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

by passing them through 30 per cent., 50 per cent., 80 per cent. and 95 per cent. alcohol, allowing 24 hours in each. After two changes of 95 per cent. alcohol the crania are left for three days in absolute alcohol, for three days in a mixture of alcohol and toluol, and, finally, in pure toluol until completely clear. Next, the crania are transferred into a melted mixture of one quarter paraffin and three quarters toluol, where they remain for 2 days. They are then transferred into a similar mixture consisting of one half paraffin and one half toluol; after another 2 days they are placed into a mixture of three quarters paraffin and one quarter toluol. Again after 2 days they are transferred into melted pure paraffin for 3 days. Finally they are placed into fresh paraffin for an additional 3 days or more. If large numbers of crania are treated, a third and even a fourth change of pure paraffin may be necessary.

This slow method of infiltration was found to be necessary, as comparatively large quantities of the preceding liquids are always carried along with the crania, thus leading to a comparatively rapid dilution of the more concentrated solutions; this in turn yields very unsatisfactory results, such as incompletely infiltrated specimens, shrivelled and distorted crania, etc.

When infiltration is completed the crania are removed from the paraffin and permitted to drip off all excess paraffin onto paper towels placed on the top of the steam radiator. This latter procedure needs careful supervision in order to assure freedom from paraffin of all foramina and at the same time prevent complete loss of the infiltrated paraffin. The finished crania are then placed on a layer of non-absorbent cotton in individual boxes.

The foregoing procedure has been applied with equal success to the crania of several species of sharks, namely *Squalus suckleyi* Gill, *Mustelus californicus* Gill, *Triakis semifasciatum* Girard and *Heptanchus maculatus* (Ayres). The only difference found was that the crania of the last-named species require approximately double the time intervals indicated above to insure complete infiltration.

The spinal column of sharks may be cleaned in the manner described for the crania, except that great care must be exercised in the hot-water treatment. If the spinal column is left but a few minutes too long, the interdorsal arches will fall out. No time can be specified, as it seems to depend, possibly, on the age of the shark. However, it was found that satisfactorily clean specimens are obtained even if not all adhering muscles can be blown off, since these fibers may be picked away readily with forceps after completed infiltration with paraffin, before cooling off of the specimen.

The same procedure is followed in infiltration of the spinal columns as indicated for the crania. The spinal column is broken into pieces about four inches long. After completed infiltration a lateral portion extending over three to four vertebrae is removed at one end of the piece of column, exposing a sagittal view of the vertebrae. At the other end of the piece of column a transverse section is made through the middle of a vertebra. These operations may be executed with a scalpel or razor blade. However, if large numbers of such pieces must be prepared, the work may be speeded up by using a power jigsaw. It was found that a very slow speed and a fine blade yield best results. The saw marks are easily removed by shaving off the exposed surface layer with a sharp scalpel. The student may be given a portion of spinal column from the trunk region and a portion from the tail region. If it appears desirable, sections through the cranium may be cut also on the jigsaw.

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A METHOD FOR MEASURING THE EFFECTS OF ACID BEVERAGES ON THE TEETH OF SMALL LABORATORY ANIMALS[1]

IN the course of studying the *in vivo* effects of various acid beverages (cola drinks, synthetic lemonade, etc.) on the molars of rats, it became necessary to devise a rapid, reliable method for evaluating the degree of destruction of the enamel. McClure[2] has used the weights of the molars, dried at 110° C., as an index of enamel destruction. We have found, however, that considerable differences (sometimes greater than 20 per cent.) may occur in the weights of comparable molars from rats of the same litter and sex. Accordingly, a simple but accurate scoring system has been worked out based on the appearance under the dissecting microscope (about ×15) of the lingual surfaces of the molars.[3] This procedure has been found to apply equally well to other small laboratory animals, such as the hamster.

After the animals have been killed the heads are autoclaved at 115° C. for 20 to 30 minutes to soften the flesh. The jaws are then easily removed and cleaned, after which the extent of acid damage to each molar is evaluated according to the following criteria:

No effect	0
High polish of lingual enamel	1
Slight etching of lingual enamel	2

[1] The opinions and views set forth in this article are those of the writers and are not to be considered as reflecting the policies of the Navy Department.

[2] F. J. McClure, *Jour. Nutrition*, 26: 251–259, 1943.

[3] J. S. Restarski, R. A. Gortner, Jr., and C. M. McCay, *Jour. Am. Dent. Asn.*, 32: 668–675, 1945.