

Research, and computations by Contract No. N6onr-269, T.O. 16, with the Office of Naval Research, Physics Branch. We are much indebted to Professors Y. Saito and V. Vand and Dr. Y. Takeuchi for helpful discussions.

- ¹ Y. Okaya, Y. Saito, and R. Pepinsky, *Phys. Rev.*, **98**, 1857, 1955.
- ² Y. Saito, Y. Okaya, and R. Pepinsky (in press); cf. also *Phys. Rev.*, **100**, 970, 1955.
- ³ T. Doyne and R. Pepinsky (in press).
- ⁴ Y. Okaya and R. Pepinsky (to be published).
- ⁵ A. H. Compton and S. K. Allison, *X-rays in Theory and Experiment* (New York: D. Van Nostrand Co., 1935).
- ⁶ R. W. James, *Optical Principles of the Diffraction of X-rays* (London: G. Bell, 1950).
- ⁷ C. H. Dauben and D. H. Templeton, *Acta Cryst.*, **8**, 841, 1955.
- ⁸ H. Eisenlohr and G. L. J. Müller, *Z. Physik*, **136**, 511, 1954.
- ⁹ L. G. Parratt and C. F. Hempstead, *Phys. Rev.*, **94**, 1593, 1954.
- ¹⁰ R. Pepinsky, Report to O.N.R., Physiology Branch, Contract No. Nonr-656(09), December 1, 1955 (unpublished).
- ¹¹ R. Pepinsky, *Phys. Rev.*, **100**, 971, 1955. Cf. also American Association for the Advancement of Science, Abstracts (Atlanta, Ga., December 27, 1955), Sec. C.
- ¹² V. Vand, P. F. Eiland, and R. Pepinsky (in press).
- ¹³ G. Shirane, R. Pepinsky, and B. C. Frazer, *Phys. Rev.*, **97**, 1179, 1955.
- ¹⁴ R. Pepinsky, *Rev. Sci. Instr.*, **24**, 403, 1953.
- ¹⁵ R. Pepinsky, Progress Report to Office of Ordnance Research, Contract No. DA-36-061-ORD-478, December 15, 1955 (unpublished).
- ¹⁶ J. M. Bijvoet, *Nature*, **173**, 888, 1954.
- ¹⁷ Y. Saito, K. Nakatsu, M. Shiro, and H. Kuroya, *Acta Cryst.*, **8**, 729, 1955.
- ¹⁸ M. J. Buerger, *Acta Cryst.*, **3**, 87, 1950; **4**, 531, 1951.
- ¹⁹ R. Pepinsky, Y. Takeuchi, and Y. Okaya, Progress Report to O.N.R., Physics Branch, Contract No. N6onr-269, T.O. 16, February 1, 1956 (unpublished).
- ²⁰ H. Hönl, *Z. Physik*, **84**, 1, 1933; *Ann. Physik*, 5th ser., **18**, 42, 1933.
- ²¹ Cf., e.g., J. M. Robertson, *Organic Crystals and Molecules* (Ithaca, N.Y.: Cornell University Press, 1953), pp. 147-150.

SOUND TRANSMISSION IN THE TURTLE'S EAR*

BY ERNEST GLEN WEVER AND JACK A. VERNON

DEPARTMENT OF PSYCHOLOGY, PRINCETON UNIVERSITY

Communicated March 10, 1956

Though a number of writers have expressed doubts about the hearing abilities of the turtle, and some have gone so far as to regard its ear as degenerate and largely or even wholly unresponsive to sounds, it is easy to show by both behavioral and electrophysiological methods that an auditory function is present. We have recently recorded the electrical potentials produced in the inner ear in response to sounds for a number of turtle species and have made detailed measurements on three of these: the wood turtle, *Clemmys insculpta*; the painted turtle, *Chrysemys picta picta*; and the "Cumberland" turtle, *Pseudemys scripta*.¹ These results show an excellent auditory function within certain limits of frequency and intensity. For tones up to 700 cycles, the sensitivity, when expressed as the sound pressure required to produce some small value of inner-ear potential, is of the same order of magnitude as that similarly measured in the mammals, such as the cat, for the same

tones. As the sound intensity is increased, the inner-ear potentials rise proportionately, but only for a short range as compared with those observed in the higher animals. For sounds that to our ears are only moderately loud these potentials depart from linearity, and they continue in their nonlinear course over a considerable range, until finally at a high level of intensity they reach a maximum from which a further increase in stimulation causes a decline. At these high levels the ear is endangered, just as has been found to be true in the higher animals, and the maintenance of sounds at these levels for a few seconds will produce a grave impairment of the sensitivity.

Thus we find in the turtle's ear the same basic phenomena that have been observed in more detail in the highly developed ears of the mammals, though with limitations of range along the frequency and intensity dimensions. We need to consider both the similarities and the differences in relation to the particular anatomy and physiology of the turtle's ear.

The turtle has a well-developed middle ear (see Fig. 1). There is a superficial tympanic membrane, a relatively large tympanic cavity, and an ossicular mechanism composed of two elements, the columella and extracolumella. The extra-

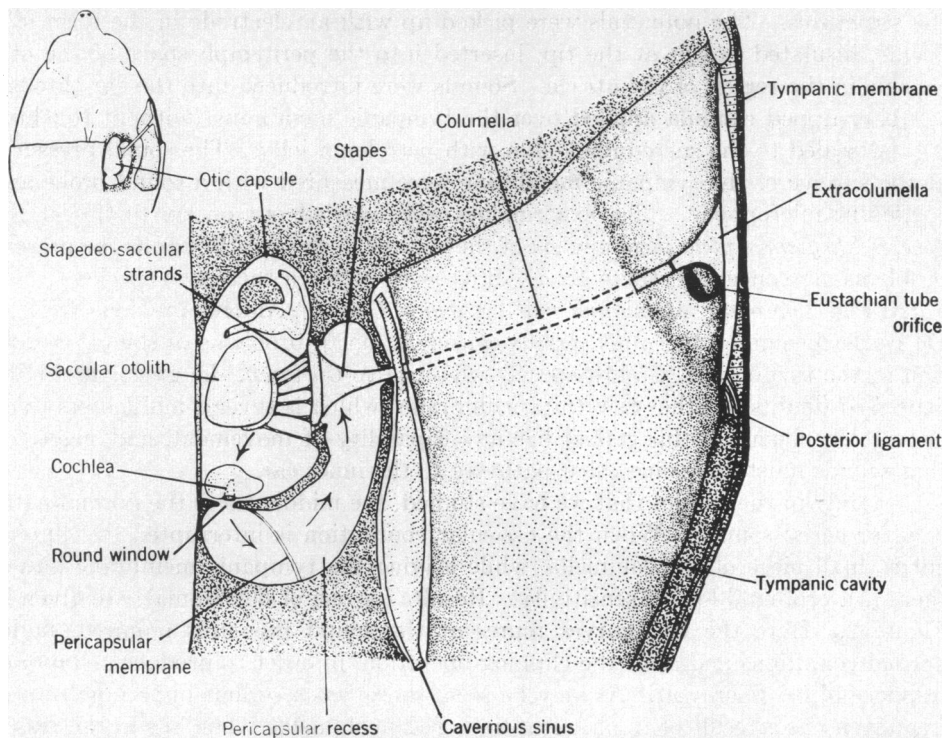


FIG. 1.—The right ear of the turtle *Pseudemys scripta*, seen from above. The insert on the upper left gives an outline of the head, about one-half natural size, with the auditory structures located.

columella consists of a cartilagenous disk that forms the inner surface of the tympanic membrane, and a short process that extends inward to articulate with the columella. The columella is a slightly curved bony rod that enters a canal in the

medial wall of the tympanic cavity and runs to the otic capsule, where it expands to form a funnel-shaped stapes. The stapes lies in the oval window of the capsule. A Eustachian tube extends from the anterior portion of the tympanic cavity to the lateral angle of the pharynx.

An unusual feature of this ear is the absence of any contact between the tympanic cavity and the otic capsule. These two are separated by bone and by a special fluid-filled cavity, the pericapsular recess. This recess extends along the lateral and posterior borders of the otic capsule and incloses both the oval and round windows of the capsule. Near the posterior end of the recess is a thin membrane that has usually been called the "round-window membrane" or "secondary tympanic membrane" but has no connection with the round window; we shall call it simply the "pericapsular membrane." There is no covering of the round window itself, though the cochlea lies opposite this window and forms a partial obstruction to the free flow of fluid. Only the pericapsular membrane separates the fluid of the recess from that of the otic capsule.

In our experiments we have tried to determine the contributions of some of these structures to the hearing process. Our method was to record the inner-ear potentials produced by sounds under normal conditions and after various alterations of the structures. The potentials were picked up with an electrode in the form of a needle, insulated except at the tip, inserted into the perilymph space of the otic capsule in the region of the utricle. Sounds were introduced into the ear through a rubber-tipped cannula applied over the tympanic membrane (without touching it) and sealed to the surrounding skin with petroleum jelly. The sound pressures at the surface of the tympanic membrane were measured with a sound probe and condenser microphone. The experiments were carried out on six turtles of the species *Chrysemys picta picta* and six of the species *P. scripta*. So far as the present problems are concerned, there are no differences between these two species.

1. *The Tympanic Membrane and Ossicular Chain.*—Among previous writers, De Burlet has given the most serious consideration to problems of sound conduction in the turtle's ear.² With special reference to *Chelonia*, the green turtles, he expressed doubts whether the drum membrane (which is several millimeters thick in large specimens of this genus) has any capability of movement, and suggested that sounds must find some other pathway to the inner ear.

Certainly in the species that we have studied, the middle ear is the normal pathway for aerial sounds. When the ossicular connection is interrupted by clipping out a small piece of the columella, while leaving the tympanic membrane intact, there is a profound loss of sensitivity. Results for one of our animals are given in Figure 2. Here the two curves represent the sound pressures required, under normal conditions and after the clipping operation, in order to produce a standard response of 0.3 microvolt. As may be seen, there was a serious impairment at all frequencies as a result of the interruption of the columella. The loss in the region of 700–1,000 cycles was particularly great in this animal.

More representative results are given in Figure 3. Here we show directly the loss of sensitivity resulting from clipping the columella, and the curve represents an average of observations on 8 ears. In the low frequencies the loss is around 40 db., indicating a reduction in the amplitude of the sounds reaching the inner ear to 1 per cent of their normal value. This reduction is somewhat greater at 700–1,000 cycles, and then less for the higher frequencies.

2. *The Tympanic Cavity.*—The tympanic cavity consists of two connected portions, an anterior portion that lies immediately behind the tympanic membrane and a posterior portion that extends backward as a tubular pocket in the squamous

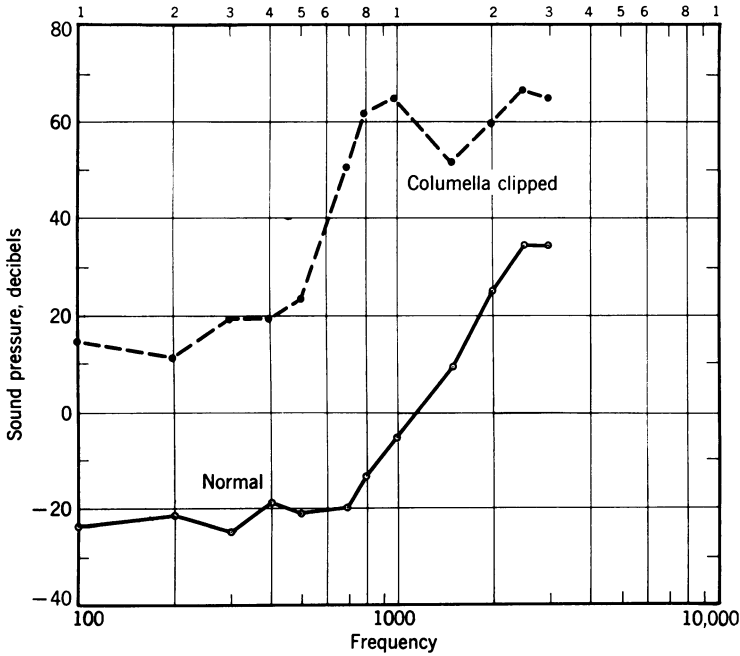


FIG. 2.—Sensitivity curves before and after interrupting the ossicular chain. The curves show the sound pressure, in decibels relative to 1 dyne per square centimeter, required to produce a response of 0.3 microvolt.

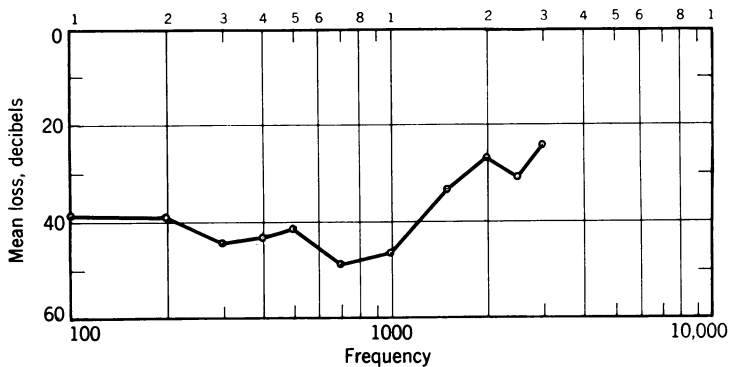


FIG. 3.—Losses of sensitivity caused by interrupting the ossicular chain, averaged for eight ears.

bone. For the procedures next to be described, it was necessary to take away the lateral wall of the posterior portion of the cavity, and it was therefore of interest to discover whether this opening of the tympanic space had any effect upon sound transmission.

The upper curve of Figure 4 shows some of the results, expressed as a loss of sensitivity after opening the tympanic cavity. At no point does the curve depart

significantly from the zero line, and hence this procedure does not have any important effect upon the transmission of sounds. Evidently this cavity does not resonate to any of the frequencies within the turtle's range, as indeed its small size would lead us to expect. (If the cavity is treated as a closed tube, a calculation indicates a fundamental resonance close to 6,000 cycles.)

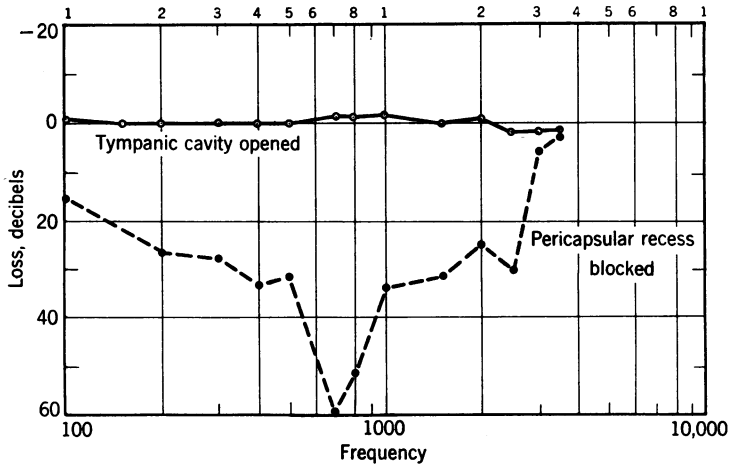


FIG. 4.—Effects on sensitivity of opening the tympanic cavity (*solid line*) and blocking the pericapsular recess (*broken line*).

3. *The Pericapsular Recess.*—After the posterior portion of the tympanic cavity is opened by removing its lateral wall, it is possible to gain access to the pericapsular recess through the medial wall. A useful landmark on the bony wall is a dark line representing the shadow of the cavernous sinus, a vessel containing the external carotid artery, internal jugular vein, and facial nerve. This sinus lies just lateral to the floor of the pericapsular recess and is separated from it by a thin lamina of bone. We penetrated the bony wall above this sinus, using great care not to damage the sinus itself.

When a small opening is made in the pericapsular recess, its fluid does not immediately flow out. The effects on the hearing are then slight, as the solid-lined curve of Figure 5 will indicate. Most tones showed losses of sensitivity, but these losses

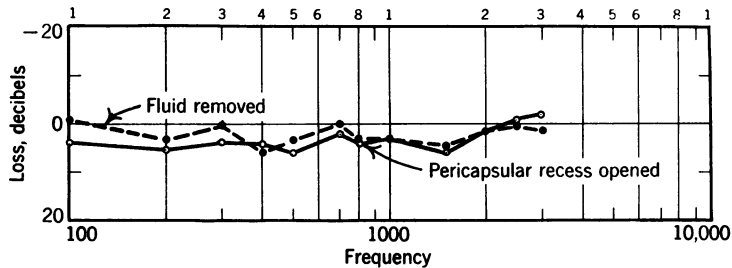


FIG. 5.—Effects on sensitivity of opening the pericapsular recess (*solid line*) and removing its fluid (*broken line*).

were never greater than 6 db., and at the two highest frequencies there were slight gains; the average was a loss of 3 db.

The fluid contained in the recess was completely removed by inserting small pledgets of absorbent cotton. The effects of this procedure on sensitivity were slight also, as the broken line of Figure 5 indicates. Again the losses varied up to 6 db., with several values on or close to the zero line; the average was 2 db. Note that the effect shown by this curve is simply that of removing the fluid, and to obtain the whole effect of opening the recess and removing its fluid we must add the two curves of Figure 5; this total loss averaged 5 db.

The fluid of the otic capsule that is displaced by movements of the stapes must find relief in some manner. In birds and mammals this relief is afforded by a bulging or retraction of the round-window membrane, which adjoins a middle-ear cavity containing air. In the turtles there is no air cavity to permit this form of relief of the moving fluid, but a circular path for the fluid is provided to achieve the same result. When the stapes moves inward, the fluid movement extends through the otic capsule to the round window; then, by displacing the pericapsular membrane, it continues through the recess to the lateral surface of the stapes, as shown by the arrows of Figure 1. An outward thrust of the stapes of course reverses the direction of this circular movement.

De Burlet suggested that this arrangement in the turtle ought to give an especially good utilization of the stapelial movements. This suggestion hardly withstands scrutiny from an acoustical standpoint, and the results seen in Figure 5 do not bear it out in any substantial way.

The fluid of the recess in its motion encounters a frictional resistance at the bony walls and in the fluid itself by reason of its viscosity, and for rapid oscillations this resistance might be expected to reduce the transmission. Removal of the fluid then would give an improvement. This does not happen, which signifies that the friction is small, at least in relation to other friction encountered by the system.

The fluid of the recess also contributes mass to the moving system, and the observations likewise signify that this mass is small relative to other mass present in the system.

Another yielding place that would give freedom for the movements of the inner-ear fluid needs to be considered. De Burlet regarded this yielding place for many amphibians as what he called the "skull-base membrane," a membranous region of the cranial capsule bordering on the perilymphatic sac. He described a similar condition in many reptilian forms also. In the turtles a portion of the floor of the otic capsule consists only of thin cartilage, and this thin region lies just above the lateral angle of the pharynx. When the mouth and pharyngeal cavities contain air, this region could serve as a yielding place for the fluid vibrations. To test this possibility, we filled these cavities with petroleum jelly. No significant differences of sensitivity were observed as a result of this obstruction.

On the other hand, an obstruction of the pericapsular membrane, produced by filling the posterior part of the pericapsular recess with bone wax, had a profound effect. The lower curve of Figure 4 shows some of the results. The losses of sensitivity were especially grave at 700 and 800 cycles and were serious for all other tones except 3,000 and 3,500 cycles. These results prove that for tones up to 3,000 cycles the oscillatory path extends through the pericapsular recess, as already suggested.

Our results do not show what sensory endings of the turtle's ear are responsible for the potentials produced by sounds. There is reason to believe that the papilla basilaris of the cochlea plays a large role in this action. It lies opposite the round window, where the passage between otic capsule and pericapsular recess is particularly narrow. As all the fluid displaced by the stapedial motion must pass through this narrow opening, there is a marked increase in the amplitude of motion here. In an average specimen the area of the stapedial footplate was measured as 12.1 sq. mm. and that of the round window as 1.5 sq. mm. Consequently there is an amplification in the motion at the round window of about 8-fold. We need further study of the relations between the cochlear endings and the fluid flow in this region to determine whether this amplification is fully effective.

Other endings in the otic capsule are not so favorably situated. The lagenar macula lies in a blind pocket of the same structure that contains the cochlea, and it is not in the immediate path of the movement. The saccular macula is in the main part of the capsule, directly opposite the stapedial footplate and probably in the path of the oscillatory movements. But this part of the capsule is wide, and the amplitude of fluid motion here will be no greater than at the footplate, and probably less. The utricular macula is situated in the superior division of the capsule, remote from the main path of the movements.

For the saccular endings there is a further means of stimulation by the stapedial motions. Extending from the footplate of the stapes to the lateral edge of the saccular macula are a great many fibrous strands, which we call the stapedeo-saccular strands. These strands are surprisingly strong and are under considerable tension. When pushed aside with a fine needle, they spring back at once, and they can be broken only by the exertion of a good deal of force. When the stapes is displaced inward or outward by microscopically visible amounts, the motion is communicated to the edge of the macula and to a lesser extent to the otolithic mass. We have not found any reference to this structure in the anatomical literature, and evidently it has escaped notice heretofore. We hope to study it further, but at present we can conceive of two possibilities for its function. It may serve an auditory function by communicating the vibratory motions of the stapes to the saccular macula. Or it may serve a hydrostatic function and represent the simple displacement of the stapes as the animal swims to different depths and the tympanic membrane is exposed to different water pressures.

It is well known that efficiency in the reception of aerial sounds is achieved in the mammals by two forms of mechanical transformer action. These are a lever action of the ossicular chain and a hydraulic action arising from the difference in areas of tympanic membrane and stapedial footplate. Exact measurements of these actions have been made in the cat³ and show a lever ratio of 2.5 and a hydraulic ratio of 24.3, thus providing a total transformer ratio of 60.7. The acoustical resistance of the air is therefore multiplied by the square of 60.7, or 3,644, and hence is more suitably matched to the resistance of the fluids of the inner ear.

In the turtle a lever system is lacking, and the areal ratio is small. The area of the extracolumellar disk was measured as 103.5 sq. mm. and that of the stapedial footplate as 12.1 sq. mm., giving a ratio of 8.5. If we assume that the condition in the cat is an efficient one, this factor of 8.5 is clearly inadequate by itself; if it operated alone, we should have difficulty in accounting for the high degree of sensi-

tivity that the turtle shows for the low tones. Our problem becomes easier if we consider also the other sort of hydraulic factor referred to above, the ratio between the areas of the stapedial footplate and the round window. If we use the value of 8 for this latter ratio as suggested, the total transformer ratio becomes 68, which is close to the figure obtained in the cat. This second factor needs more precise determination, but it appears from this preliminary consideration that the turtle's ear is well adapted to the reception of aerial sounds, at least in the low-frequency range.

* This investigation was supported in part by the Office of Naval Research, under Contract N6-onr-270-3, and by Higgins funds allotted to Princeton University. Permission is granted for reproduction and use by the United States Government.

¹ E. G. Wever and J. A. Vernon, "The Sensitivity of the Turtle's Ear as Shown by Its Electrical Potentials," these PROCEEDINGS, 42, 213-220, 1956.

² H. M. De Burlet, "Vergleichende Anatomie des statoakustischen Organs," in Louis Bolk, E. Göppert, E. Kallius, and W. Lubosch, *Handbuch der vergleichenden Anatomie der Wirbeltiere*, Berlin and Wien: Urban and Schwarzenberg. 2, Part II, 1293-1432, 1934.

³ E. G. Wever and M. Lawrence, *Physiological Acoustics* (Princeton, N. J.: Princeton University Press, 1954).

NEW EXPERIMENTS ON CHEMICAL PHENOCOPIES*

BY RICHARD B. GOLDSCHMIDT AND L. K. PITERNICK

UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA

Communicated March 5, 1956

In his early work on heat-induced phenocopies Goldschmidt¹ had shown that the relative frequency and the type of phenocopies produced were dependent upon the specific genetic background of the experimental material, *Drosophila*. Data were presented for the different responses to the same treatment of three wild-type and five mutant stocks. They showed that some reacted preferably with wing effects, some showed phenocopies otherwise not encountered, while others had a tendency to asymmetrical effect. Additional important findings were, in one case, the presence of a subthreshold (isoallelic) mutant of vestigial which was enhanced to high penetrance by the phenocopic treatment, and, further, two cases of production of a dominant effect of the recessive, which might also be described as enhancement of a subthreshold action of the heterozygous mutant locus. Although in later work by different authors the genetic element in the production of the phenocopic effect was noted and the enhancing effect upon subthreshold or low-penetrance genetic action was also encountered (e.g., Goldschmidt;² Child, Blanc, and Plough;³ Plaine and Glass;⁴ Sang and McDonald⁵), no specific importance was attributed to the facts, which could be regarded as more or less expected in view of the known presence in all stocks of widely recombining genetic differences of the type usually described as modifiers.

Since Rapoport⁶ discovered the specific phenocopies produced by different chemicals, especially metal salts (later found for still other chemicals; see, e.g., Gloor,⁷ Bodenstein and Abdel-Malek,⁸ Hinton *et al.*⁹), a more quantitative study