critical antigens could in each case have been derived from the co-twin. (3) It has been demonstrated, by a simple immunological technique developed for this purpose, that there is a mixture of two distinct types of erythrocytes in certain twins.

These facts are consistent with the conclusion that an interchange of cells between bovine twin embryos occurs as a result of vascular anastomoses. Since many of the twins in this study were adults when they were tested, and since the interchange of formed erythrocytes alone between embryos could be expected to result in only a transient modification of the variety of circulating cells, it is further indicated that the critical interchange is of embryonal cells ancestral to the erythrocytes of the adult animal.[8] These cells are apparently capable of becoming established in the hemapoietic tissues of their co-twin hosts and continuing to provide a source of blood cells distinct from those of the host, presumably throughout his life.

Several interesting problems in the fields of genetics, immunology and development are suggested by these observations. Most of them are still largely speculative and will not be considered here. An application that may be mentioned is the tool now provided by the blood tests for selecting, with a high degree of reliability, those heifers, born twin with bulls, that are potentially not freemartins but normal, fertile individuals. A heifer whose blood type is the same as her twin brother's will very probably be a freemartin, while a difference in even a single antigen between twins of opposite sex may indicate that vascular anastomosis did not occur, and therefore that the heifer will be normal. Thus clinical observations on the heifer alone, probably not always reliable when the heifer is young, can be supplemented by an objective laboratory test applicable as soon as the twins are born. Possible limitations of this application, as well as a more complete presentation of the data and further discussion of the implications of the present study, will be included in another paper.

RAY D. OWEN

GROWTH INHIBITION BY ANALOGUES OF PANTOTHENIC ACID. II. α- AND β-SUBSTITUTED PANTOTHENIC ACIDS AND RELATED COMPOUNDS[1]

VARIOUS analogues of pantothenic acid which contain the pantoyl (α,γ-dihydroxy-β,β-dimethylbutyryl) group inhibit growth of organisms which do not synthesize pantothenic acid.[2-5] Such inhibition is competitive in nature.

Since isoserine and β-aminobutyric acid have been reported to prevent the growth-promoting effect of β-alanine for yeast,[6] the pantoyl derivatives of these compounds were prepared to determine if they would inhibit growth of bacteria by interfering in a similar fashion with utilization of pantothenic acid. A related compound, N-pantoyl-β-aminoisobutyric acid[7] has been included for comparison and testing on additional organisms. A homologue of pantothenyl alcohol,[2] N-pantoyl-4-amino-2-butanol, was also prepared and tested.

All these compounds have an asymmetric carbon atom in both the lactone and non-lactone portion of the molecule; consequently the products synthesized contained a mixture of two to four stereoisomers. To avoid separation of diastereoisomers, crude reaction mixtures were tested throughout. In all cases, adequate controls showed that the parent lactone and substituted amine from which the analogues were prepared did not show the physiological properties of the condensates.

All the above compounds inhibited the growth of microorganisms which require pantothenic acid. This inhibition was competitive in nature, since it became apparent only when the ratio of the concentration of the analogue to that of pantothenic acid surpassed a definite value, but was independent of the absolute amount of the analogue present. Two of the compounds seemed to show a slight growth-promoting activity in the absence of pantothenic acid, but inhibited growth induced by added pantothenic acid to the level of their own activity.

Details of these findings are presented below.

EXPERIMENTAL

dl-Pantothenyl Alcohol. This product was prepared as previously described.[2]

N-Pantoyl-4-amino-2-butanol. Hydrogenation of aldoxime (40 gm in 150 cc of ethanol) proceeded readily at a pressure of 2,000 lbs./sq. in. and 120° in the presence of Raney's nickel catalyst. After filtering to remove the catalyst and distilling the solvent, the residue was fractionally distilled under reduced pressure to obtain dl-4-amino-2-butanol (yield, 25 gm). The aminobutanol was condensed with dl-pantolactone in the manner described for the preparation of pantothenyl alcohol[2] from 3-amino-1-propanol. The product thus prepared contained 88 per cent. of the various stereoisomeric forms of N-pan-

[8] *Cf.* H. E. Jordan, *Physiol. Rev.*, 22: 375–384, 1942.

[1] For paper I of this series, see Snell and Shive (footnote 2).

[2] E. E. Snell and W. Shive, *Jour. Biol. Chem.*, 158: 551, 1945.

[3] E. E. Snell, *ibid.*, 139: 975 and 141: 121, 1941.

[4] J. W. Barnett and F. A. Robinson, *Biochem. Jour.*, 36: 357, 364, 1942.

[5] R. Kuhn, T. Wieland and E. F. Moeller, *Ber. dtsch. chem. Ges.*, 74: 1605, 1941.

[6] N. Nielson and G. Johansen, *Naturwissenschaften*, 31: 235, 1943.

[7] M. A. Pollack, *Jour. Am. Chem. Soc.*, 65: 1335, 1943.

toyl-3-butanol-1-amine as calculated from Van Slyke amino-nitrogen determinations before and after hydrolysis.

Sodium N-pantoyl-β-aminobutyrate.[8] Reaction of 0.52 gm of β-amino-butyric acid in 5 cc of isopropyl alcohol with an equivalent amount of sodium isopropoxide in 5 cc of isopropyl alcohol gave sodium-β-aminobutyrate. To the alcoholic solution, 0.65 gm of dl-pantolactone, dissolved in a small amount of isopropyl alcohol, was added. After standing several hours, the mixture was refluxed for one hour and evaporated to dryness. The resulting white crystalline solids were freed of solvent by heating at 50° for 30 minutes under reduced pressure. The yield (or purity of the product) was 58 per cent. as calculated from amino-nitrogen determinations before and after hydrolysis.

N-Pantoylisoserine (Sodium salt). The sodium salt of N-pantoyl-isoserine was prepared from 0.65 gm of dl-pantolactone and 0.53 gm of isoserine in the manner described above for sodium N-pantoyl-β-aminobutyrate. The yield (or purity of the product) determined in the same manner was 75 per cent.

Sodium N-pantoyl-β-aminoisobutyrate. A sample of sodium N-pantoyl-β-aminoisobutyrate, previously prepared in this laboratory[7] from (-)-pantolactone and sodium β-aminoisobutyrate by refluxing in absolute alcohol, was used in this investigation. As the compound is hygroscopic, it was dried at 50° under reduced pressure. The purity of the sample was 71 per cent., based on amino-nitrogen determinations before and after hydrolysis.

Testing methods. The techniques of biological testing have been described previously.[2] Temperature of incubation for *Lactobacillus casei* and *Lactobacillus fermentum* was 37°, for *Lactobacillus arabinosus* 17–5 and *Leuconostoc mesenteroides* P–60, 30°. The time of incubation was 20 hours, unless otherwise noted. The data in Table 1 were obtained by adding 0.2 γ calcium pantothenate per 10 cc of medium, and varying the amount of inhibitor to determine the concentrations at which inhibition of growth resulted.

TABLE 1

COMPARATIVE SUSCEPTIBILITY OF VARIOUS ORGANISMS TO INHIBITION BY PANTOTHENIC ACID ANALOGUES

Compound	*Leuconostoc mesenteroides* P–60		*Lactobacillus casei*		*Lactobacillus arabinosus* 17–5		*Lactobacillus fermentum*	
	Molar ratios (analogue/pantothenic acid) for indicated inhibitions*							
	half maximum	maximum	half maximum	maximum	half maximum	maximum	half maximum	maximum
dl-Pantothenyl alcohol	330	700	4,000	20,000	1,000	10,000	36,000	200,000
N-Pantoyl-4-amino-2-butanol†	280	600	6,000	ca. 100,000	4,000	50,000	.....	.....
Sodium N-pantoyl β-amino-butyrate†	850	2,000	25	250	100	1,500	375	5,000
Sodium N-pantoyl-β-amino-isobutyrate‡	350	ca. 1,000§	40	1,000	75	1,000	725	> 50,000
N-Pantoylisoserine (Sodium salt)†	1,500	ca. 5,000§	75	2,500	80	ca. 2,500§	.....	.....

* These ratios are somewhat variable depending upon testing conditions. Use of heavy inocula and long incubation times tend to give higher ratios, especially for maximum (or complete) inhibition.

† Prepared from dl-pantolactone and the racemic amino compound. With pantoyltaurine and pantothenyl alcohol, only the product derived from (-)-pantolactone shows significant inhibitory properties. (See footnotes 2 and 3). This is probably also true for these products; hence, in comparing their activity with that of sodium N-pantoyl-β-aminoisobutyrate, which was prepared from (-)-pantolactone, this fact should be considered.

‡ Prepared from (-)-pantolactone and sodium dl-β-aminoisobutyrate. (See footnote 7.)

§ These compounds never inhibited growth completely (cf. Table II); ratios listed are for minimum growth approaching complete inhibition; higher ratios resulted in slightly increased growth.

Results. The results of the tests are summarized in Table 1, which lists the molar ratios of inhibitor to metabolite at which both half and complete inhibition of growth resulted. Large amounts of any of these compounds (more than 5 mg per 10 cc) did not inhibit growth if sufficient pantothenic acid was present. Susceptibility of various organisms to a given analogue varied greatly. For example, N-pantoyl-4-amino-2-butanol completely inhibited growth of *Leuconostoc mesenteroides* when the ratio of analogue to pantothenic acid was only 600, whereas for complete inhibition of *L. casei,* a ratio of about 100,000 was required. *L. casei* is very sensitive to some of the other analogues, however, especially to N-pantoyl-β-aminobutyric acid. Thus the inhibitory properties of these two antimetabolites for one organism lie in the reverse order found with the other organism. This is not an unusual situation: several similar examples are apparent in the table, and similar behavior has been previously noted for other analogues.[2] Of the analogues so far prepared, N-pantoyl-β-aminobutyric acid (β-methylpantothenic acid) appears most versatile, since it has relatively great inhibitory powers for a wide variety of organisms. Even *Lactobacillus fermentum,* whose growth is extremely resistant to inhibition by pantothenyl alcohol and pantoyltaurine,[2] is readily inhibited by this compound.

In some cases, complete inhibition of growth was

[8] This product was previously synthesized by a different method and reported to be incapable of supporting growth in the absence of pantothenic acid for organisms which require the latter (H. H. Weinstock, E. L. May, A. Arnold and D. Price, *Jour. Biol. Chem.,* 135: 343, 1940).

not obtained regardless of the amount of inhibitor added. This was true in the case of the α-substituted pantothenic acids, N-pantoylisoserine and N-pantoyl-β-aminoisobutyric acid, and appeared due to the fact that these compounds themselves stimulated growth of some organisms at high concentrations. An example of this type of behavior is shown in Table 2. Growth

TABLE 2

EFFECT OF N-PANTOYLISOSERINE ON GROWTH OF *Lactobacillus arabinosus* 17–5
Incubated 22 hours at 30°

Amount of material added: N-Pantoylisoserine* (Sodium salt)	Amount of material added: Calcium pantothenate	Galvanometer reading†
0	0.0	7.0
10	0.0	11.5
100	0.0	11.7
1000	0.0	15.2
10000	0.0	22.0
0	0.2	91.2
30	0.2	76.0
100	0.2	36.0
300	0.2	22.0
1000	0.2	13.5
3000	0.2	19.5
30000	0.2	20.0

* Product prepared from dl-pantolactone and sodium salt of dl-isoserine.
† A measure of culture turbidity; distilled water reads zero, an opaque object 100.

induced by pantothenic acid was readily inhibited by the compound to the level corresponding to its own stimulatory effect, but not further. In all cases of this type, inhibition was almost complete before stimulation by the antimetabolite became apparent. This "double action" has been previously noted for N-pantoyl-β-aminoisobutyric acid;[7] if confirmed with the purified compounds, it would be of some theoretical importance. Also of interest are the relative concentrations of the antimetabolite required to produce half and complete inhibition of the various organisms (Table 1). With *Leuconostoc mesenteroides,* slightly more than twice as much of any given compound is required for complete inhibition as is required for half-maximum inhibition, with most of the other organisms, considerably more than this excess is required for complete inhibition.

In Table 3 are listed the most effective antimetabolites which interfere with pantothenic acid metabolism for a variety of organisms. In each of these, the pantoyl moiety of the pantothenic acid molecule is present intact; variation is in the remainder of the molecule. It has been shown[2, 3] that only those compounds derived from (-)pantolactone show inhibitory action, and the molar inhibition ratios have been calculated on this basis. In each of the compounds reported herein, the amino compound condensed with the lactone contained an asymmetric carbon atom; hence, two stereoisomers would be formed which might differ in their physiological action. Preparation of the two stereoisomeric forms of N-pantoyl-β-aminobutyric acid is in progress to settle this point.

TABLE 3

EFFECTIVE GROWTH-INHIBITORY ANALOGUES OF PANTOTHENIC ACID FOR VARIOUS MICROORGANISMS

Organism	Inhibitor	Molar inhibition ratio for maximum inhibition*
Leuconostoc mesenteroides P–60	Pantothenyl alcohol	175–350†
	N-Pantoyl-4-amino-2-butanol	300
Lactobacillus arabinosus 17–5	N-Pantoyltaurine[2, 3]	500–1000†
	N-Pantoyl-β-amino-butyric acid	750
Lactobacillus casei	N-Pantoyl-β-amino-butyric acid	125–250†
	N-pantoyl-β-amino-isobutyric acid	1000
Lactobacillus fermentum	N-Pantoyl-β-amino-butyric acid	5000

* Determined or calculated on the basis of amino compound (racemic if asymmetric) condensed with (-)-pantolactone.
† Ranges indicate values found under varying conditions.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

PREPARATION OF SHARK CHONDROCRANIA FOR CLASS USE

IN connection with the study of the shark in courses in general zoology or comparative anatomy, a careful examination of the cranium is desirable. The usual method of storing cleaned crania for this purpose in formalin or alcohol leads to a high rate of destruction of the specimens because the cartilaginous material remains very fragile. To overcome this difficulty the writer has developed the following method which has been used successfully at the University of California.

Heads of sharks are severed from the body and placed into hot but not boiling tap water (about 60° C) for from one half to two hours. Then the heads are roughly cleaned by hand; the skin, the visceral skeleton and most of the muscles are thus removed. The partially cleaned crania are then placed into a fresh quantity of hot water and after another half hour they are vigorously shaken out. Small fragments of tissues still adhering to the crania are then blown off by directing intermittent jets of compressed air (35 pounds pressure was found satisfactory) through a fine nozzle over the surface of the crania.

The meticulously clean crania are then dehydrated