variants have acquired the ability to synthesize their own PGA or C.F. This possibility seems unlikely for the same reasons given in (a) above, (c) the leukemic cells have an increased ability to detoxify the PGA antagonist. From the studies reported here, it is apparent that toxic effects of antagonist do not appear in mice growing the transformed leukemic cells because the antagonist is metabolized in supporting optimal growth. If this were a case of detoxification of antagonist, there would not be expected a differential in the growth capacities of resistant leukemic cells supplied with or lacking antagonist. That is, detoxification could be an explanation for the resistance but not for the dependence characteristic, or (d) the transformed leukemic cells have acquired the ability to use 4-amino-N^{10}-methyl PGA without conversion to PGA or C.F., employing a different mechanism for the synthesis of nucleic acids than that suggested as occurring normally(11,12). This would seem the most likely explanation available at present, although recently Skipper and Burchenal(13) have presented evidence which indicates that the differences in formate fixation in sensitive and resistant strains of the Ak-4 leukemia is only a relative one. It seems improbable, however, from the evidence presented(14), that the resistant Ak-4 cells which were used in their studies are dependent upon PGA antagonist for optimal growth.

Summary. 1. The effect of a PGA antagonist, 4-amino-N^{10}-methyl PGA on resistant leukemic cells of the mouse has been studied. The development of a florid leukemia with profuse localized growth of lymphomatous tissue, leukocytosis with escape of leukemic cells into the blood and extensive infiltration into various organs occurs at dosage levels as high as the LD_{50}. 2. Symptoms of vitamin deficiency did not appear in mice growing the resistant leukemic cells and supplied with a total dose of antagonist as high as 20 mg./kg, indicating that the resistant leukemic cells are metabolizing the antagonist in some manner. Tolerance to 4-amino-N^{10}-methyl PGA has been increased approximately 50-fold. 3. Citrovorum factor did not influence the growth of resistant leukemic cells but partially blocked the optimal growth-promoting effect of the PGA antagonist. 4. Possible mechanisms of action of 4-amino-N^{10}-methyl PGA on resistant and dependent leukemic cells are considered.

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Non-Specificity of Biotin Activity for Leuconostoc.* (18774)

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Literature evidence(1-5) indicates that micro-organisms possess definite configurational specificity with regard to biotin activity.

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It is commonly reported that compounds such as dl-oxybiotin† and dl-desthiobiotin possess not more than 50% of the activity of d-biotin, indicating that only the d-forms are effective. Because of our previous demonstration(6) of the unusual nature of biotin activity in *Leuconostoc mesenteroides* and *L. dextranicum*, we investigated the response of these organisms to dl-oxybiotin, dl-desthiobiotin, and to a typical inhibitor of the vitamin, γ-(3,4 ureylenecyclohexyl) butyric acid. Also, since the biosynthesis of aspartic acid is prominent among the numerous functions currently ascribed to biotin(7-10), we studied the effect of biotin and certain of its analogues on the growth of *Leuconostoc* in the presence and absence of added amounts of this amino acid.

Experimental. *L. dextranicum* elai was the major organism employed. It was carried in sucrose nutrient agar. The composition of the basal medium is given in Table I. It was made up in three-halves strength and distributed in 2 ml amounts in tubes closed with aluminum caps. The remaining components were added in 1 ml volume, and the tubes sterilized 10 minutes at 15 lb pressure. Inocula were prepared by transferring the cultures into 5 ml of the basal medium containing 2% sucrose and incubating 24 hours at 25°C. The cells were separated by centrifugation, washed with saline and resuspended in saline to give a reading of 97% transmission in the Klett-Summerson colorimeter with filter No. 42. On the average this corresponded to a dilution of approximately 1:30. One drop of the washed, diluted inoculum was added to each 3 ml tube of experimental medium. Incubation of experimental tubes was at 25°C. Growth rate was followed by titration with alkali at suitable intervals, bromthymol blue being used as indicator. After correction for the blank titration of uninoculated controls, the results were expressed in terms of ml of 0.01 N acid produced per ml of medium. All measurements were made in triplicate. Assays for aspartic acid content of dried cells were made with *L. mesenteroides* 8042, growth being measured turbidimetrically(11).

TABLE I. Composition of Basal Medium.

Constituent	Conc. per L
Carbohydrate*	25-50 g
L-Glutamic acid	500 mg
DL-Valine	500
DL-Isoleucine	500
L-Leucine	500
DL-Alanine	400
DL-Methionine	400
L-Cysteine	400
DL-Lysine	200
DL-Threonine	200
DL-Phenylalanine	200
DL-Tryptophane	200
DL-Serine	200
Glycine	200
L-Arginine	100
L-Histidine	100
L-Tyrosine	100
L-Proline	100
Adenine	10
Xanthine	10
Guanine	10
Thymine	10
Uracil	10
Nicotinic acid	1
Riboflavin	.5
Thiamin chloride	.5
Calcium pantothenate	.5
Pyridoxal	.2
Pyridoxamine	.2
p-Aminobenzoic acid	.1
Folic acid	.01
NaOAc	25 g
KOAc	5 g
$MgSO_4$	100 mg
NaCl	10
$FeSO_4$	10
$MnSO_4$	10
KH_2PO_4	500
K_2HPO_4	500

* When the carbohydrate used was glucose or fructose, the level was 25 g per l; when sucrose, 50 g per l.

Results and discussion. In Table II are presented results for the effect of d-biotin, dl-

† We wish to thank Dr. K. Hofman, University of Pittsburgh, for a sample of dl-oxybiotin and Dr. Jackson P. English, Am. Cyanamid Co., Stamford, Conn., for γ-(3,4 ureylenecyclohexyl) butyric acid.

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TABLE II. Effect of Biotin and Various Analogs on Growth of *L. dextranicum* elai in a Synthetic Amino Acid-Fructose Medium Containing No Added Aspartic Acid.

Conc. in μg/ml	Acid production in ml .01 N NaOH per ml medium			
	D-Biotin	DL-Desthiobiotin	DL-Oxybiotin	γ-(3,4-Ureylene-cyclohexyl) butyric acid
0	4.9	4.9	4.9	4.9
1×10^{-6}	6	6	5.4	—
1×10^{-5}	7.4	7.4	8	—
4×10^{-5}	—	—	—	5.4
5×10^{-5}	10	10	10	—
1×10^{-4}	10	10.7	10	—
4×10^{-4}	—	—	—	6.7
1×10^{-3}	10.7	10.1	10.6	—
4×10^{-3}	—	—	—	7.4
2×10^{-2}	—	—	—	9.4
4×10^{-2}	—	—	—	9.4

desthiobiotin, dl-oxybiotin and γ-(3,4 ureylenecyclohexyl) butyric action on the growth of *L. dextranicum* elai in a fructose medium containing no added aspartic acid; each derivative allowed maximal growth to occur. The unusual aspect of the results lies in the fact that dl-oxybiotin and dl-desthiobiotin were found equal to d-biotin in activity, as contrasted with the 50% maximum level of activity reported for these compounds in the case of organisms such as *Lactobacillus arabinosus* and *Saccharomyces cerevisiae* (12-16). Since in other experiments we found dl-desthiobiotin also to be equal in activity to d-biotin for *L. mesenteroides* 683, *L. mesenteroides* 9135, and *L. dextranicum* 8086, it appears that *Leuconostoc* are unique in not showing the configurational specificity demonstrated by other organisms. The fact that the growth curves obtained with the dl-forms showed no increased lag and paralleled that from d-biotin suggests that oxybiotin and desthiobiotin are utilized directly and without conversion to the vitamin. Direct utilization has been established (5,12) for oxybiotin in the case of several micro-organisms. However, evidence has accumulated (13-15) that organisms which utilize desthiobiotin do so only after conversion to biotin. The point is being further investigated for *L. mesenteroides* and *L. dextranicum,* but it appears that the ability to utilize desthiobiotin directly may be more widespread than commonly believed. Lilly and Leonian (16) early demonstrated that desthiobiotin, although it could not substitute for biotin in the metabolism of various lactobacilli, was strongly stimulatory to the organisms if minimal amounts of biotin were present. Thoma and Peterson (17), and Pearlman (18), have reported similar results for various clostridia. The reported functions of biotin are many and diverse, and while for certain organisms the vitamin itself may be required for all these functions, in others it is likely that some of them may be taken over by related compounds. The unexpected finding that a compound reported to be a biotin-blocking agent, γ-(3,4-ureylenecyclohexyl) butyric acid, could stimulate growth in an aspartic acid-free medium further points up the unusual nature of the spectrum of biotin activity in *Leuconostoc.*

The fact that aspartic acid synthesis by *L. dextranicum* elai actually occurred was proven in the cases of media containing d-biotin, dl-desthiobiotin or Tween 80. Tween 80, when used, was present in a concentration of 10 mg per ml. Cells grown under these various conditions were harvested, hydrolyzed, and assayed for their aspartic acid content by accepted methods (11,19). The assay organism used was *L. mesenteroides* 8042,‡ a strain which cannot synthesize (11) aspartic

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TABLE III. Effect of Biotin, Tween 80, Pyridoxal and Pyridoxamine on Growth of *L. dextranicum* elai in Carbohydrate Media with and without Aspartic Acid.

Additives	Acid production ml .01 N NaOH per ml medium			
	Sucrose		Fructose	
	No A*	Added A	No A	Added A
0	7.8	10	2.4	3.5
Pyridoxal, pyridoxamine	12.5	12.5	4	4.2
Biotin	7.8	10	2.8	5.2
Pyridoxal, pyridoxamine, biotin	12.5	12.5	8.3	8.8
Tween	7.8	10.7	3.2	5.5
Pyridoxal, pyridoxamine, Tween 80	12.2	12.2	8.3	8.7

* A = Aspartate.

acid under the influence of either biotin or Tween 80. It was found that the aspartic acid content of the dried cells was essentially the same regardless of whether they were grown in media containing d-biotin, biotin plus aspartic acid, dl-desthiobiotin, or Tween 80, the values obtained ranging from 4.4 to 5%. It seems safe to assume that the amino acid was also formed in the media in which dl-oxybiotin or γ-(3,4-ureylenecyclohexyl) butyric acid was employed.

Literature evidence(9,10,21) indicates that microorganisms which synthesize aspartic acid under the influence of biotin do so via the beta carboxylation of pyruvic acid to yield oxalacetic acid, pyridoxamine then being required to complete the formation of the amino acid. It appeared to be of interest to determine whether aspartic acid synthesis by *L. dextranicum* elai also required the presence of pyridoxamine. Demonstration of such a requirement was rendered difficult by the known (22) ability of *Leuconostoc* to synthesize the B_6 group, although at a rate insufficient to meet the full needs of the organisms. For *L. dextranicum* elai, pyridoxal and pyridoxamine have been shown(23) to be essential only under special conditions. The results obtained are presented in Table III, from which it is seen that in the sucrose medium, addition of pyridoxal and pyridoxamine allowed maximal growth in the aspartic-free medium. Biotin and Tween 80 were without effect. In the sucrose medium containing added aspartic acid, nearly full growth resulted in the absence of pyridoxal and pyridoxamine, although a slight requirement for the B_6 group was still evident. In the fructose medium pyridoxal and pyridoxamine, plus either biotin or Tween 80, were required for full growth in either the presence or absence of added aspartic acid. However, a higher level of growth was observed in the absence of pyridoxal and pyridoxamine when added aspartate was present, again suggesting that synthesis of the amino acid proceeded with oxalacetic acid as an intermediate.

Many investigators have reported(24,27) that oleic acid, or certain of its derivatives, can satisfy the biotin requirement of various organisms only when aspartic acid is furnished in the medium, thus suggesting that oleic acid cannot replace the vitamin in the synthesis of this amino acid. Recently Broquist and Snell (19) found that *Lactobacillus fermenti* and *Clostridium butyricum* represented exceptions to these findings, since the organisms were able to synthesize aspartic acid under the influence of oleic acid. The present study constitutes another case in which the presence of either biotin or an oleic acid derivative allows growth of a microorganism in an aspartate-free medium. However, the results obtained do not offer evidence for the direct participation of biotin or Tween 80 in aspartic acid synthesis by *Leuconostoc*.

Summary. It has been found that *L. dextranicum* elai is unique in that the dl-forms of oxybiotin and desthiobiotin are equal in activity to d-biotin in allowing maximum growth

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in an aspartate-free fructose medium. Since no increased lag period was observed the results suggest that these biotin analogs are utilized directly and without prior conversion to the vitamin. It was also found that γ-(3,4-ureylenecyclohexyl) butyric acid was not a blocking agent for this organism, but instead possessed biotin activity.

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Radioactive Sodium Clearance as a Test of Circulatory Efficiency of Tubed Pedicles and Flaps.* (18775)

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It has been the objective of earlier studies (1-6) on the development of circulation in tubed pedicles and flaps to achieve an accurate test of efficiency of circulation and to encourage its substitution for the current practice of clinical decision as to the interval of time between operative stages in the transplantation of soft tissues. It is apparent that an accurate and dependable test, by shortening the waiting periods between operative stages, would shorten the time of hospitalization and would prevent the complication of tissue necrosis in those cases in which there is uncertainty as to the viability of the pedicle. It is of interest that the clinical problem in cases of peripheral vascular disease is not unlike that presented in the management of tubed pedicles and flaps whose circulation has been artificially contrived. In the former, the clinician seeks to determine the degree of circulatory impairment and to measure the efficiency of the collateral circulation; in the latter, the surgeon is concerned with determination of the earliest date upon which an artificially contrived soft tissue circulation achieves the normal physiological level. Recently, Kety(7,8) showed that the

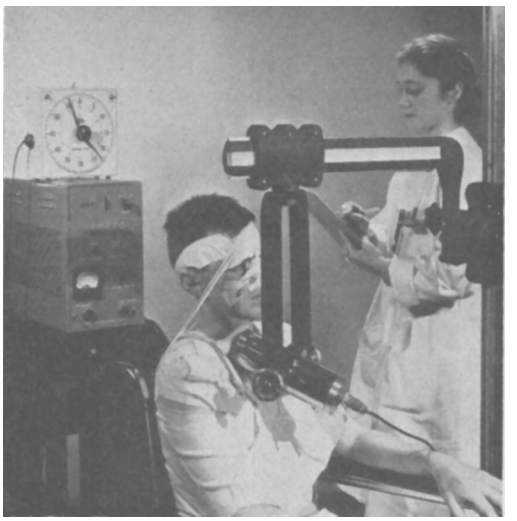

FIG. 1. Mica window of Geiger-Mueller counter placed over end of thoracoacromial pedicle to be transplanted for reconstruction of the nose, upper lip, and chin. Efficiency of circulation of tubed pedicle is determined by comparing clearance of radioactive sodium injected intradermally with that of control area. Relative clearance is determined by comparing half-life of radiosodium in the two areas. If clearance is as rapid from the end of pedicle to be transplanted as it is from the control area, the pedicle should withstand surgical transplantation successfully.

* Reviewed in the Veterans Administration and published with approval of the Chief Medical Director. Statements and conclusions published by authors are result of their study, and do not necessarily reflect the opinion or policy of the Veterans Administration.

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