remaining, bound SO<sub>4</sub><sup>-</sup> groups. Amidemodified 0.5-µm particles were ingested in the same proportion (100 percent efficiency) at which they were in suspension (treatment 4, Table 2), in contrast to the 0.5-µm unmodified polystyrene particles (treatment 1, Table 1). Addition of a surfactant caused a statistically significant reduction in capture efficiency of 0.5-µm unmodified particles (treatment 3, Table 1) to 44 percent and of amidemodified particles (treatment 5, Table 2) to 71 percent. These experiments indicate that capture efficiencies of the smallest particles can be affected by changing surface charge of the particles and by changing wettability. Neutral particles were captured more readily than particles with a net negative charge, and addition of a surfactant, which increases the wettability of both particles and animals, caused more particles to escape the filtering apparatus of the Daphnia.

Differential particle capture on the basis of charge and wettability has general significance for freshwater and marine filter feeding. Natural particles have a range of surface properties that affect their adsorption to surfaces and movement through fluids (16). Anomalous selective feeding by zooplankton may be explained on the basis of surface chemistry, in that the animals' filtering appendages may have had greater affinities for some particles than for others (17). Selective filter feeding by copepods (18) may in fact be due to surface chemistry interactions rather than size selection or taste selection. Surface charge is affected by pH(16), so we may expect that environments with extreme pH values will affect filter-feeding capabilities of small invertebrates. The elimination of certain zooplankton species from systems with elevated pH due to high rates of photosynthesis, or lowered pH due to dissolved humic substances or acid rain (19), may be due to a reduced ability of certain species to capture food as well as other effects mediated by pH. Finally, we may expect surface adaptations of filter-feeding animals and their prey to enhance or reduce particle capture. An example of this might be the nonwettability of the exoskeleton of cladocerans, which frequently imprison small individuals in the surface tension of the water, but may aid in particle capture. The interactions between surface chemistry and feeding may change some of our models in aquatic ecology.

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- ter feeding.
  Healthy adult animals (> 2 mm) were starved in particle-free water for 24 hours before the experiments. Particle suspensions were in equal concentrations by particle volume (Tables 1 and Concentrations were verified by microscopical counts of stock suspensions followed by

dilution to the required concentration. Animals (N = 25) were placed in 125 ml of feeding sus-pension on a revolving plankton wheel (1 rev/ min) to prevent settling. Animals were sieved, washed in particle-free water, anesthetized in CO<sub>2</sub>, and preserved in formaldehyde. Guts of eight to ten animals were dissected on slides and relative abundances of moticles determined relative abundances of particles determined from at least ten nested subsamples, where 5.7- $\mu$ m particles were counted at 160× magnifica-tion, and smaller particles were counted at 1000× with Nomarski interference contrast. Accuracy was verified with a focal-plane micrometer. Expected proportions of particles were cal-culated from the known concentrations in suspension and the known relative magnification. Proportions of each size class present in the gut

- 12. Proportions of each size class present in the gut of each animal were transformed (arcsin-square root) to degrees for comparison to expected proportions. The probability levels for the multi-ple comparisons by *t*-tests, were controlled at the  $\alpha$  level by use of  $\alpha/k$ , where *k* is the number of comparisons. In Tables 1 and Table 2 *k* = 3. The 0.5- $\mu$ m size class was then dropped from analysis nonportions of the larger naricles were analysis, proportions of the larger particles recalculated, retransformed, and analyzed with a *t*-test to determine if there was any difference in relative ingestion, independent of the smallest oarticles
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## **Deep Oxygenated Ground Water: Anomaly or Common Occurrence?**

Abstract. Contrary to the prevailing notion that oxygen-depleting reactions in the soil zone and in the aquifer rapidly reduce the dissolved oxygen content of recharge water to detection limits, 2 to 8 milligrams per liter of dissolved oxygen is present in water from a variety of deep (100 to 1000 meters) aquifers in Nevada, Arizona, and the hot springs of the folded Appalachians and Arkansas. Most of the waters sampled are several thousand to more than 10,000 years old, and some are 80 kilometers from their point of recharge.

The geochemical and hydrogeologic literature provides a broad spectrum of notions regarding the occurrence of dissolved oxygen (DO) in ground water. The views range from the idea that DO is absent below the water table (1, 2) to the idea that DO is purportedly generated by the radiolysis of water at depths of 2 to 3 km (3). The prevailing opinion (1, 4, 5) is

that the bulk of DO in recharge water is consumed in the soil and unsaturated (or vadose) zones by microbial respiration and the decomposition of organic matter, or rapidly thereafter in the aquifer by various mineral-water and organic oxidative reactions. Despite the multitude of studies of ground-water geochemistry in the last decade, measurements of DO in

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Fig. 1. Variation in the dissolved oxygen content of ground water along an approximately 80-km flow path in the Ash Meadows ground-water basin, south-central Nevada. The first number in parentheses is the temperature of the water (in degrees Celsius); the second is the number of measurements at the site, or at Ash Meadows the number of springs sampled. Error bars represent 1 standard deviation for Cold Creek Spring and seven Ash Meadows springs; the standard deviation is too small to show for the other three stations. Highly fractured Paleozoic carbonate rocks comprise the aquifer; hydrogeologic, hydrochemical, and isotopic studies of this ground-water flow system and location of the line of section and sampling sites are given in (10).



Fig. 2. Dissolved oxygen in deep ground waters versus the approximate distance from recharge areas in five intermontane basins of southern Arizona. Numbers on the index map identify the following locations: 1, Vekol Valley; 2, Ranegras Plain; 3, Butler Valley; 4, San Pedro Valley; and 5, San Simon Wash. Numbers next to the well symbols are, from top to bottom: the well depth (in meters), the depth to water (in meters), the depth to the top aquifer (in meters), and the temperature (in degrees Celsius). Letters designate well locations, based on the U.S. Geological Survey and state of Arizona township, range, section, system: well A, (D-7-2)18ABA; B, (D-8-1)31CBC; C, (D-7-1)10CBC\_2; D, (D-8-1)14BAA; E, (D-9-1)13BBD; F, (D-8-1)35ABD; G, (B-4-15)18BBB; H, (B-6-16)33AAA; I, (B-6-16)26AAD; J, (B-3-15)2DAB; K, (B-8-14)29CDD; L, (D-9-17)24DCC; M, (D-8-17)32DAA; and N, (C-16-1)10CCA.

water from shallow (< 100 m) aquifers (6) are not routine and such measurements have rarely been made for deeper ground water (7). It is our intent here to document the widespread presence of DO in significant (2 to 8 mg/liter) concentrations in water several thousand to more than 10,000 years old from deep aquifers of several lithologies in both arid and humid climates, and at distances as great as 80 km from recharge areas.

Because of the ease with which anoxic well waters can be oxygenated, special precautions were taken during sampling. The pumping water levels, in the highcapacity production wells chosen for sampling, were several meters to tens of meters above the pump intakes. In addition, many of these wells tap confined aquifers; in such wells, entrainment of air by pumping is unlikely because of the absence of unconfined flow. The sample bottles were purged and sealed within the full flowing discharge pipe. Sampling in the flowing artesian wells and springs was accomplished by filling and purging the sample bottles under water. We carried out replicate analyses in the field by using either a modified Winkler method or a dissolved oxygen meter (8). That our sampling techniques did not introduce  $O_2$  is best shown by the absence of DO in water sampled in similar ways from deep aquifers (9) which contain organic detritus and in which DO should, intuitively at least, be absent.

Dissolved oxygen occurs at concentrations of 2 to 8 mg/liter in water from a variety of aquifers in the south-central Great Basin, Nevada. These aguifersprincipally Paleozoic carbonate rocks, Tertiary welded tuffs, and Quaternary valley fill-occur at depths of 200 to 1000 m (10). Water table (more correctly potentiometric surface) depths in the region range from 200 to 660 m below the surface (10). The residence time of water from most of these aquifers is on the order of thousands to more than 10,000 years (11). Figure 1 shows the variation in DO along an 80-km flow path in the Paleozoic carbonate-rock aquifer of the Ash Meadows ground-water basin (12).

Dissolved oxygen has been observed in all shallow (< 100 m) and deep ground water from valley-fill aquifers in the southern Arizona portion of the Basin and Range Province. Unequivocal evidence of DO at depths of hundreds of meters was obtained from wells in several of the agriculturally less developed basins where well construction, thickness of aquifer tapped, location of recharge areas, and the absence of oxygenated irrigation return flow could be documented. The basins sampled and our results are shown in Fig. 2. In many of the areas sampled, the aquifers are overlain by a thick (> 100 m) clay stratum, which effectively precludes the possible mixing of deep water with shallow oxygenated ground water. Hydraulic and <sup>14</sup>C data (13) indicate water ages in excess of 10,000 years for most of the water sampled.

The DO content of thermal spring waters in the Valley and Ridge Province from western Georgia to eastern New York and the waters of Hot Springs National Park, Arkansas, ranges from 2 to 7 mg/liter (14). Water temperatures are between 30° and 60°C, and the minimum depths of water circulation are 250 to 2300 m. Hydrogeologic and isotopic evidence suggest relatively short flow paths (from recharge to discharge areas), on the order of a few kilometers to at most tens of kilometers (14). The flow to some springs is chiefly through carbonate rocks, whereas flow to other springs is entirely in siliceous reservoir rocks.

The <sup>3</sup>H data for some of these hot springs suggest that their DO content may represent a mixture of deeply circulating thermal water and relatively shallow, cooler, and younger ground water (14). However, several spring waters that have a DO content of 2 to 7 mg/liter have negligible  ${}^{3}H$  (< 1 ± 1 tritium unit). The low <sup>3</sup>H content is a clear indicator that these waters are, at the least, predominantly of pre-H-bomb (before 1952) age. The Arkansas waters have a  $^{14}$ C age of about 4000 years (14).

The presence of DO in the deep carbonate-rock aquifers of the Great Basin and the folded Appalachians (14), like that in the shallower carbonate aquifers of Great Britain [Edmunds (6); Morgan-Jones and Eggboro (6)] did not completely surprise us, despite the great difference in the age of the shallow and deep waters. Commonly, recharge to such aquifers is oxygenated, after passage through soils, by flow through fissures and caverns in the unsaturated zone.

More importantly, flow through the dense carbonate-rock aquifers that we sampled is predominantly by way of solution-modified fractures rather than through intercrystalline pore space; relatively rapid flow through, and the low ratios of rock surface area to water volume in, such fractured aquifers would not favor removal of DO by chemical reactions. Moreover, in the middle and distal portions of regional flow systems comprised of carbonate-rock aquifers, oxidizable minerals, if originally present along fracture surfaces, are likely to be coated with calcite or dolomite precipitated from the ground water.

The presence of DO contents of 2 to 8 mg/liter in the deep valley fill and tuff aquifers of Nevada and Arizona, although unexpected, appears in hindsight to be qualitatively explainable. Valleyfill sediments of the Basin and Range Province were deposited under generally oxidizing conditions and probably remained exposed to oxidizing arid and semiarid climates for tens to hundreds of years prior to burial; after burial they commonly remained in oxidizing unsaturated zones for tens of thousands to perhaps hundreds of thousands of years, depending upon the rates of basin subsidence and the depth to the regional water table. Such depositional environments hardly favor the preservation of readily oxidizable organic or mineral matter. Moreover, recharge to such aquifers is commonly by way of the infiltration of oxygenated runoff along the bottoms of major arroyos; such recharge may have little contact with readily decomposable or relatively unoxidized soil organic matter.

More puzzling is the presence of DO in those Arkansan and Appalachian hot springs (14) whose water has passed principally through fractured siliceous rocks. Recharge to these humid-zone aquifers probably had to traverse an organic-rich soil zone; moreover, the reservoir temperature (30° to 60°C) should certainly have favored both the outgassing of the DO and mineral-water reactions. Perhaps all pertinent reactions (organic and inorganic) involving DO have gone to completion within the aquifer prior to the entry of the extant ground water, as hypothesized by Galloway (7) for the oxidative "tongues" found in sandstones containing roll-front uranium deposits.

We hope that this report will stimulate a systematic appraisal of DO in future geochemical studies of shallow and deep ground water. Such measurements, which can readily be made in the field, are essential for predictions of the movement of toxic transition metals (15) and actinide radionuclides in aquifer environments (15). The common assumption that reducing conditions prevail in deep aquifer environments must be tested on a case-by-case basis.

Note added in proof: Mineralogic evidence for deep oxidizing conditions in an 1800-m-thick Tertiary ash-flow and ashfall tuff sequence in the Jackass Flats area of southern Nevada is given by Bish et al. (16). They found highly oxidized iron-titanium minerals in cores (test hole USW-G1, Yucca Mountain) from the upper 1600 m of this tuffaceous sequence. The water table is about 580 m deep at the site of the test hole. We measured DO (6 to 7 mg/liter) in ground water, from the upper 120 m of the saturated zone in this volcanic sequence, at nearby water-supply wells J-12 and J-13. We are not suggesting that the extant ground water caused the deep oxidation noted by Bish et al., because such oxidation might well have occurred several millior. years ago. Rather we cite their work tc suggest that oxidizing conditions were once, and may still be, present within this volcanic rock sequence at considerably greater depths than the water we sampled.

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- deep ground water has been inferred by severa authors. J. D. Hem [U.S. Geol. Surv. Wate Supply Pap. 1473 (1970), p. 221] and Freeze and Cherry (4) suggested that, in the absence o organic matter and in areas where little soi overlies fractured rock, DO might be retained in solution by ground water in detectable (> 0mg/liter) concentrations for a long time. Eco nomic geologists [H. C. Granger and C. G Warren, U.S. Geol. Surv. Open-file Rep. 79 1603 (1979); W. E. Galloway, in Depositiona and Ground Water Flow Systems in the Explora tion for Uranium, W. E. Galloway, C. W tion for Uranium, W. E. Galloway, C. w Kreitler, J. H. McGowen, Eds. (Texas Bureau of Economic Geology, Austin, 1979), chap. 7 pp. 177-180; A. E. Saucier, N.M. Bur. Mine Mineral Resour. Mem. 38 (1980), pp. 116-121 have inferred the former presence of a few to perhap ground waters at distances of a few to perhap 30 km from presumed paleorecharge areas it sandstones containing roll-front uranium depos . Based on the distribution of oxidized ore: F. Park, Jr., and R. A. MacDiarmid [O Deposits (Freeman, San Francisco, ed. 3, 1975) pp. 85–91 and 468–469] inferred that locally along highly permeable fracture zones, oxygen ated ground water once descended to depths o 960 m at the Tsumeb mine in southwest Africa However, they also subscribe to the notion tha the depth of mineral oxidation is controlled b the water table, which, they point out, ma occur at great depths in arid terrane. The only actual measurements and detection of DO deep ground water that we are aware of are the following: A. I. Germanov, G. A. Volkov, A. K Lisitsin, V. S. Serebrennikov, *Geochemistr* Lisitsin Serebrennikov Geochemist (U.S.S.R.) 3, 322 (1959); I. J. Winograd and F

J. Pearson, Jr., *Water Resour. Res.* **12**, 1125 (1976); J. C. Vogel, A. S. Talma, T. H. E. Heaton, J. Hydrol. **50**, 191 (1981); K. Wolfs-berg, Los Alamos Sci. Lab. Rep. LA-8739-PR (1980), pp. 21-22; (14). Beneath uplands, such as there diverged by Commercial and Science and Scien those discussed by Germanov et al., oxygenated young ground water is not unexpected at depths of hundreds of meters, because in recharge areas flow is dominantly downward. The deep waters sampled in the other studies cited were not exclusively from recharge areas. Unique evidence for the presence of deep oxygenated water comes from the distal portions of the Edwards aquifer system in the San Antonio Texas, area. There, blind catfish (*Trogloglanis*) *pattersoni*) live in caverns in this aquifer at depths of hundreds of meters in 27°C water containing DO at concentrations up to 4 to 5 mg/ liter [G. Longley and H. Karnie, Jr., report prepared for the U.S. Fish and Wildlife Service, Albuquerque, N.M., under contract 14-16-0002-77-035 (1978)]. The <sup>3</sup>H content of water in this portion of the Edwards aquifer is as low as 1 portion of the Edwards adulter is as low as a tritium unit [F. J. Pearson, Jr., P. L. Rettman, T. A. Wyerman, U.S. Geol. Surv. Open-file Rep. 74-362 (1975)], suggesting a water age, at the least, on the order of decades. A Hach model OX-2P field kit (a modified Winkler method) and a YSI model 57 dissolved ovveen meter were used to measure the DO

- 8. oxygen meter were used to measure the DO contents in our ground waters. The YSI meter is accurate to 0.1 mg/liter and the Hach field kit to 0.2 mg/liter. Measurements of DO (12 replicates) at a deep well in the Vekol Valley, Ariz., made with the Hach kit gave a mean of 4.3 mg/liter with a standard deviation of 0.2 mg/liter. This This compared with a value of  $4.1 \pm 0.1$  mg/liter. measured with the YSI meter on water from the same well. C. E. Boyd [J. Environ. Qual. 6, 381 (1977)] compared the Hach field kit DO values with standard laboratory methods; in the range of interest to us (3 to 7 mg/liter), the Hach kit
- of interest to us (3 to 7 mg/liter), the Hach kit results were only 3 to 13 percent higher than those obtained by standard laboratory methods. D. C. Thorstenson, D. W. Fisher, M. G. Croft, *Water Resour. Res.* 15, 1479 (1979); I. J. Wino-grad and G. M. Farlekas, in *Isotope Techniques* in *Groundwater Hydrology 1974* (International Atomic Energy Agency, Vienna, 1974), vol. 2, pp. 69–93. Indirect geochemical evidence that peither our pumping nor our sampling proce-9 neither our pumping nor our sampling proce-dures caused the oxygenation of naturally reduced ground waters consists in the virtual absence of iron in all our waters. The dissolved iron content of the southern Nevada ground waters, for example, varies from 15 to 40  $\mu g/$  liter. If we had aerated moderately reduced [*Eh*

(oxidation-reduction potential) -100 to +200 mV] waters, we would have expected iron contents-either dissolved or as precipitates on our filters-in the milligrams per liter range, in at least some of our waters, all of which have pH

- Jeast some of our waters, all of which have pH values in the range of 6.5 to 8. See Winograd and Pearson (7); I. J. Winograd and W. Thordarson, U.S. Geol. Surv. Prof. Pap. 712-C (1975); W. W. Dudley, Jr., and J. D. Larson, U.S. Geol. Surv., Prof. Pap. 927 (1976); I. J. Winograd and G. C. Doty, U.S. Geol. Surv. Open-file Rep. 80-569 (1980); I. J. Winograd and J. Friedman Geol Soc. Am. Bull. 83 3691 10 Friedman, Geol. Soc. Am. Bull. 83, 3691 1972
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## Induction of Crisis Forms in Cultured Plasmodium falciparum with Human Immune Serum from Sudan

Abstract. Serums from 90 individuals from three areas in Sudan were tested for inhibitory activity against cultures of Plasmodium falciparum. In addition to inhibitory activity against merozoite invasion, all of the serums demonstrated, in varying degrees, the ability to retard intraerythrocytic development, leading to crisis forms and parasite deterioration. These retardation factors could be removed by absorption of immune serum with parasite-infected erythrocytes and were demonstrable in purified immunoglobulin fractions. Serum from donors in hypoendemic Khartoum did not retard parasite development.

Nearly four decades ago, Taliaferro and Taliaferro (1) reported that infections of Plasmodium brasilianum in Cebus capucinus monkeys progressed at a predictable rate and pattern until the host's immune response began to resolve the infection. The parasite's highly synchronous development then became severely retarded, and "crisis forms" of the parasite appeared (1). The crisis was characterized by significant changes in the synchrony of the parasite's developmental cycle, a reduced average number of merozoites per segmenter, and a retardation of the periodicity, resulting in many deteriorating schizonts within the infected erythrocytes. Since this early report, the term crisis form has become synonymous with obviously degenerating intraerythrocytic parasites seen in hemoprotozoan infections with Babesia and Plasmodium sp. (2). Experimental induction of crisis forms is not always consistent and, in rodent infections with Babesia and Plasmodium sp., nonspecific factors associated with Corynebacteri-

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um parvum, Mycobacterium bovis BCG, or endotoxin-stimulated macrophages appear to be important (3).

Studies of immunity to primate malaria-including malaria due to P. falciparum in man-have demonstrated that serums collected from experimentally infected animals, or from humans living in regions of malaria hyperendemicity, contain humoral factors that inhibit parasite development in vitro (4). Since the merozoite is the only extracellular stage of the blood infection, it is especially susceptible to immunologic attack, and numerous studies have confirmed that malariaimmune serum appears to act by blocking invasion of erythrocytes by the merozoites (5). Attempts to demonstrate inhibition of intracellular parasite development or to identify additional protective actions for malaria-immune serum have been, up to now, unsuccessful (6). One result of these studies has been to emphasize the merozoite as the source of protective antigen. We now report that serums collected from individuals living in malarious regions of the Sudan not only contain merozoite-blocking antibodies, but also cause intracellular parasite deterioration and classical crisis forms in cultures of *P. falciparum*.

We have collected more than 300 serum samples from three different regions in Sudan, and of these, 90 have been tested for parasite inhibition in continuous cultures of P. falciparum. Since in some areas, particularly Blue Nile Province, the villagers have access to chloroquine, all serums were dialyzed 1:1000 against RPMI 1640 medium. This procedure removes 98 percent of the chloroquine from serum (7). Because dialysis also removes hypoxanthine, a required nutrient not found in RPMI 1640, complete medium was supplemented with hypoxanthine to give a final concentration of  $5 \times 10^{-5} M$  (8). All serums were heat-inactivated at 56°C for 30 minutes. Parasites of P. falciparum, strain FCR<sub>3</sub>/Gambian, were synchronized with a modification of the sorbitol method (9); cultures were washed with 5 percent (weight to volume) aqueous sorbitol, cultured for 12 hours, washed again with sorbitol, returned to culture for 24 hours, then concentrated to 80 to 90 percent parasitemia by the gelatin-RPMI 1640 method (10). This procedure results in highly synchronous schizonts with a 6hour age differential. The synchronized schizont-infected red cells were diluted to a 0.5 to 1.0 percent parasitemia with freshly washed O+ erythrocytes and dispersed into 96-well microculture plates so that each well received 3 µl of packed erythrocytes. The dialyzed serum was