found in tubes containing 0.833 μg/ml of chloramphenicol and 0.050 μg/ml of aureomycin, indicating that *L. monocytogenes* was considerably more susceptible *in vitro* to aureomycin than to chloramphenicol.

The survival rates of animals inoculated with strains 447 and 2597 are shown in Table I. Thus, a significantly greater number (80-100%) survived after being treated with aureomycin as compared with those receiving chloramphenicol (18 to 35%). Only 2% of the untreated controls survived.

During the course of the study it was noted that mice, both treated and untreated, which died more than 10 days after being infected, often exhibited a marked and progressive paralysis. The first apparent symptom was a dragging of one hind leg, followed by partial immobilization of both lower quarters and eventual paralysis of the entire body except for the head. Three of these paralyzed mice were sacrificed and cultures taken from the brain and heart. In 2 instances *L. monocytogenes* was recovered, once from both brain and heart and once from the brain only.

Under the conditions of the experiments reported, five consecutive daily doses of 50 mg of aureomycin per kg of mouse body weight are necessary to obtain 90 to 100% survival of mice infected with *L. monocytogenes*. With the same dosage of chloramphenicol, however, only 30 to 35% of the mice survived. Treatment of the mice with 100 mg/kg of chloramphenicol did not increase the percentage of survivors.

Summary. 1. Aureomycin is more effective than chloramphenicol in inhibiting growth of three strains of *Listeria monocytogenes in vitro*. 2. More mice survived infection with *L. monocytogenes* when treated with aureomycin than with chloramphenicol.

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Succinate Stimulation of Normal and Tumor Tissue Slice Metabolism Measured by Reduction of Tetrazolium Chloride.* (18516)

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Roskelley *et al.*(1) reported that the addition of succinate or p-phenylenediamine to slices of normal tissues resulted in a marked increase in oxygen uptake. In contrast to this, only a minimal stimulation was observed when different neoplastic tissues were studied. In an extension of this type of investigation, Rosenthal and Drabkin(2) found that some normal tissues also tended to yield a relatively low percentage stimulation by these 2 substrates. At the same time, they confirmed the finding of a low response in diverse tumor tissues. By the clever device of studying the response to the substrate in the presence of added cytochrome c Greenstein was able to show that when tissues change from the normal to the malignant state, cytochrome c is diminished to a much greater extent than is cytochrome oxidase. In short, the limiting factor in the substrate stimulation in the experiments discussed appears to be the components of the cytochrome system(3,4). It has recently been shown that tetrazolium salts may be used to measure dehydrogenase activity of tissues both in the absence and presence of substrates(5-7). While the exact site of

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1. Roskelley, R. C., Mayer, N., Horwitt, B. N., and Salter, W. T., *J. Clin. Invest.*, 1943, v22, 743.

2. Rosenthal, O., and Drabkin, D. L., *Cancer Research*, 1944, v4, 487.

3. Greenstein, J. P., Werne, J., Eschenbrenner, A. B., and Leuthardt, F. M., *J. Nat. Cancer Inst.*, 1944, v5, 55.

participation of the tetrazolium in the respiratory chain of enzymes is not precisely defined as yet, it would appear that its reduction reflects the participation of dehydrogenase and/or flavoprotein activity(8).

In the course of studies which will be reported elsewhere, we had found that the intensity of the *in vitro* endogenous oxygen uptake by slices of different tissues as measured by conventional manometric technics is paralleled by the intensity of their reduction of tetrazolium chloride. However, under varied experimental conditions the two procedures may be shown to reflect the activity of different components of the respiratory chain of enzymes. It, therefore, appeared worthwhile to test the effect of succinate on the reduction of tetrazolium salts by various normal and tumor tissues and to compare these findings with the previous reports of oxidative stimulation by succinate. The results of the present investigation would indicate that normal and tumor tissue slices may show an identical percentage increase in metabolism upon the addition of succinate. In addition, the presence of a malignant tumor was not shown to produce any change in the endogenous or succinate stimulated metabolism in respect to tetrazolium reduction by kidney, liver or spleen slices of mice as compared with the values found in control animals of the same strain.

Materials and methods. The human tissues consisted of operative specimens obtained within 30 minutes of removal, while the mouse tissues were obtained by rapid removal from animals killed by crushing of the cervical cord. The mice used were of the CFW strain (Carworth Farms, New York) with and without spontaneous breast carcinomas, as well as C57 black mice with and without implanted sarcoma 180. The transplantable sarcoma 180 was kindly furnished by Dr. K. Sugiura of the Sloan-Kettering Institute. The tumor was implanted into adult female C57 black mice and allowed to grow to the size of at least 1 cm in diameter before the animals were sacrificed for analysis. The CFW mice were all females weighing more than 22 g and only the grossly non-necrotic portions of the spontaneous breast tumors were taken for analysis. The tissues to be studied were sliced free hand with a razor to a thickness of approximately 1 mm. In the case of the normal colon and stomach, the mucosa was carefully dissected free and the sheet of tissue then cut into segments approximately 1 cm square. The measurement of the metabolism was performed according to the technic indicated in a previous publication(6). In essence it consists of the following steps: a. The tissue slices are incubated with continuous gentle agitation at 37°C, pH 7.2 in individual wide mouthed test tubes. The incubation solutions consisted of 3 ml of a phosphate buffered 1% solution of 2, 3, 5-triphenyl tetrazolium chloride (TTC) in distilled water plus 1 ml of 0.9% saline for the measurement of the endogenous metabolism, or 1 ml of sodium succinate solution, equivalent to 2×10^{-4} M of succinate. b. After one hour's incubation the solution is decanted and the reduced TTC, which is red in color, is extracted by repeated washings with acetone. c. The acetone washings are added to a colorimeter tube, diluted to the 5 ml mark with acetone and readings taken of the light transmission, using the Klett-Summerson photoelectric colorimeter with the No. 54 green filter. d. The tissues are dried, weighed on an analytical balance and the intensity of color produced per mg of acetone dried tissue per hour is calculated. e. This value is converted to micrograms of TTC reduced per mg of acetone dried tissue per hour by the use of a standard curve obtained by the chemical reduction of known amounts of TTC. It should be mentioned that each determination was run in duplicate.

Results. The results of our studies on a wide variety of normal and tumor tissue types are indicated in Table I. It is readily apparent that the percentage increase in TTC

4. Shack, J. J., *J. Nat. Cancer Inst.*, 1943, v3, 389.

5. Kun, E., and Abood, L. G., *Science,* 1949, v109, 144.

6. Black, M. M., and Kleiner, I. S., *Science,* 1949, v110, 660.

7. Rutenberg, A. M., Gofstein, R., and Seligman, A. M., *Cancer Research,* 1950, v10, 113.

8. Kuhn, R., and Jerchel, D., *Ber. Deut. Chem. Ges.,* 1941, v74B, 949.

TABLE I. Reduction of TTC by Normal and Tumor Tissues with and without Added Succinate.

Species	Tissue	No. cases	μg TTC reduced/mg Ro.	μg TTC reduced/mg Rs.	% succinate stimulation
Human	Stomach mucosa	11	1.0	1.9	90
	Carcinoma	2	.2	.8	300
	Colon mucosa	9	.8	1.7	110
	Carcinoma	8	.5	1.5	200
	Breast, control	4	1.1	2.1	91
	Carcinoma	26	.6	1.6	160
	Lymph node	1	.3	.9	200
	Lymphosarcoma	2	.3	1.7	460
	Lung carcinoma	1	1.2	2.4	100
	Ovary carcinoma	1	.4	.8	100
	Metastatic ca., primary site unknown				
	Epidermoid	1	.6	1.7	180
	Adenocarcinoma	1	.3	.6	100
	Anaplastic	1	1.3	2.0	54
	Teratoma, benign, embryonic	1	1.0	2.3	130
	Prostate, hypertrophic	1	.2	.6	200
	Ileum, mucosa	2	1.8	3.0	66
	Thyroid, control	6	.5	1.0	100
	Ewing's tumor	1	.5	1.6	220
Mouse	Breast, ca., spontaneous	30	1.2	3.0	150
	Sarcoma 180	1	.4	2.3	470
CFW	Liver, control	33	2.3	7.3	218
	Tumor mice*	52	2.4	7.2	200
	Kidney, control	47	4.3	9.8	128
	Tumor mice	67	4.0	9.5	137
	Spleen, control	24	.7	1.7	140
	Tumor mice	24	.7	2.1	200
C57 black	Liver, control	21	2.6	7.3	180
	Tumor mice†	4	2.3	6.2	170
	Kidney, control	22	4.2	10.2	143
	Tumor mice	6	3.1	8.6	178

* Spontaneous breast carcinoma.
† Transplanted sarcoma 180.

reduction caused by the addition of succinate is not constant in the different tumor types, nor is the range of values more limited than that found in the comparable control tissues. It is especially interesting to compare the succinate stimulation obtained in the human tissues from the stomach, colon, breast and lymph node since in these instances we have had available the corresponding malignancy arising in these areas. In none of these instances was the percentage succinate stimulation less in the tumor tissue than it was in the homologous control. In fact, the reverse appears to be the case. This is in direct contrast to the results reported when succinate stimulation is evaluated on the basis of increased oxygen uptake as found with the conventional manometric technics.

It will be noted that the absolute values for the endogenous TTC reduction are often less in the tumor as compared to the corresponding normal tissue. It should be remembered that particularly in human material, sections of "tumor tissue" actually contain but a small fraction of tumor cells infiltrating the stroma, which may be extensive due to desmoplastic reaction.

The endogenous reduction of TTC by liver, kidney and spleen slices from mice was found to be the same in the tissues from the control animals as compared to those bearing spontaneous mammary carcinomas. Similar agreement of values was also found when the TTC reduction in the presence of succinate was compared for these tissues in the tumor and non-tumor bearing animals. Parallel studies performed on C57 black mice with and without implanted sarcoma 180 failed to demon-

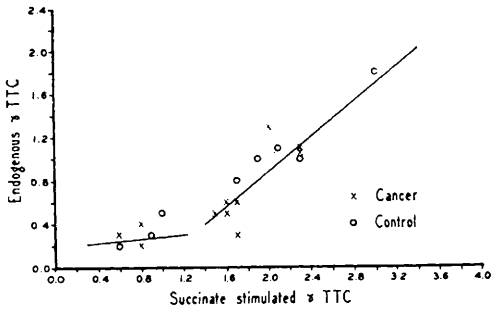

FIG. 1.

Plot of values of μg of TTC reduced per mg tissue in presence and absence of succinate. × = cancer tissue; ○ = control tissue.

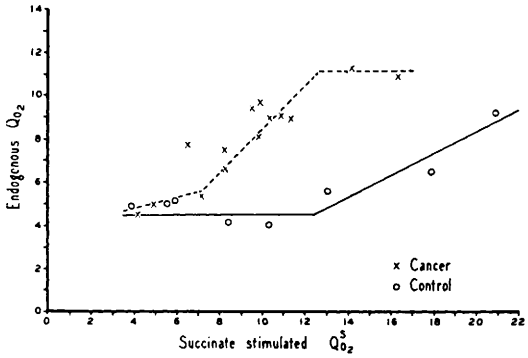

FIG. 2.

Plot of endogenous and succinate stimulated oxygen uptake (Q_{O_2}) for human and cancer tissues. Data of Roskelley *et al.*(1) and Rosenthal and Drabkin(2). ○ = control tissue; × = cancer tissue.

strate any change in the endogenous or succinate stimulated metabolism in liver and kidney slices. These findings are in accord with observations of Riehl and Lenta(9,10) who were unable to demonstrate a significant difference in the tissues of normal and tumor bearing mice in respect to total, malic and lactic dehydrogenase activity as measured by the Thunberg technique.

Discussion. In Fig. 1, we have plotted the values for the TTC reduction with and without succinate for a variety of control and tumor tissues. It appears from the distribution of the various values that a definite relationship obtains between the endogenous activity and the succinate stimulated activity. It is evident that the value for the endogenous reduction of TTC by a tissue is of greater significance in determining its response to succinate than is the benignancy or malignancy of the tissue. This observation suggests that both normal and tumor tissues tend to show a rather fixed relationship between dehydrogenase activity in the "resting" and stimulated state.

In an effort to evaluate this type of relationship in regard to endogenous and succinate stimulated Q_{O_2} values we have plotted these values as reported by Roskelley *et al.* (Fig. 2). It is evident that such a plot of the control tissues yields a curve very similar in shape to that obtained by the TTC technique, and is also indicative of a relationship between the endogenous Q_{O_2} value and the succinate stimulation. While the curve for the various tumor tissues is distinctly separated from the control except at the lower end of the curve it may be inferred that the succinate stimulated oxygen consumption of tumors is also a function of the endogenous respiration. The separation of the two curves as the Q_{O_2} values rise would indicate that a different member of the respiratory chain of enzymes functions as the limiting factor in tumors as compared to normal tissues. The similar type curve obtained for the oxygen consumption of normal tissues and the TTC reduction (dehydrogenase activity) of control and tumor tissues would indicate that it is not the dehydrogenase activity *per se* which is the limiting factor in the decreased oxidative stimulation of some tumors by succinate. In tumor tissues possessing a relatively high Q_{O_2} the limiting factor in succinate stimulation would be expected to occur in the cytochrome-cytochrome oxidase system. This is in complete agreement with Greenstein's demonstration that tumors show not only a lowered cytochrome c content but also the greatest disparity between cytochrome c and cytochrome-oxidase. From his data as well as our own it must follow that a disparity also exists between the cytochrome c and the components of the respiratory chain of enzymes preceding it; *viz.* dehydrogenases and flavoproteins.

Finally, it is particularly significant to note

9. Riehl, M. A., and Lenta, M. P., *Cancer Research*, 1949, v9, 42.

10. Riehl, M. A., and Lenta, M. P., *Cancer Research*, 1949, v9, 47.

that in regard to succinate stimulation of TTC reduction and oxygen uptake, tumor tissues behave not in an autonomous, unpredictable fashion, but rather in accord with equally rigid control as obtains in representative normal tissues.

Summary. Studies were made of the endogenous and succinate stimulated *in vitro* reduction of TTC by tissue slices from various human normal and cancer tissues as well as liver, kidney and spleen slices of control and tumor bearing mice. The per cent stimulation by succinate was not found to differ significantly in normal and tumor tissues. These findings were compared with previous studies of oxidative stimulation by succinate. It was also found shown that the presence of a tumor did not change the endogenous or succinate stimulated dehydrogenase activity of the liver, kidney or spleen of mice as measured by the TTC technique.

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Interrelationships of Vitamin B_6, Niacin, and Tryptophan in Pyridine Nucleotide Formation.* (18517)

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Since an increased excretion of niacin and some of its derivatives have been shown to result from the administration of tryptophan (1-4), tryptophan probably functions as a metabolic precursor of niacin. These studies have been implemented considerably by the work of Heidelberger *et al.*(5,6). It has also been observed that rats deficient in vitamin B_6 (pyridoxine) do not metabolize tryptophan normally as shown by the excretion of xanthurenic acid in the urine(7-10). Other workers(11,12) have observed that deficiency of combinations of various B vitamins besides pyridoxine also markedly interfere with tryptophan metabolism. Spector(13) reported that pyridoxine apparently had little effect upon the conversion of tryptophan to niacin or N-methylnicotinamide as measured by excretion of the expected metabolites. Recently it has been reported from this laboratory(14, 15) that dietary tryptophan appears to be more important in the maintenance of tissue pyridine nucleotides (DPN and TPN) than dietary niacin although one might expect the reverse to be true because of the presence of the niacin residue in DPN and TPN.

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1. Singal, S. A., Briggs, A. P., Sydenstricker, V. P., and Littlejohn, J. M., *Fed. Proc.*, 1946, v5, 154.

2. Dann, W. J., *Fed. Proc.*, 1946, v5, 231.

3. Rosen, H., Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, 1946, v163, 343.

4. Singal, S. A., Briggs, A. P., Sydenstricker, V. P., and Littlejohn, J. M., *J. Biol. Chem.*, 1946, v166, 573.

5. Heidelberger, C., Abraham, E. P., and Lepkovsky, S., *J. Biol. Chem.*, 1948, v176, 1461.

6. Heidelberger, C., Abraham, E. P., and Lepkovsky, S., *J. Biol. Chem.*, 1949, v179, 151.

7. Lepkovsky, S., and Nielsen, E., *J. Biol. Chem.*, 1942, v144, 135.

8. Lepkovsky, S., Roboz, E., and Haagen-Smit, A. J., *J. Biol. Chem.*, 1943, v149, 195.

9. Miller, E. C., and Baumann, C. A., *J. Biol. Chem.*, 1945, v157, 551.

10. Miller, E. C., and Baumann, C. A., *J. Biol. Chem.*, 1945, v159, 173.

11. Ginoulhiac, E., *Acta Vitaminol.*, 1947, v1, 49.

12. Porter, C. C., Clark, I., and Silber, R. H., *Arch. Biochem.*, 1948, v18, 339.

13. Spector, Harry, *J. Biol. Chem.*, 1948, v173, 659.

14. Williams, Jr., J. N., Feigelson, P., and Elvehjem, C. A., *J. Biol. Chem.*, 1950, v137, 597.

15. Williams, Jr., J. N., Feigelson, P., Shahinian, S. S., and Elvehjem, C. A., *J. Biol. Chem.*, in press.