

electrophoresis and total protein by the biuret method, did not appear to influence Gm phenotype. In 21 animals (11 tuberculous and 10 healthy) gamma globulin concentrations and Gm<sup>a</sup> type were determined on the same serum aliquots. The upper limit of the gamma globulin concentration for Gm (a-) individuals was 4 times the lower limit observed among Gm (a+) individuals. The mean gamma globulin concentration was 1.50 g/100 ml among the Gm (a+) animals and 1.60 g/100 ml among the Gm (a-) animals. Several animals with Gm (a+b+) phenotype had smaller concentrations of gamma globulin than the two chimpanzees with Gm (a-b-x-), that is, "Gm-less" phenotype. The single Gm (a+b+x+) individual had a gamma globulin concentration of 2.01 g/100 ml. This was exceeded by the gamma globulin concentrations of three other animals, two of which were Gm (a-). The individual with an intermediate Gm<sup>b</sup> score had a gamma globulin concentration of 0.54 g/100 ml. Lack of correspondence of gamma globulin concentration with Gm phenotype is in accord with observations in man (7). Such findings suggest that the Gm substances are specific proteins rather than a variable feature of all gamma globulins. Further evidence for this view is provided by the failure of large increases in gamma globulin, after immunization of chimpanzees with ovalbumin, to alter either a Gm (a-b-x-) or a Gm (a-b+x-) phenotype.

Our results confirm the earlier observation of Podliachouk (8) who found that of various animals studied the chimpanzee alone possessed the Gm (a+) character. All of 24 chimpanzees examined were Gm (a+). Reagents for typing other allelic products were not then available. The universality of the Gm (a+) character in the earlier study may be the result of inbreeding in small ape isolates.

The appearance of Gm polymorphism in both man and chimpanzee might be due to two independent sets of mutation resulting in at least three similar allelic products in two species. A simpler explanation would be to assume a common origin for the polymorphism in which case the system probably originated at least as early as the Oligocene period. If the latter explanation is correct, then it, in turn, indicates that the polymorphism in both species is balanced rather than transient and subject to rather ubiquitous selective forces.

The observation of heterogeneity of specific gamma globulins which are common to man and chimpanzee suggests a possible means of demonstrating in man the allotype described by Oudin

(9) and others (10) in rabbits. The chimpanzee may be useful as a surrogate man for the purpose of creating isoprecipitins which can then be tested against human sera for reaction with isoantigens. Such an attempt is currently in progress (11).

SAMUEL H. BOYER  
WILLIAM J. YOUNG  
*Departments of Medicine and Anatomy,  
Johns Hopkins University School of  
Medicine, Baltimore, Maryland*

#### References and Notes

1. R. Grubb and A. B. Laurell, *Acta Pathol. Microbiol. Scand.* **39**, 390 (1956).
2. M. Harboe and J. Lundevall, *ibid.* **45**, 357 (1959).
3. M. Harboe, *ibid.* **46**, 191 (1959).
4. S. H. Boyer, in preparation.
5. The cooperation of Prof. David Bodian in providing samples of chimpanzee and cynomolgous monkey blood is gratefully acknowledged. Dr. James Wright of the National Zoological Gardens, Washington, D.C., provided blood samples from gibbons.
6. Agglutination with each RA dilution was scored on a scale of 0-4. Complete neutralization by animal serum results in a score of 0 and classification as Gm+.
7. R. Grubb [*Ciba Symposium on Biochemistry of Human Genetics* (Boston, Mass., 1959), p. 264] has observed that the human Gm a substance migrates as a gamma<sub>2</sub> globulin. Grubb also reported that Gm grouping in kindreds with hypergammaglobulinemia gave no evidence of correlation between gamma globulin concentration and Gm (a+) character.
8. L. Podliachouk, *Ann. Inst. Pasteur* **96**, 362 (1959).
9. J. Oudin, *Compt. rend.* **242**, 2606, 2489 (1956); *J. Exptl. Med.* **112**, 107, 125 (1960).
10. S. Dubiski, A. Dudziak, D. Skalba, A. Dubiska, *Immunology* **2**, 84 (1959); S. Dray and G. O. Young, *J. Immunol.* **81**, 142 (1958); —, *Science* **131**, 738 (1960).
11. This work was supported in part by U.S. Public Health Service grant No. B2053 from the National Institute of Neurological Disease and Blindness.

21 November 1960

### Fossil and Living Conchostracan Distribution in Kansas-Oklahoma across a 200-Million-Year Time Gap

*Abstract.* Fifty-nine of 493 ponds sampled in the Wellington fossil conchostracan belt contained *Cyzicus mexicanus* (Claus). Persistent habitat preference and faunal association were also found for four orders of insects (Odonata, Ephemeroptera, Neuroptera, and Homoptera). Comparative limnology is detailed. Greater geographic fractionation of Permian conchostracan gene-pools is attributed to a more arid climate indicated by evaporites.

For the past 3 years, as part of a paleolimnological research project, one of us (P.T.) has been tracing the conchostracan-bearing beds of the Wellington formation (Permian, Leonardian) of Kansas and Oklahoma (1). It occurred to him that a survey of living clam shrimp distribution in the entire area of mapped Wellington conchostracan-bearing beds might provide useful in-

formation for comparison. Accordingly, Zimmerman was assigned to the project as limnologist-entomologist. Well over 550 ponds were sampled during the summers of 1958 to 1960. Collecting during 1958-59 was sporadic, and only during June, July, and August of 1960 was systematic and persistent daily sampling carried out. It is this last sampling of 493 ponds that is reported on here.

Of 493 ponds sampled in and near the Wellington outcrop belt [Fig. 1, Table 1, and (2)], 59 contained clam shrimps. An over-all percentage of 12.1 percent of all ponds sampled contained clam shrimps, the range being from 6.4 percent to 16.4 percent.

Weather records (3) show that, for the belt of investigation, average temperatures and rainfall were similar for the 3-year sampling period.

The conchostracan, *Cyzicus mexicanus* (Claus), was the only species present in all collections. It was found to be presently distributed, though more irregularly, in the same general area as the Wellington fossil-conchostracan beds. A persistence of habitat-preference from Paleozoic time to the present has thus been demonstrated. In fact, several ponds bearing clam shrimp were discovered *within* the outcrop belt of fossil occurrences (Marion County line, northern Kay County, northwest Noble County; see Fig. 1).

In addition, most other clam shrimp ponds were found to be adjacent or proximate, or both, to such outcrop belts. A few exceptions were noted. Most present-day clam shrimp ponds in southern Dickinson County, for example, occur to the north and east of the fossil belt in this region. The relatively rugged topography in the fossil belt compared to the low-lying terrain to the north and east may account for this distribution (4).

Modern ponds in the sampled area were found to possess very specific characteristics. Alongside of these, fossil occurrences in the Wellington will be cited.

Duration of modern ponds is relatively short. In fossil occurrences, conchostracans found on any given bedding plane are separated from younger and older occurrences by rock intervals ranging from millimeters to meters. Hence, we may conclude that Leonardian ponds, like modern ones, were temporary and sporadic in occurrence.

Currents are generally absent in modern ponds. This is also true of Leonardian ponds. However, there is some evidence of microcross-bedding due to currents in clam shrimp-bearing argillaceous limestone. Similarly, highly fossiliferous cross-bedded siltstones were found.

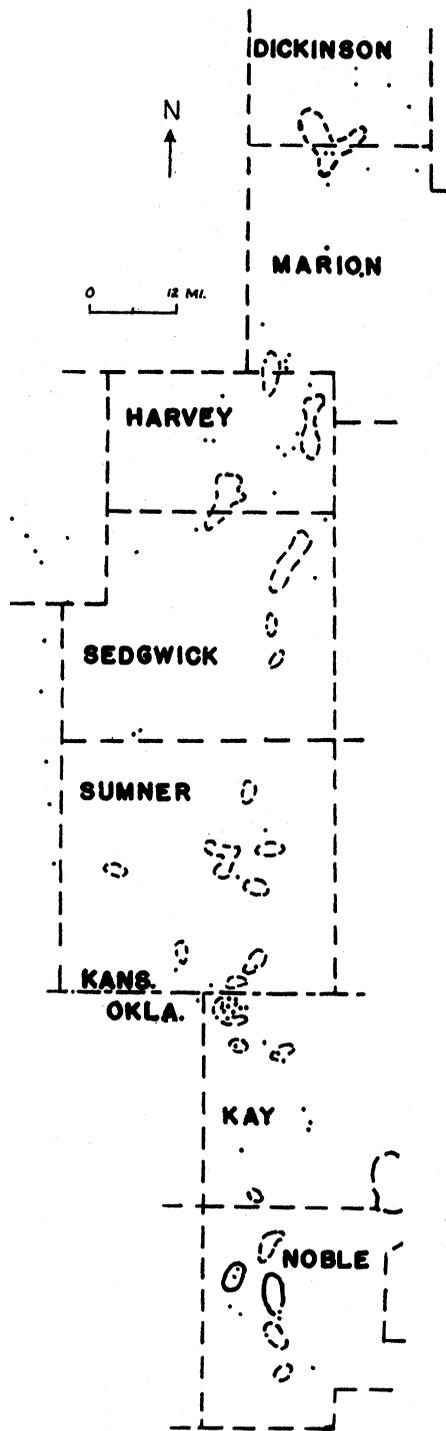


Fig. 1. Distribution of the living conchostracan, *Cyzicus mexicanus* (Claus), in the outcrop belt of Leonardian (Wellington) fossil conchostracans. Dashed lines embrace area of fossil clam shrimp localities (data by Tasch). Solid line (Noble County) embraces fossil clam shrimp localities (data by Raasch) explored but not sampled by Tasch. Dots represent localities of ponds bearing *C. mexicanus* (data by Zimmerman). Isolated dots to west of Sedgwick and Harvey counties are clam shrimp-bearing ponds that parallel some of the Ninnescah formation clam shrimp beds. Drafting by Bernard Shaffer (assistant to Tasch in fossil collections).

The bottom of modern ponds consists of soft clay mud, often mixed with gravel. In fossil occurrences, the lithology of clam shrimp-bearing beds was most often argillaceous limestone. Indurated red, green, and gray clay muds and siltstones also bore fossil conchostracans at specific localities.

The depth of modern ponds is generally less than 12 in. In fossil occurrences, the depth is also generally very shallow. In one instance (many could be cited to illustrate the basis for this conclusion), a thin fissile shale 0.2-foot thick bore 25 distinct clam shrimp generations completely separated from each other by an interval of sediment 1.0 mm to 5.0 mm thick.

Turbidity was found to be present in all but two of the modern ponds containing clam shrimps. This turbidity was due to clay mud in suspension. In fossil occurrences, turbidity may be inferred by the frequently appearing indurated clay muds containing fossil clam shrimps and by the highly argillaceous composition of clam shrimp-bearing limestones which are extremely fine-textured.

Modern ponds have indefinite margins, probably due to depression of the strandline during drying. In fossil occurrences, there are numerous small irregularly shaped bio- and lithofacies bearing clam shrimp fossils that have been mapped. One instance of an actual three-dimensional pinch-out of a wedge-shaped body bearing clam shrimps was found.

Modern ponds are generally barren of submergent or emergent vegetation with the exception of allochthonous materials. By far the most common situation in fossil occurrences is the absence of all vegetal debris. In some portions of the Wellington, however, there were abundant plant debris, carbonized and silicified wood, whole logs (autochthonous), and leaves. Charophytes and spores are also found in some clam shrimp beds.

In modern ponds, a mud margin of several yards generally intervened between the water and encircling vegetation. In fossil occurrences, clam shrimp valves abounded in some mud-crack beds. This conforms to field observations of the mud margins of some modern ponds studded with conchostracan shells.

Modern ponds are small, approximately 500 ft<sup>2</sup>. In the Wellington, many clam shrimp found as fossils occupied puddle-sized water bodies. Several instances of occupancy of larger water bodies as well were indicated when it was possible to sample for fossil conchostracans laterally for many hundred feet.

Modern ponds were found in open fields, roadside ditches, sloughs, and

Table 1. Distribution of modern conchostracan-bearing ponds in a seven-county area embracing mapped outcrop belts of the Permian Wellington conchostracan beds (sampled during June, July, and August 1960).

| Region* | Total No. of ponds sampled | No. of ponds containing clam shrimps | Total No. of sampled ponds bearing clam shrimps (%) |
|---------|----------------------------|--------------------------------------|---|
| I       | 73                         | 11                                   | 15.0  |
| II      | 79                         | 13                                   | 16.4  |
| III     | 91                         | 6                                    | 6.5   |
| V       | 78                         | 5                                    | 6.4   |
| VI      | 101                        | 16                                   | 15.8  |
| VII     | 71                         | 8                                    | 11.3  |
| Total   | 493                        | 59                                   | 12.1 (av.)  |

\* I, Dickinson County, Kan.; north and central Marion County, Kan.; II, southern Marion County, Kan.; Harvey County, Kan.; III, Sedgwick County, Kan.; V, Sumner County, Kan.; VI, Kay County, Okla.; VII, Noble County, Okla.; Region IV embraced sampled ponds in Reno, Harper, and Kingman counties, Kan., but it is not included since it parallels the outcrop belt of the Ninnescah shale (9). Of 47 ponds sampled in this region, 11 bore clam shrimps.

flood plains. While details are still to be worked out, the picture that emerges for the Wellington is of a series of relict ponds and puddles in a coastal swamp area where the sea occasionally invaded. This last conclusion is indicated by the occurrence of algal reefs over and below clam shrimp-bearing beds and by related data.

Modern ponds have a pH ranging from neutral to slightly alkaline. Ponds with reducing conditions do not contain clam shrimps. In fossil occurrences, an equivalent pH seems to have prevailed in most instances. However, the finding of fossil conchostracans in green shales (formed under reducing conditions) and the presence of organic debris that could yield humic acids indicate some reducing condition in Leonardian bottom muds of the mapped area. In such events, clam shrimps probably migrated to the upper reaches of the water. Aquarium populations of *Cyzicus mexicanus* raised by one of us (P.T.) were found to behave in this way when bottom muds were fouled. In general, living clam shrimps, and presumably fossil forms during their lifetime, require well-aerated waters.

No clam shrimps were found in brackish waters of modern ponds. No salt flats were observed in dried-out modern ponds. By contrast, gypsum, salt casts, and casts of hopper crystals—some very large—were not infrequent in Wellington clam shrimp-bearing beds (5).

It was found that four of the several orders of insects (Odonata, both damselflies and dragonflies; Ephemeroptera, mayflies; Neuroptera, lacewings; and Homoptera, leaf hoppers) that were fossilized with Wellington conchostra-

cans (6) were also present in or on the surface of modern clam shrimp ponds in the sampled area. In this instance, as with the conchostracans, it is clear that habitat-preference and adaptation which were established or operative in Leonardian time still persist.

While the fossil collections have yet to be thoroughly analyzed for clam shrimp populations, certain observations, based on field and laboratory notes are possible at present. A one-species spread such as that for *Cyzicus mexicanus* seems, at any given time, to have characterized only portions of the Leonardian outcrop belt in Kansas and Oklahoma. Thus, one of the oldest clam shrimp zones, some 10 feet above the Annelly gypsum, contained three different generic types: pemphicyclids (bearing a tubercle or spine on the initial valve), typical estheriids (lacking valve structures), and, at one Oklahoma locality, leaiid conchostracans (bearing two ribs on the valve). Or, in Kansas localities, three related but distinct genera were found in contemporaneous beds: *Pemphicyclus* (initial valve with central minute tubercle), *Gabonestheria* (large anterodorsal conical spine on initial valve), and *Curvacornutus* (with large anterodorsal, looped or hooked spine on initial valve). Multiple instances of this kind of differentiation (speciation) in contemporaneous ponds could be cited (7).

Thus, the fossil record in the mapped area (Fig. 1) indicates a greater incidence of genetic variability at specific times and at several different times of clam shrimp appearance during the Wellington. In turn, this refers to a more frequent geographic fractionation of the common gene pool with attendant reproductive isolation and speciation in Leonardian situations than is found in modern ponds in the sampled area. The evaporites noted earlier denote a more arid climate with consequently greater alternation of drying and wetting. This might well be a critical factor in the "more frequent geographic fractionation" referred to above (8).

PAUL TASCH

Department of Geology, University  
of Wichita, Wichita, Kansas

JAMES R. ZIMMERMAN

Department of Biology, Indiana  
Central College, Indianapolis

#### References and Notes

1. P. Tasch, "Microstratigraphy and the search for Permian freshwater biofacies," American Association of Petroleum Geologists-Society of Economic Paleontologists and Mineralogists program (Atlantic City, N.J., 1960), p. 83 (abstr).
2. Constructed farm ponds are not included in this sample.
3. Data were obtained from the U.S. Weather Bureau, Wichita, Kan., for the years 1957 through June 1960. The belt of investigation lies in and occupies most of the 30-35 in. rainfall zone.

4. The spotty distribution of *Cyzicus mexicanus* in the sampled belt recorded during the 1960 sampling confirms field observations made during the less intense collecting of the summers of 1958 and 1959. During the 1960 field season, some areas that contained no clam shrimp ponds in June or July were re-explored during the early weeks of August. Of 35 such ponds sampled, clam shrimps were found in four, or in 11.4 percent of the total sample. This figure confirms the 12 percent over-all average (Table 1). Thus, while a few more scattered clam shrimp ponds might be located by continued visitations to the same areas, it is unlikely that the over-all average will be importantly increased.
5. Data on faunal associates, multiple generations per season, special cases, and so forth, are too detailed for inclusion and analysis here.
6. P. Tasch and J. R. Zimmerman, *Science* **130**, 1656 (1959).
7. P. Tasch, *J. Paleontol.*, in press.
8. This is a progress report of a paleolimnological research project supported by National Science Foundation grant No. G-7320.
9. P. Tasch, "Newly discovered conchostracan-bearing beds of the Ninescah formation of Kansas," Geological Society of America program (Pittsburgh, Pa., 1959), p. 12 (abstr).

15 August 1960

### Metabolism of Adrenaline after Blockade of Monoamine Oxidase and Catechol-O-methyltransferase

**Abstracts.** Experiments in cats infused with 5  $\mu$ mole of *dl*-adrenaline-2- $C^{14}$  showed that blockade of either monoamine oxidase or catechol-O-methyltransferase is largely compensated for by the activity of the intact enzyme system; combined blockade of both enzyme systems results in the formation of a new adrenaline catabolite and in the increased production of acidic, mainly conjugated, catabolites, the identity of which remains to be established.

Previous studies on the metabolism of adrenaline and noradrenaline have shown these hormones to be inactivated mainly by oxidative deamination and O-methylation (1, 2). In order to evaluate more fully the role of monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) in the inactivation of adrenaline and noradrenaline, the work described below was undertaken.

Cats anesthetized with Nembutal were infused over a 5-minute period through a cannula in a femoral vein with 5  $\mu$ mole of *dl*-adrenaline-2- $C^{14}$  (specific activity, 1.25  $\mu$ C/ $\mu$ mole) dissolved in physiological saline. The animals were killed 5 minutes after the infusion. Blood, heart, liver, and kidneys were removed, homogenized in 10 percent trichloroacetic acid, and stored at  $-15^{\circ}\text{C}$  until assayed. After the homogenates were filtered on a suction flask, the residue was extracted three times more by homogenizing in 5 percent trichloroacetic acid and filtering. The combined filtrates were then extracted three times with ether to remove the acid. The residual ether was evaporated *in vacuo*, and a sample of the aqueous solution was taken for determination of total radioactivity. The

remainder was concentrated in a rotating flash evaporator at  $35^{\circ}\text{C}$ , and the pH of the concentrate was adjusted to 6.8.

The radioactive products which appeared in the blood and tissues after the infusion of *dl*-adrenaline-2- $C^{14}$  were separated by a modification of the procedure previously described for urine (2). The metabolic pattern was much more complex in blood and tissues than in urine, and resolution of the various fractions was not as clearly defined.

The various catabolites were essentially identified by paper chromatographic analysis of the various fractions, with and without previous acid or enzymatic hydrolysis, with three different solvent systems: butanol saturated with 1N HCl; isopropanol, ammonia, and water (8:1:1); and butanol, acetic acid, and water (4:1:1).

Table 1 shows the pattern of metabolism of *dl*-adrenaline-2- $C^{14}$  in controls; in cats after treatment with iproniazid (3) (100 mg/kg, intraperitoneally, 24 hours and 4 hours prior to the infusion); in cats after treatment with pyrogallol (4) (150 mg in 30 ml of physiological saline, intravenously) immediately before the infusion; and in cats after combined treatment with iproniazid and pyrogallol (same doses). It should be mentioned that at this time it is not our intent to present mathematically significant data on the distribution of adrenaline and its catabolites in the various tissues, but to present semiquantitatively the general aspects of adrenaline metabolism to serve as an orientation for future studies. The data in Table 1 are discussed in the sense that they show the major metabolic changes undergone by adrenaline and the effects of inhibition of the enzyme systems involved.

Adrenaline was found to disappear very rapidly from the blood and, except in one instance, it constituted only a small fraction of the total radioactivity recovered. The highest concentrations of free adrenaline occurred in the liver. Metadrenaline was by far the most important basic fraction. In some cases an additional, hitherto unidentified, basic catabolite was found having an  $R_f$  value slightly less than that of adrenaline in butanol and 1N HCl.

Among the neutral and acidic catabolites, peak 1 of Table 1 has been tentatively identified as a mixture of 3-methoxy-4-hydroxyphenylethylglycol, 3,4-dihydroxyphenylethylglycol, and possibly conjugates of metadrenaline and adrenaline; peak 2 is still unidentified; peak 3 is 3-methoxy-4-hydroxymandelic acid; and peak 4 is 3,4-dihydroxymandelic acid. Peak 5 is a mixture of various fractions; there is evidence that