

In vivo studies. Acute toxicity of 2-sulfanilamido-5-carboxythiazole in mice gave the following results: by oral administration, LD₅₀, 8.0 grams/kg.; intraperitoneal, LD₅₀, 5.0–6.0 grams/kg.; subcutaneous, LD₅₀, 8.0 grams/kg. The chronic toxicity studied in mice, rabbits, and dogs was found to be much less than that of the readily absorbed sulfonamides and sulfaguanidine and comparable with succinylsulfathiazole.

Studies on the absorption following oral administration were carried out in mice, rabbits, dogs, and men. Blood levels obtained in all species were low (e.g. in man given 0.25 grams/kg./day for five days, the maximum blood level was less than 1.0 mg. per cent).

Absorption and excretion studies in man revealed that from 3 to 11 per cent of the 2-sulfanilamido-5-carboxythiazole administered orally was excreted in the urine, the average being 6.1 per cent.

The effect of the compound on reducing the number of coli in the feces of dogs and man was very striking. Comparison of the data with those of Poth, *et al.* (3) indicates that 2-sulfanilamido-5-carboxythiazole reduces the number of coli more rapidly and at a lower dose level than either succinylsulfathiazole or phthalylsulfathiazole. In a study of more than 200 patients on succinylsulfathiazole therapy, Poth and his co-workers found that 38 per cent showed less than 1,000 *E. coli*/gram of wet feces within three days of treatment, and that 79 per cent had less than this number within five days of treatment and 93 per cent within seven days. Poth explained that the remaining 7 per cent failed to respond to therapy within seven days because of some condition interfering with the action of the drug. The dosage used by Poth consisted of 0.25 gram/kg. of succinylsulfathiazole as an initial dose, followed in four hours by 0.25 gram/kg. daily, divided in six equal amounts and administered at four-hour intervals. Although the number of subjects on 2-sulfanilamido-5-carboxythiazole was small, it is worthy to note that the most refractory case studied had less than 1,000 coli/gram of feces within 48 hours of treatment at the same dosage employed by Poth. One subject, given one-half the dosage, showed less than 10 coli/gram of feces within 48 hours of treatment. With two subjects on 0.25 gram/kg. daily, omitting the initial dose of 0.25 gram/kg., a count of less than 1,000 coli/gram of feces was obtained within the first 24 hours of therapy. At the end of the second day, both counts were below 10 coli/gram of feces. Thus, in each of the four instances where 2-sulfanilamido-5-carboxythiazole was administered in doses either equal to or decidedly less than that employed by Poth for succinylsulfathiazole, coli counts of less than 1,000/

gram of wet feces resulted within two days of treatment.

In an independent clinical study of the compound, Harris and Finland (2) report findings that are in general agreement with these. They used the drug in treating cases of bacillary dysentery and state that, in the amounts given, it is poorly absorbed and that, as far as could be determined it is nontoxic. They found the drug to be effective in the cases of dysentery studied and state that it may deserve a place with the other drugs used in enteric infections and in bowel surgery.

Extensive clinical trials at a number of medical centers are now in progress which will establish the value of 2-sulfanilamido-5-carboxythiazole relative to that of other drugs used for intestinal antiseptics.

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Pectoral Girdles vs. Hyobranchia in the Snake Genera *Liotyphlops* and *Anomalepis*

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The Serpentes have always been considered to differ from all other reptiles in having lost the pectoral girdle completely. Recently, however, Dunn and Tihen (1) reported the discovery of a shoulder girdle in a primitive burrowing snake, *Liotyphlops albirostris*. This report seems to warrant further investigation before the interpretation of this structure can be accepted or rejected.

The specimens studied are, in part, the same as those used by Dunn and Tihen. Two specimens of *Liotyphlops albirostris* stained with alizarin red and cleared in glycerine were obtained from Dr. J. A. Tihen. Mr. K. P. Schmidt provided a stained specimen of *Anomalepis dentatus*. Finally, one specimen of *Anomalepis aspinosus*, used for gross dissection, was loaned by Mr. A. Loveridge. The writer is greatly indebted to these authorities for providing the necessary material.

Anomalepis, which is considered a close relative of *Liotyphlops*, possesses the same structure that Dunn and Tihen described in *Liotyphlops*. It is here considered valid, therefore, to apply findings in the

former genus to *Liotyphlops* and to the problem originally posed by it.

Dunn and Tihen present evidence to show that (1) a structure at the anterior end of the trachea is the hyobranchial apparatus and that (2) a more posterior element is a vestigial pectoral girdle. The present author believes that there is other, almost incontrovertible evidence that an alternative explanation suggested but not adopted by Dunn and Tihen is the proper one: that the anterior element is the cricoid cartilage of the larynx and the posterior element the hyobranchial apparatus.

The posterior element of Dunn and Tihen was found by gross dissection in a specimen of *Anomalepis aspinosus* measuring 178.4 mm. in total length. The anterior portion of the structure is extremely superficial, while the posterior parts are found imbedded progressively deeper in muscle. The structure is thread-like and shaped roughly like an M whose legs have been bent back upon themselves. It is situated 3.6 mm. posterior to the tracheal opening. The anterior horizontal part is 1 mm. broad and depressed 2 mm. medially. On either side it curves posteriorly to form two somewhat divergent posterior processes. These extend posteriorly for 1.3 mm., at which level they are 1.9 mm. apart, and then recurve sharply to assume an anterior course parallel to the posterior processes and separated from them by .2 mm. The anterior course can be seen for only .6 mm. in this specimen, although in the stained specimen it can be seen to extend as far forward as the level of the central depression in the horizontal portion. The extreme thinness of the apparatus probably accounts for the difficulty of demonstration by gross dissection.

M. geniohyoideus, M. sternohyoideus, M. omohyoideus, and M. hyoglossus are the muscles which Gnanamuthu (3) describes as having attachments to the ophidian hyobranchial apparatus. M. geniohyoideus has its origin on the mandible and its insertion on a median raphe, the basihyal and the anterior borders of the thyrohyals (= posterior cornua of the hyobranchium). M. sternohyoideus and M. omohyoideus have cutaneous origins, and have insertions on the thyrohyals. M. hyoglossus has its origin on the hind end of the posterior cornua of the hyoid and passes forward to form the greater part of the musculature of the tongue.

In *Anomalepis aspinosus* all the above muscles are found attached to the structures mentioned. M. geniohyoideus originates on the mandible and inserts on the anterior and anterolateral borders of the horizontal portion and posterior processes of the structure. M. sternohyoideus inserts on the posterior border of the horizontal portion and on the median surface of the posterior processes. It extends caudad

to a cutaneous origin. The two remaining muscles—M. omohyoideus and M. hyoglossus—are attached to the portion of the posterior process where it recurves sharply and extends anteriorly. M. omohyoideus inserts on the posterior margin of the bend and runs posterolaterally to its cutaneous origin. M. hyoglossus has its origin on the anterior margin of the bend and runs anteromedially; its right and left divisions converge anteriorly and join each other at the level of the horizontal portion of this element. At this level the two divisions of the M. hyoglossus are firmly bound together by connective tissue and form, with the M. genioglossus, the bulk of the tongue musculature.

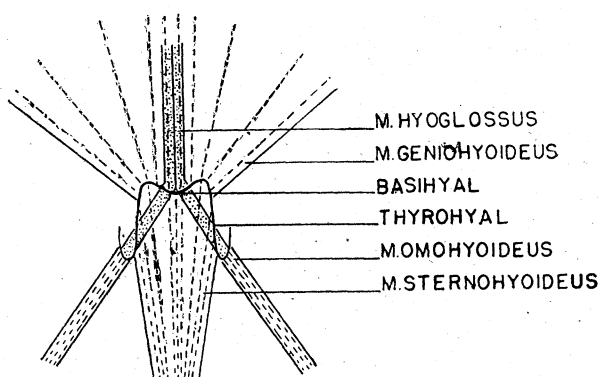


FIG. 1. Hyobranchium and associated muscles of *Anomalepis aspinosus*.

The laryngeal apparatus of serpents consists of dorsal paired arytenoid cartilages which are narrowly continuous posteriorly with the cricoid cartilage. Edgeworth (2) cites as exceptions the genera *Boa* and *Python* in which, according to him, the arytenoids articulate with the cricoid instead of being fused with it. The cricoid cartilage is a single structure having a striking \wedge -shaped anterior extension on the ventral surface and a more posterior and smaller \wedge or Ω configuration on the dorsal surface of the larynx. The cricoid cartilage extends posteriorly and is fused on either side with a variable number of tracheal rings.

Although the arytenoid cartilages cannot be discerned in the stained specimens of *Liotyphlops albirostris*, the cricoid cartilage is distinct. It is the anterior element which Dunn and Tihen interpreted as the hyobranchial apparatus. This cricoid cartilage is in the usual position and has the structure typical of all ophidians. It should be regarded as such.

The combined existence of hyoidean muscular attachments on the posterior element and the demonstration of typical cricoid form of the anterior element indicate that the concept of a pectoral girdle in *Liotyphlops* and *Anomalepis*, intriguing though it is, should be discarded. The structure incorrectly inter-

puted as a girdle should rather be regarded as a hyobranchial apparatus. It is different from any other hyobranchium ever recorded in snakes, but not different enough to cause concern regarding its identity. A further discussion of its significance is reserved for a later paper.

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Interrelations Between Choline, Betaine, and Methionine¹

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In nutrition there are many examples in which one dietary component reduces the required intake of another which is necessary to maintain normal life. Among these examples two principal classes may be distinguished:

Class 1. Substances which act as precursors for a physiologically required substance, permitting a synthesis of the required substance to any extent necessary. A few examples of this class are: (a) the several vitamin A-active carotenoids which are precursors for vitamin A; (b) methionine as a precursor of cystine; and (c) dimethylethanolamine in replacing dietary choline.

Class 2. Substances which may assume or diminish certain, but not all, functions of a required nutrient and which are not related to the required nutrient as a precursor or complete substitute. Examples of this class are: (a) the well-known effect of fat in reducing the requirement for thiamine; (b) the similar effect of Ca and P on vitamin D, especially in the fowl; and (c) the value of dietary cystine in reducing the necessary methionine level.

These two classes do not include other types of substances which affect the stability, absorption, transport, and utilization of nutrients and for which there already are specific and descriptive designations such as antioxidants and pro-oxidants, poisons, carriers, enzymes, etc. In the present discussion the term "sparing action" is reserved for examples as in Class 2, to the first of which it was applied quite some years ago (7).

In examples of Class 2, the "sparing" substance is able either to (1) partially reduce the need for, or (2) provide some, but not all, of the biological func-

tions of the "spared" compound. It is characteristic of these examples, in contrast to those of Class 1, that the replacement does not continue to be effective as the intake of the spared compound decreases, but often becomes sharply limited. In other words, a significant quantity of the spared compound must be present in order that a sparing action may become evident.

In dogs, fat may spare no more than approximately two-thirds of the thiamine requirement (4). In promoting calcification, Ca and P are interchangeable with vitamin D over a considerable range, yet all the vitamin D cannot be replaced by Ca and P without incurring additional symptoms of vitamin D deficiency (8, 19). Steps in the biological synthesis of cystine from methionine and other precursors have been demonstrated and reviewed in considerable detail (6). The reversal of this process does not take place, however, since a definite minimal level of methionine, which cannot be reduced further by surplus amounts of cystine, is needed both by rats (24) and by chicks (2).

In the rat, choline synthesis from betaine or methionine proceeds readily, *i.e.* the known symptoms of choline deficiency may be relieved by using sufficient of these substitutes (5, 10, 21). On the other hand, in the chick, choline synthesis is extremely limited. Methionine does not replace choline in chicks (9, 11, 13, 20) or in turkeys (12) for preventing perosis when the deficiency of choline is *severe*. A similar statement may be made for betaine in the case of *severe* choline deficiency (1, 13, 15, 16). Synthesis of choline in the chick may evidently take place from dimethylaminoethanol, which promotes growth and prevents perosis, but the total synthesis fails somewhere in the stages leading to this compound (14).

Chicks grew very slowly on a purified diet which involved a combined severe choline and partial methionine deficiency (1, 9). Upon the addition of methionine, the chicks made improved gains which reached a plateau at about two-thirds normal rate. It is noteworthy that the effect of methionine was definitely limited and, with respect to the choline requirement, probably consisted of the provision of methylating capacity but no extensive synthesis of choline. In the presence of ample choline and cystine and a partial deficiency of methionine in the basal diet, improved but limited growth rate was again observed. The evidence suggested that the methionine-sparing actions of cystine and choline were additive under the experimental conditions.

With other sources of labile methyl (methionine and betaine) present in the diet not more than 0.02 per cent choline was needed to support a two-thirds optimal rate of gain. Any choline synthesis which may take place in the chick is probably less than this figure,

¹ Condensed from an address delivered at AAAS-Gibson Island Research Conferences, 1945.