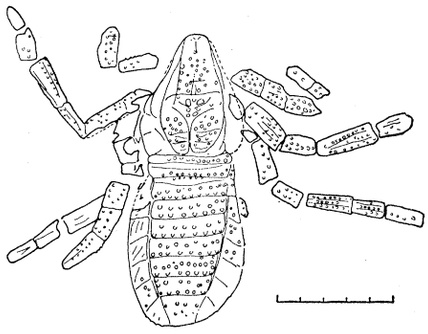

Fig. 2. A restoration of the arachnid based on the structures shown in the specimen (Fig. 1) and its counterpart. Scale is 5 mm.

Besides the rather complete specimen a few abdominal shields have been found (Fig. 3) which evidently belong to a different species. The moderately inflated shields (3.8 to 5.6 mm long) have a nearly circular outline. A median axis has seven segments, the frontal one being rather broad. The lateral portions of the shield are divided into eight pairs of segments, the hind ones forming a single plate behind the axis.

The eight segments of the abdomen are characteristic of the family Trigonotarbidae of the order Trigonotarbida. However, with its subcircular opisthosoma and an axis not reaching the hind border, the species does not fit into previously described genera. The shape of the opisthosoma might recall species of the Carboniferous order Anthracomarti, which, however, have an extra row of plates outside the lateral ones. The present forms may possibly have been related to the ancestors of the Anthracomarti.

The fossils described reveal the existence of advanced nonscorpionid arachnids in the Lower Devonian. The previously known arachnids from Rhynie in Scotland may possibly have been of the same early age, but the accurate dating is uncertain. The German arachnids are much bigger than the Scottish

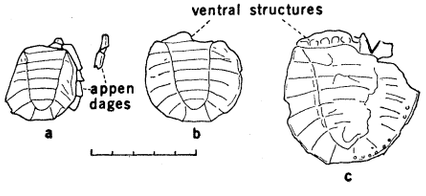

Fig. 3 (a–c). Specimens of the abdominal shield (opisthosoma) of another arachnid from the Lower Devonian (Emsian) of Alken and der Mosel, Germany (Nos. SMF VIII 32–34 of the Senckenberg Museum und Forschungstelle, Frankfurt am Main, Germany). Scale is 5 mm.

ones, and the morphological structures, notably the strong ornamentation of the integument of the large form (Fig. 1), indicate specialized and advanced features. The general structure of the legs conforms well with that of Carboniferous arachnids, which in turn correspond to recent terrestrial forms. The same terrestrial type of legs is found in the minute arachnids from the Devonian Rhynie Chert. This indicates that the German forms were terrestrial as mentioned above.

When did the arachnids become permanent land dwellers? They probably had their first development in aquatic environments, and it is reasonable to assume that before they invaded the land, members of the group passed a transition stage in which the aquatic forms were able to spend a short time on land. This is to some extent the case in the living horseshoe crabs, which can spend a short while on dry land because the gills are kept moist under the cover of the plate-shaped abdominal appendages. Among the Silurian and Devonian eurypterids, the Stylonuracea with their long and powerful legs might also have been able to crawl about on the tidal flats, perhaps taking advantage of the primitive swamps and land vegetation. As in the horseshoe crabs the gills were protected by the ventral plates. As shown by Wills and Kjellesvig-Waering (*9*) the early scorpions had similar ventral plates, which here too probably protected softer gills between the body and the plates. Also the legs of the primitive scorpions were eurypterid-like, the tarsal region of the legs not being so well adapted to carry the larger weight of the body on land (*8*). The first scorpion with stigmata, corresponding to those in recent forms, is known from the Carboniferous (*10*), a fact which suggests that the majority of the scorpions went on land in Devonian, perhaps late Devonian time, more or less at the same time as the first vertebrates. The nonscorpion arachnids seem to have been well established on land already in Lower Devonian time. The same may have been the case with the insects (*11*). Terrestrial forms of both these groups developed contemporaneously with the early land plants and probably depended on their presence. We do not know which of the various arthropod groups was the first to produce terrestrial forms, nor do we know when this happened. Certain forms described as diplopods (Myriapoda) have been described from the Silurian (Wenlockian) (*12*), but knowledge of these forms is too limited for one to decide whether or not they were terrestrial animals.

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References and Notes

1. The assumption of an aquatic habitat was suggested rather early by some authors. [See E. N. Kjellesvig-Waering, *J. Paleontol.* **40**, 361 (1966).]
2. S. Hirst, *Ann. Mag. Nat. Hist.* **12**, 455 (1923).
3. Dr. C. D. Waterston, Royal Scottish Museum, Edinburgh, informs me that the age of the Rhynie Chert of the Scottish Old Red is still uncertain, ranging from Givetian down to Emsian (Middle and Lower Devonian). See *The Fossil Record, a Symposium with Documentation* (Geological Society of London, London, 1967), p. 500.
4. B. Kräusel and H. Weyland, *Senckenbergiana Lethaea* **43**, 249 (1962); ———, *ibid.* **49**, 241 (1968).
5. K. Fahlbusch, *ibid.* **47**, 165 (1966).
6. The collections were brought together by J. Hefter in Kolbenz and the material is preserved in the Senckenberg Museum in Frankfurt am Main.
7. A. Petrunkevitch, *Treatise on Invertebrate Palaeontology, Arthropoda 2* (Geological Society of America, Boulder, Colorado, 1955), p. 1.
8. L. Størmer, *Norske Vid. Akad. Skr. I. Mat. Nat. Kl. (new ser.)* No. 8, 86 (1963).
9. "Eurypterid" ventral plates in Carboniferous scorpions have been described by R. J. Pocock [*Quart. J. Microscop. Sci.* **44**, 291 (1901)] and by L. J. Wills [*Palaeontology* **3**, part 3, 330 (1960)]. A probable aquatic habitat of the early scorpions has been advocated by Kjellesvig-Waering (*1*) and Størmer (*8*).
10. B. R. Vogel and C. J. Durdon, *J. Paleontol.* **40**, 655 (1966).
11. Apterygote insects of the Order Collembola are known from the Rhynie Chert (Lower-Middle Devonian), the first pterygote insects probably from the Upper Devonian. [*The Fossil Record, a Symposium with Documentation* (Geological Society of London, London, 1967), p. 515.]
12. *Ibid.*, p. 507.

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Nitrogen Fixation in Some Anoxic Lacustrine Environments

Abstract. *Low rates of acetylene reduction to ethylene in water samples from two dystrophic lakes indicate the presence of nitrogenase and* in situ *nitrogen fixation. Highest rates were found in anoxic water from the aphotic zone. Environmental conditions in these lakes suggest the agents of fixation were bacteria.*

Nitrogen fixation in the surface waters of lakes and oceans is well documented (*1–4*). Numerous studies on ^{15}N have reported rates of fixation and have associated fixation with the presence of certain blue-green algae, primarily in the order Nostocales. Possible nitrogen fixation in aphotic zones of natural waters appears to have been either neglected or considered insig-

nificant. In support of this view, Stewart *et al.* (*5*) reported that fixation decreased rapidly with depth in Lake Mendota, Wisconsin. However, the presence of potential nitrogen fixers in aphotic or anoxic zones is well established (*6*); nitrogen-fixing aerobic bacteria, anaerobic bacteria, and photosynthetic bacteria have been identified and isolated from various lacustrine environments. Presence of these agents does not necessarily imply that they are fixing nitrogen *in situ*.

Nitrogen fixation was measured at different depths in two dystrophic lakes by the acetylene reduction method (*5*), modified by use of 60-ml bottles to accommodate larger samples, and by longer incubations which were necessitated by the low rates encountered. Briefly, the method measures the production of ethylene from acetylene by nitrogen-fixing organisms. Acetylene is a competitive inhibitor of molecular nitrogen for the enzyme nitrogenase. Nitrogen gas was purged from anoxic waters with helium before addition of acetylene; a compressed gas mixture of 20 percent O_2, 80 percent Ar, and 0.035 percent CO_2 (*7*) was used to remove nitrogen gas from oxygenated samples. Samples were incubated from 4 to 24 hours in the laboratory. Biological activity was stopped after incubation by addition of trichloroacetic acid. Controls in each experiment consisted of samples to which trichloroacetic acid was added to precipitate protein before addition of acetylene and incubation. All controls showed either no ethylene or only a trace (evidently a very minor contaminant of the acetylene). Separation of ethylene from acetylene and measurement of ethylene production was made by gas chromatography with a flame ionization detector and Porapak T column (1.8 m by 0.3 mm) (*8*).

Two lakes in different climatic zones were chosen on the basis of their extensive anoxic environments. Lake Mary, Wisconsin (*9*), is a small (1.2 ha), deep ($D_{max} = 21.5$ m) lake with a rather unusual limnology (*10*). The lake water is colored—about 150 to 300 parts per million (ppm) on the chloroplatinate scale (*11*)—and displays other characteristics of dystrophy. Lake Mary is meromictic, and the water below about 5 m in depth is permanently anoxic. Lake Mize, Florida (*9*), is similar to Lake Mary in that it is small (0.91 ha) and deep ($D_{max} = 25$ m). However, it is more cone-shaped than Lake Mary. The lake has a high but variable color value (30 to 300 ppm) and has other characteristics of dystrophy (*12*). The morphology of Lake Mize does not promote good vertical circulation, but the lake is monomictic rather than meromictic. Slow circulation during winter resupplies the bottom water with oxygen. This oxygen is lost during the long period of stratification, and the lake is anoxic below about 5 m from June to September.

Table 1. Acetylene reduction in Lake Mary and Lake Mize.

Depth (m)	Total nitrogen* (mg/liter)	Ethylene production (nmole liter^{-1} hr^{-1})	Nitrogen fixation † (ng^{-1} liter^{-1} hr^{-1})
	Lake Mary, 28 June 1968		
0‡	0.93	0.54	5.0
2	3.1	0.36	3.3
5	1.8	0.89	8.3
7	2.9	0.98	9.2
10	2.5	1.70	15.8
15	3.3	1.16	10.8
20	4.5	5.00	46.6
	Lake Mize, 11 July 1968		
0‡	0.56	17.8	166
1.5‡	0.56	14.3	133
3.0‡	0.63	12.5	117
6.1‡	0.65	11.6	108
6.1	0.65	8.9	83
9.1	0.70	33.0	308
18.3	0.69	10.7	100
	Lake Mize, 21 August 1968		
0‡	0.38	0	0
2‡	0.60	0	0
5‡	0.69	0	0
7	0.73	2.98	27.8
10	0.77	3.28	30.6
15	1.29	8.93	83.3
20	0.66	2.68	25.0

* Total Kjeldahl nitrogen in Lake Mary and Lake Mize (21 August), total organic nitrogen in Lake Mize (11 July). † Calculated from nanomoles of ethylene produced per liter of water per hour if we assume a theoretical ratio of 1.5 mole of ethylene produced per mole of ammonia fixed. ‡ Samples purged with oxygen-argon mixture before incubation; all other samples purged with helium before incubation.

Lake Mary was sampled 28 June 1968. Temperature and dissolved oxygen decreased rapidly with depth; surface values were 19.3°C and 7.2 mg/liter, respectively. Corresponding values at 3 m were 6.6°C and 0.1 mg/liter. From 4 m to the bottom, there was no dissolved oxygen and the temperature was between 4.0° and 5.0°C. Surface samples were incubated at room temperature (about 22°C) in sunlight for 5 hours; depth samples were incubated at 4°C in the dark for the same length of time. The results (Table 1) indicate a slight increase in ethylene production with depth from the surface to 15 m and a marked increase at the bottom (20 m). The rate of production in the upper water of Lake Mary was only slightly higher than the detection limit of our procedure, which was about 0.1 nmole of ethylene per liter of water per hour.

Nitrogen fixation in Lake Mize was determined on 11 July and 21 August 1968. Conditions in the lake were similar on both dates. Dissolved oxygen was present only in the upper 3 m. Water temperature ranged from 30°C at the surface to 11.5°C at the bottom, and the thermocline occurred between 3 and 7 m. Samples were incubated for 4 hours on 11 July and for 24 hours on 21 August. Relatively high nitrogen-fixing activity (Table 1) was found at all depths in July, but the maximum occurred in anoxic waters at a depth of 9 m. Rates of ethylene production were considerably lower in August, and no production was observed in the epilimnion. Maximum production occurred at 15 m.

The rates shown in Table 1 are generally low compared with nitrogen-fixation rates measured by ^{15}N techniques in some eutrophic surface waters with blooms of blue-green algae. Goering and Neess (*2*) found fixation rates from 0.07 to 8.49 μg of nitrogen per liter of water per hour in surface waters of Lake Mendota, Wisconsin. Dugdale and Dugdale (*3*) reported fixation rates in Sanctuary Lake, Pennsylvania, ranging from 1 to 6 μg per liter of water per hour, and Billaud (*4*) found rates as high as 3 μg per liter of water per hour in Smith Lake, Alaska. Stewart *et al.* (*5*) recently reported ethylene production rates as high as 2.3 nmole per 1-ml sample per 30-minute period in surface blooms from Lake Mendota. If we assume a theoretical ratio of 1.5 moles of ehylene produced per mole of ammonia fixed (*5*), this rate is equivalent to 43 μg of nitrogen per liter of water per hour. However, it is unclear whether or not their sample was concentrated.

It should be noted that the rates of nitrogen fixation in Table 1 are only apparent. The acetylene reduction method is an indirect assay for fixation, and the rates have not been verified with ^{15}N. However, reduction of acetylene to ethylene is not known to be a natural biological process. The only enzyme now known to reduce acetylene to ethylene is the nitrogen-fixing enzyme (nitrogenase). Results of controls in each experiment imply that the reduction of acetylene to ethylene is enzymic, since no reduction oc-

curred in bottles preserved with trichloroacetic acid prior to acetylene introduction.

Agents of the apparent fixation in the hypolimnia of Lake Mary and Lake Mize would seem to be heterotrophic since ethylene production occurred in anoxic waters where light penetration was negligible. Fixation in the surface waters of these lakes may have been algal, but we detected no blue-green algae in either lake by microscopic examination. Fixation under these environmental conditions has not been previously reported, although low rates of fixation have been noted in surface samples incubated in the dark (*2*, *5*). The immediate ecological significance of these low rates is probably minor; that is, fixation evidently supplies only a small portion of the nitrogen requirements of aquatic microorganisms. However, the biogeochemical significance may be considerable. If the rates of ethylene production in Lake Mary are representative of nitrogen-fixing activity in the lake, the annual fixation of nitrogen in the anoxic region (5 m and below) of this small lake is approximately 4.6 kg (*13*). If fixation is found to be widespread in anoxic lake water and lacustrine sediments, estimates of the role of nitrogen fixation in the global nitrogen budget (*14*) will have to be increased.

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References and Notes

1. R. Dugdale, V. Dugdale, J. Neess, J. Goering, *Science* **130**, 859 (1959); R. Dugdale and V. Dugdale, *Limnol. Oceanogr.* **6**, 13 (1961); R. Dugdale, J. Goering, J. Ryther, *ibid.* **9**, 507 (1964).
2. J. Goering and J. Neess, *Limnol. Oceanogr.* **9**, 530 (1964).
3. V. Dugdale and R. Dugdale, *ibid.* **7**, 170 (1962).
4. V. Billaud, *Proceedings of the Symposium on Environmental Requirements of Blue-Green Algae* (Univ. of Washington, Seattle, 1966), pp. 35–53.
5. W. D. Stewart, G. Fitzgerald, R. Burris, *Proc. Nat. Acad. Sci. U.S.* **58**, 2071 (1967).
6. L. Pshenin, *Rep. Acad. Sci. U.S.S.R.* **129**, 930 (1959); ———, *Microbiologia* **28**, 927 (1959); ———, in *Symposium on Marine Microbiology*, C. H. Oppenheimer, Ed. (Thomas, Springfield, Ill., 1963), chap. 36; M. B. Allen, *ibid.*, pp. 85–92; S. Waksman, M. Hotchkiss, C. Carey, *Biol. Bull.* **65**, 137 (1933); J. Lackey, E. Lackey, G. B. Morgan, *Bull. Series No. 119*, Industrial Engineering Experiment Station, University of Florida, Gainesville (1964).
7. Acetylene (purified) and compressed gas mixture were obtained commercially (Matheson).
8. A Varian Aerograph 600D gas chromatograph (hydrogen flame ionization detector) was used. Column and operating conditions as recommended by O. Hollis and W. Hayes, *Proc. 6th Int. Symp. Gas Chromatography and Associated Techniques*, Rome (1966).
9. Exact locations of the lakes are: Lake Mary, Vilas County, Wisconsin, 46°04′N, 90°09′W, Lake Mize, Alachua County, Florida, 29°44′N, 82°13′W.
10. C. Juday and E. Birge, *Trans. Wis. Acad. Sci. Arts Lett.* **27**, 415 932); ———, V. Meloche, *ibid.* **29**, 1 (1935); R. Allgeier, B. Hafford, C. Juday, *ibid.* **33**, 115 (1941); G. E. Hutchinson, *Treatise on Limnology* (Wiley, New York, 1957), vol. 1; K. Stewart, K. Maleug, P. Sager, *Verh. Int. Verein. Limnol.* **16**, 47 (1966).
11. *Standard Methods for the Examination of Water and Wastewater* (Amer. Public Health Ass., New York, ed. 12, 1965), pp. 127–29.
12. W. Harkness and E. L. Pierce, *Quart. J. Fla. Acad. Sci.* **5**, 6 (1940); F. Nordlie, Final Report to the Federal Water Pollution Control Administration, grant No. WP-00530, 1967 (unpublished).
13. Volume of Lake Mary below 5 m is 46,170 m^3 [C. Juday and E. Birge, *Trans. Wis. Acad. Sci. Arts Lett.* **33**, 21 (1941)]. Since the lake is meromictic, temperature and other conditions remain relatively constant in the bottom water year round. Thus seasonal changes in fixation in the bottom water are probably small.
14. G. E. Hutchinson, *Amer. Sci.* **32**, 178 (1944); C. Delwiche, in *Microbiology and Soil Fertility*, C. Gilmour and O. N. Allen, Eds. (Oregon State Univ. Press, Corvallis, 1965), pp. 29–58.
15. Supported by FWPCA grant WP-01203. We thank G. Ashley for assistance in sampling and chemical analyses.

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Protein-Bacteriophage Conjugates: Application in Detection of Antibodies and Antigens

Abstract. *Covalent attachment of proteins to bacteriophage yielded modified phage preparations with which it is possible to detect antibodies to proteins at concentrations as low as 0.5 to 2.0 nanograms per milliliter. Similarly, antibodies may be linked covalently to phage, and the resulting antibody-phage conjugate is useful in detecting proteins. An alternative method for quantitative determination of proteins is suggested, in which the inactivation of protein-phage by antibodies to protein is inhibited by the protein tested. With rabbit immunoglobulin G as the protein, as little as 0.3 nanogram per milliliter could be determined.*

Immunospecific inactivation of bacteriophage is the basis of a sensitive method for the detection of antibodies (*1*). Antibodies with specificity directed toward peptides (*2*) and haptens such as the 3-iodo-4-hydroxy-5-nitrophenylacetyl (*3*), penicilloyl (*4*), and 2,4-dinitrophenyl (DNP) (*5*) groups were detected and quantitated by the use of bacteriophage to which the specific molecules were covalently bound. In view of the great interest in the detection of very small amounts of antibodies to proteins, we have now linked proteins to bacteriophage T4 by covalent bonding and shown that the modified phage may be inactivated with antibodies against the protein. Moreover, chemical attachment to bacteriophage of immunospecifically isolated antibodies to protein yielded phage preparations which could be used for detection of protein antigens.

Bacteriophage T4 was grown, purified, and assayed as described (*2*). The proteins used were bovine pancreatic ribonuclease, bovine serum albumin (BSA), and rabbit immunoglobulin G (IgG). Coupling of proteins to the bacteriophage was performed by addition of a solution of the bifunctional reagent tolylene-2,4-diisocyanate (TDIC) (*6*) in dioxane (0.1 ml) to mixtures of bacteriophage and protein in 0.05*M* phosphate buffer, *p*H 7.0, containing 20 μg of gelatin per milliliter (0.6 to 1.4 ml); TDIC is a coupling agent which reacts with amino groups, producing entirely covalently linked conjugates between proteins (*6*). In order to find the optimum conditions, the concentrations of bacteriophage, proteins, and TDIC and the time of reaction were varied. The reaction was performed at 24°C and was terminated by dialysis against the same buffer. Any visible

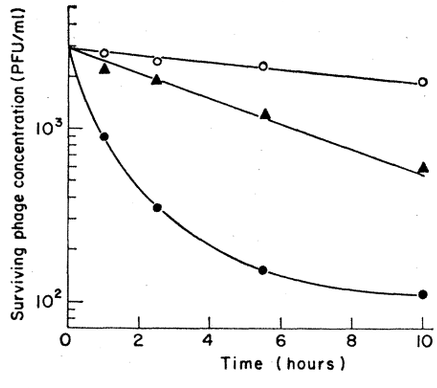

Fig. 1. Inactivation of conjugate consisting of bacteriophage T4 and rabbit immunoglobulin G by a goat antiserum to rabbit immunoglobulin G. The final dilutions of serum in the reaction mixtures were: ●, 1 to 2×10^6; ▲, 1 to 2×10^7. The serum contained 10 mg of antibody per milliliter. ○, Control experiment in which phage was kept in the absence of antiserum; *PFU*, plaque-forming units.