal lymph nodes and lungs in rabbits transplanted with Vx2 or with Vx7 are listed in Table III. Metastases were seen in inguinal lymph nodes, kidney and liver in Vx2-bearing rabbits while so far no gross

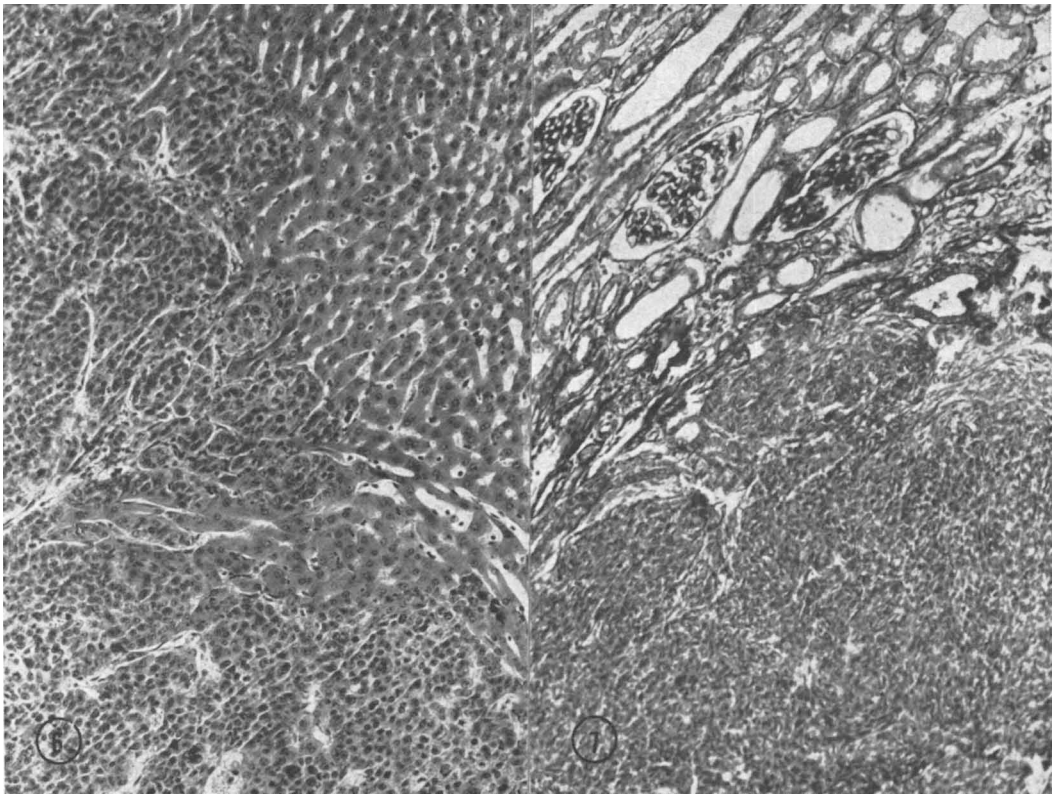


FIG. 6. Histological section of metastasis to liver in Vx2-bearing rabbit. H and E stain; × 160.
FIG. 7. Histological section of metastasis to kidney in Vx2-bearing rabbit. H and E stain; × 160.

metastasis was observed in those organs in the Vx7-bearing rabbits. The histological sections of metastatic lesions in liver and kidney of rabbits transplanted with Vx2 carcinoma are shown in Fig. 6 and 7, respectively.

TABLE III. Incidence of Metastasis to Liver, Kidney, and Other Organs in Rabbits Transplanted with Vx2 Carcinoma.

Days after transplantation	Number of rabbits	Rabbits with metastases to		
		Liver	Kidney	Inguinal lymph node
– 20	5	—	—	1
21– 30	59	—	—	—
31– 40	88	1	1	2
41– 50	35	1	3	—
51– 60	18	—	1	1
61–100	6	—	—	—
101–	2	—	—	—
Total	213	2	5	4

Discussions and Conclusion. It has been attempted in the present study to compare the malignancy of the two transplantable carcinomas of rabbit, both originating from the cutaneous papillomas induced by Shope papilloma virus some 20 years ago, from somewhat quantitative aspects. The results confirmed the generally accepted impression that the Vx2 carcinoma is more malignant than the Vx7 carcinoma. Comparative studies on the growth pattern and tendency to metastasize to remote organs from the primary site of transplantation have clearly shown that the Vx2 has a more rapid growth rate, and shows a higher frequency of formation of metastatic lesions in retroperitoneal lymph nodes, lungs, and other organs than the Vx7 carcinomas.

The authors are grateful to Dr. Peyton Rous for his generous gifts of Vx2 and Vx7 carcinomas.

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The Effect of Altering Metabolic Rate on the Mortality and Incubation Time of Rabies* (32884)

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The basis of the postexposure treatment of rabies, except for the actual elimination of the virus from the wound, depends upon the length of the incubation period; for if this is long enough, the patient can be protected by a combination of passive and active immunization. From this viewpoint, it would be significant if certain procedures would lengthen the incubation period and still allow the host to actively participate in his own defense.

The incubation period of rabies in bats may be prolonged by placing this host in low ambient temperatures and conversely may be shortened in higher than normal temepratures (1). It has been shown that the metabolic rate of bats varies directly with the ambient temperature (2). Subsequent reports (3,4) have amply confirmed this relationship. It is not known whether the incubation times for rabies in bats placed in different ambient temperatures is due to changes in metabolic rate or some other factor associated with changes in temperature.

This paper reports the results of a series of experiments designed to evaluate the effect of altering the metabolic rate of homothermic mammalian hosts on the incubation time and survival ratio of rabies.

Materials and Methods. Swiss mice of the Webster strain, Sprague-Dawley rats, golden Syrian hamsters and Duncan-Hartley guinea pigs were used in these experiments. To increase the metabolic rate of these animals, iodinated casein (CI) was added at a 0.1% concentration by weight in their feed. This concentration in the daily diet was sufficient to maintain an increased metabolic rate, without toxic effects, over a period of 30 days. The animals did not show a marked increase in metabolic rate until they had been on the diet approximately 5 days. The feeding of CI was usually started on the same day the virus was inoculated; however, in Expt. 15, the diet was started 10 days before the rats were inoculated so that they would have an increased metabolic rate when they were inoculated with the rabies virus.

To lower the metabolic rate of the animals, propylthiouracil (PTU) was added to the diet in an 0.1–1% concentration by weight. While this drug was tolerated in concentrations up to 4%, no difference in the metabolic rate was found in the higher dosages and palatability problems were encountered at dosages over 2%. The animals' metabolic rate was not noticeably lowered until PTU was given daily in the diet for approximately 10 days. In Expts. 1, 6, and 18, the PTU was given 10–15 days before inoculation. In the rest of the experiments, PTU was started the same day the animals were inoculated with the rabies virus.

Guinea pigs, hamsters, and rats were thyroidectomized at least 14 days prior to virus inoculation in order to compare the metabolic rates and incubation times with respect to rabies in these animals with those that were fed propylthiouracil. Rats were hypophysectomized to reduce the thyroid activity indirectly,

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