will tend to insulate the interior against the cooling at the palm surface, (ii) more warm arterial blood will be supplied to the hand interior than to the palmar and dorsal surfaces, and (iii) venous blood will be cooler than arterial blood and leaves the hand primarily through the dorsal veins. The measured temperatures suggest that most of the 3.3 Ghz radiation originates near the dorsum while most of the 1.3 Ghz radiation originates deeper, near the region of highest temperature. Thus in all time intervals, the microwave data indicate changing hand temperatures at different depths between the dorsum and the palm.

The results are representative of those obtained in several trials; the purpose of our experiments was to demonstrate subsurface temperature sensing, and not to conduct research in thermal physiology—a field quite outside our area of expertise.

We have constructed models of fatty tissue as described by Guy (*7*). These models have been useful in measuring and improving antenna properties. However, we have not used them in tests of subsurface thermal sensing, because of our belief that the homogeneity of their electrical and thermal properties departs too greatly from what happens with tissue.

There are many potential medical applications of microwave radiometry. By analogy with infrared thermography, we may expect these to include detection of subsurface thermal anomalies such as malignant tumors, especially in the female breast; localized inflammations, such as appendicitis; and vascular insufficiency in the limbs and in the brain. However, the usefulness of the technique is difficult to predict because detailed knowledge of the internal thermal structure of the human body is sparse. Extensive clinical evaluation, involving observations at more than one frequency, will be required. If simultaneous observations are made at two well-separated frequencies, the ability to determine the depth of a particular thermal anomaly will be improved. However, this depth resolution will still be crude, of order 1 cm at best, because of the long wavelengths involved. Experiments at other frequencies have been performed by others in the laboratory but have not been the subject of detailed clinical evaluation (*8*). Infrared thermography has been utilized in the detection of breast cancer for many years, and this is an area where microwave thermography is being evaluated. Microwave thermograms at 3.3 Ghz on some 30 to 40 female patients per week at Faulkner Hospital, Boston, are being correlated with mammography, infrared thermography, clinical, and, where appropriate, biopsy results. These data are the first microwave thermographic data taken in a systematic, routine manner in a clinical environment and should help establish the currently unknown microwave emission patterns from the breasts of normal patients. Once these patterns are known with confidence, an examination of departures can be carried out for diagnostic purposes. Our initial data indicate good agreement with infrared patterns, but insufficient data exist for any further conclusions.

ALAN H. BARRETT
PHILIP C. MYERS
Department of Physics and
Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge 02139

References and Notes

1. H. P. Schwan and G. M. Piersol, *Am. J. Phys. Med.* **33**, 371 (1954); *ibid.* **34**, 425 (1955); H. P. Schwan, *Adv. Biol. Med. Phys.* **5**, 147 (1954). An excellent review of electromagnetic wave effects in biological materials is given by C. C. Johnson and A. W. Guy [*Proc. IEEE* **60**, 692 (1972)].
2. The penetration depth is defined as that distance over which the intensity of propagating electromagnetic radiation will be reduced to $1/e$, or approximately 37 percent, of its original value. Engineers and physicists will recognize that what we call "penetration depth" is what textbooks call "skin depth." We avoid this term here because of the obvious confusion it can cause in a biomedical application. Use of the term "penetration depth" in connection with biological tissue did not originate with us (*1*).
3. The rectangular waveguide antenna has been analyzed by many, including S. Silver, in *Microwave Antenna Theory and Design*, S. Silver, Ed. (McGraw, New York, 1949), p. 169; R. C. Hansen, in *Microwave Scanning Antennas*, R. C. Hansen, Ed. (Academic Press, New York, 1964), vol. 1, p. 1; A. W. Guy, *IEEE Trans. Microwave Theory Tech.* **19**, 214 (1971).
4. S. L. Poole, thesis, Massachusetts Institute of Technology (1971).
5. A. H. Barrett and P. C. Myers, *Bibl. Radiol.* **6**, 45 (1975); *Quarterly Progress Report*, Research Laboratory of Electronics, Massachusetts Institute of Technology, No. 107 (15 October 1972), p. 14; *ibid.* No. 109 (15 April 1973), p. 1; *ibid.* No. 112 (15 January 1974), p. 39.
6. G. Bolen, thesis, Massachusetts Institute of Technology (1973).
7. A. W. Guy, *IEEE Trans. Microwave Theory Tech.* **19**, 205 (1971).
8. We are aware of measurements at 0.6, 0.9, and 1.2 Ghz by B. Enander and G. Larson [*Electronics Lett.* **10**, 37 (1974)]; at 0.8 Ghz by R. A. Porter and F. J. Wentz (Final Report, NASA-CR-114675, October 1973); and at 45 Ghz by J. Edrich and P. C. Hardee [*Proc. IEEE* **62**, 1391 (1974)]. Also, a discussion of the application of microwave radiometry to the study of communication in biological systems has been given by J. Bigu del Blanco and C. Romero-Sierra, in *Biologic and Clinical Effects of Low-Frequency Magnetic and Electric Fields*, J. G. Llaurado, A. Sances, Jr., J. H. Battocletti, Eds. (Thomas, Springfield, Ill., 1974), pp. 123–136.
9. We thank Dr. P. P. Lele for assistance with measurements requiring ultrasonic heating, G. K. Stimac for assistance in both laboratory and hospital measurements, Dr. N. L. Sadowsky for vital medical collaboration at Faulkner Hospital, and for numerous contributions, J. Orenstein, J. W. Barrett, and D. C. Papa. Supported by NIGMS grant GM-20370-02 and by NIH biomedical sciences support grant 5-SO5-RR 07047-09.

27 January 1975; revised 22 July 1975

Parietal Eyes in Lizards: Zoogeographical Correlates

Abstract. *Lizards without parietal eyes tend to be restricted to low latitudes, whereas lizards with parietal eyes are successful at higher latitudes also. These zoogeographical data, along with current theories of parietal eye physiology, strongly suggest that the overall significance of the parietal eye to lizards as a group is that it facilitates survival at higher latitudes, thus making possible the exploitation of a wider variety of habitats.*

In addition to their lateral eyes, many lizards have a small "third eye" located on top of their heads. The general morphology of this third eye is similar to that of the larger lateral eyes, except that the third eye (parietal eye) lacks muscles and an eyelid (*1*). The parietal eye of a few genera is known to be a functional photoreceptor (*2*), but no comprehensive data are available for lizards as a group. Neither has there been any comprehensive analysis of the pattern of parietal eye occurrence (*3*). In order to determine the possible significance of the parietal eye in the evolutionary and present-day success of lizards, we investigated the relationship of the pattern of parietal eye occurrence to their lifestyles and geographic distribution.

Parietal eye occurrence is relatively nonvariable in genera of the same family (*3*). Thus, either a very high percentage or a very low percentage of the members of any particular family has a parietal eye (Table 1). These data bear no apparent correlation with life-style. Instead, occurrence of parietal eyes seems to be following lines of lizard evolution at the familial level regardless of the variety of natural history types within each family (*4*). However, there is a relationship between latitudinal distribution and parietal eye occurrence.

Virtually all geckos and teiids lack parietal eyes. Since these are the largest, most successful, and best studied parietal-eyeless groups, we chose them for an analysis of geographical distribution. Centers of abundance and range were plotted for 59 of the 79 genera in Gekkonidae and for 39 of the 42 genera in Teiidae (*5*). Gekkonidae are most abundant within 10° of the equator. Among Teiidae, the equatorial concentration is more pronounced (Table 2). It is possible that teiids were restricted in their northward distribution by the instability of the land bridge between North and South America. It is unlikely, however, that this could be the sole explanation for their equatorial concentration, since they are relatively unsuccessful at high southern latitudes as well.

For comparison, we analyzed the geographical distribution of two large success-

Table 1. Parietal eye occurrence in lizards.

Infra-order	Family	Genera with visible parietal eye (%)
Gekkota	Gekkonidae	0–1*
	Pygopodidae	0
	Dibamidae	0
Scinco-morpha	Xantusiidae	100
	Anelytropsidae	0
	Scincidae	91–95
	Cordylidae	100
	Lacertidae	90
	Teiidae	0–3*
Anguino-morpha	Varanidae	100
	Helodermatidae	0
	Lanthanotidae	0
	Anniellidae	100
	Anguidae	100
	Xenosauridae	100
Iguania	Iguanidae	96–98
	Agamidae	83
	Chameleontidae	100

*Members of the families Gekkonidae and Teiidae almost certainly all lack parietal eyes. Ranges are given because the presence of a parietal eye could not definitely be ruled out in the sole specimen available for one genus in each family.

ful families in which the parietal eye is usually present, Agamidae and Iguanidae (Table 2). Of 33 genera recognized in Agamidae, we studied geographical distribution in 33 and parietal eye occurrence in 29. Of 53 genera recognized in Iguanidae, we studied geographical distribution in 38 and parietal eye occurrence in 53. Those agamid genera possessing parietal eyes are most abundant 20° to 30° from the equator, with progressively fewer centers of abundance at lower latitudes. In the family Iguanidae, genera possessing parietal eyes have established centers of abundance 32° and 21° from the equator.

Restriction of parietal-eyeless lizards to low latitudes is even more apparent from distributional analysis of specific parietal-eyeless genera within the families Agamidae and Iguanidae (Table 3). There are five genera of Agamidae (*Cophotis, Harpesaurus, Leiolepis, Lophocalotes,* and *Phoxophrys*) which are parietal-eyeless. Their center of abundance is on the equator, and four of the five range no further than 9° from it. The fifth ranges to 24°. The family Iguanidae contains only one genus (*Urocentron*) in which all specimens examined lacked a parietal eye (*3*). This genus has a range which is centered only 1° from the equator.

Most genera in the families Scincidae and Lacertidae possess parietal eyes. Although we have not thoroughly investigated ranges of lizards from these two families, others report that skinks range from latitude 48°N to 46°S (*6*), and *Lacerta*, having a prominent parietal eye, ranges farther north than the Arctic Circle, 66½° from the equator (*7*). The parietal-eyeless skinks (*Corucia, Ristella,* and *Voltzkowia*), and the parietal-eyeless lacertids (*Holaspis* and *Philochortus*), however, have centers of abundance within 10° of the equator and range to a maximum latitude of only 25° in Scincidae and 10° in Lacertidae (Table 3).

It is not likely that the abundance of parietal-eyeless lizards at low latitudes is simply a function of the fact that there are more lizards of all kinds at low latitudes. Iguanidae, for example, is a group that is not more abundant at low latitudes, and yet the range of its sole parietal-eyeless genus is centered only 1° from the equator. Approximately 41 percent of all lizard genera do not have parietal eyes (*3*). Therefore, if there is no latitudinal effect of parietal eye occurrence, 41 percent of all lizards should be parietal-eyeless regardless of the latitude. We compared the number of parietal-eyeless genera expected by chance (that is, 41 percent of the total number of genera present) with the observed number at each latitude. In Agamidae, there are more parietal-eyeless genera than expected within 10° of the equator and fewer than expected from 10° to 24° from the equator. There are no parietal-eyeless agamids above 24°.

Since the six families examined—Gekkonidae, Teiidae, Agamidae, Iguanidae, Scincidae, and Lacertidae—account for nearly 2500 of the 3000 species of lizards, a low-latitude restriction of parietal-eyeless animals is a general trend in lizards as a group. This suggests that possession of a parietal eye might facilitate survival at high latitudes. Laboratory data support this idea. The parietal eye is a functional photoreceptor capable of detecting light and discriminating wavelengths (*2*). Blocking photic input to the parietal eye causes acceleration and, thus, desynchronization of the normal reproductive cycle (*8*). The parietal eye may also play an important role in temperature tolerance and thermoregulation (*9*). Reproductive synchronization and thermoregulation are critical for poikilotherms living in harsh climates and varying photoperiods at high latitudes (*10*). Therefore, the overall significance of the parietal eye to lizards as a group may be that it facilitates survival at high latitudes through reproductive or thermoregulatory modulation, thus opening a wider variety of habitats for exploitation.

Table 2. Latitudinal distribution of lizard families north or south of the equator. Numbers in parentheses next to centers of abundance indicate how many genera overlap at that particular latitude; *N* is the number of genera in which the parietal eye is known to be absent or present.

Family	Centers of abundance	Range
	Without parietal eyes	
Gekkonidae (N = 67)	9°N (10), 5°N (9), 0° (9), 22°S (9), 25°S (10)	46°N–45°S
Teiidae (N = 31)	0° (18)	43°N–40°S
	With parietal eyes	
Agamidae* (N = 24)	24°N (8), 24°N (8), 12°N (8), 10°N (7), 0° (7), 30°S (7)	50°N–44°S
Iguanidae* (N = 52)	32°N (10), 21°N (9)	48°N–55°S

*Parietal-eyeless genera of this family are listed in Table 3.

Table 3. Latitudinal distribution of parietal-eyeless genera of lizards north or south of the equator. The number of parietal-eyeless genera in each family is given by *N*. Center of abundance here does not conform precisely with the definition in (*5*). In Iguanidae, it indicates the center of range of only one genus. In Scincidae, it indicates the latitude around which the three ranges cluster, since they do not overlap.

Family	Center of abundance	Range
Agamidae (N = 5)	0°	24°N– 8°S
Iguanidae (N = 1)	1°S	10°N–12°S
Scincidae (N = 3)	6°S	13°N–25°S
Lacertidae (N = 2)	9.5°N	10°N– 4°S

G. Craig Gundy*
Charles L. Ralph
Gloria Z. Wurst*
Department of Zoology and Entomology, Colorado State University, Fort Collins 80523

References and Notes

1. R. Eakin [*The Third Eye* (Univ. of California Press, Berkeley, 1973)] gives the best review of parietal eye literature.
2. D. I. Hamasaki, *Vision Res.* **9**, 1453 (1969).
3. G. C. Gundy and G. Z. Wurst (*J. Herpetol.*, in press) detail parietal eye occurrence and methods used in its determination. Histological confirmation is reported for 65 genera. Table 1 was prepared from data presented therein. Further comparative morphological data will be presented (G. C. Gundy and G. Z. Wurst, in preparation).
4. This "primary" pattern of parietal eye occurrence depends on its presence or absence in the form ancestral to each family. Subsequent "secondary" losses have also occurred and are responsible for the absence of a parietal eye in members of smaller taxonomic categories. These data will be discussed by G. C. Gundy and G. Z. Wurst (in preparation).
5. A center of abundance is that geographical location where the ranges of the greatest number of genera overlap. If the number of genera declines moving away from this center of abundance and then increases again, this second concentration is also termed a center of abundance if it represents range overlap of at least one less than the number of genera found in the first center of abundance. Distributional data were taken from N. De Rooij, *The Reptiles of the Indo-Australian Archipelago. Part I, Lacertilia, Chelonia, Emydosauria* (Brill, Leiden, 1915); J. Peters and R. Donoso-Barros, *Catalogue of the Neo-tropical Squamata. Part II* (Smithsonian Institution, Washington, D.C., 1970); H. Smith, *Handbook of Lizards* (Comstock, Ithaca, N.Y., 1946); H. Smith and E. Taylor, *An Annotated Checklist and Key to the Reptiles of Mexico Exclusive of the Snakes* (Government Printing Office, Washington, D.C., 1950); H. Wermuth, *Das Tierreich* **80**, 1 (1965); *ibid.* **86**, 1 (1967); E. Worrell, *Reptiles of Australia Crocodiles-Turtles Tortoises-Lizards Snakes* (Angus & Robertson, Sydney, 1963).
6. A. Greer, *Bull. Mus. Comp. Zool. Harv. Univ.* **139**, 151 (1970).
7. K. Porter, *Herpetology* (Saunders, Philadelphia, 1972), p. 250.

8. R. C. Stebbins and N. W. Cohen [*Copeia* **1973**, 662 (1973)] review the theory of parietal eye regulation of gonadal recrudescence in lizards.
→ R. Kosh and V. Hutchison, *Herpetologica* **28**, 183 (19[illegible] → V. Hutchison and R. Kosh, *Oecologia (Berlin)* **16**, 173 (1974); B. Firth, thesis, University of New England, New South Wales, Australia (1974).
10. In an attempt to distinguish between photoperiod and temperature effects, we gathered some preliminary data on elevations at which low-latitude parietal-eyeless animals were found. Data were sparse, but two parietal-eyeless forms (*Cophotis* and *Ristella*) were reported to occur up to 7000 feet (> 2000 m) which argues for a photoperiod rather than a temperature effect.
11. Data presented here were part of G.C.G.'s thesis, University of Pittsburgh (1974). An abstract covering part of these data appeared in G. C. Gundy, C. L. Ralph, C. J. McCoy, *Am. Zool.* **14**, 1271 (1974). Supported in part by NIH grants NS08554 and NS12257 to C.L.R.
* Present address: Department of Zoology, University of California, Berkeley 94720.

15 July 1975; revised 8 September 1975

α-Lactalbumin Production in Human Mammary Carcinoma

Abstract. *α-Lactalbumin was isolated from mature human milk and utilized as an immunogen in rabbits. A radioimmunoassay was developed that was capable of detecting nanogram quantities of the antigen. α-Lactalbumin synthesis was detected in human breast cancer cells (MCF-7) cultivated as a continuous cell line in vitro. Other human carcinoma epithelial cell lines (throat and cervix) failed to react in this assay. The ability to synthesize α-lactalbumin by breast carcinoma cells appeared to be independent of the addition of prolactin to the culture medium.*

α-Lactalbumin (α-LA) is a protein specifically synthesized in functionally differentiated mammary epithelial cells. It is recoverable in large quantities from their secretory products, milk and colostrum. The protein has been identified by Brodbeck and Ebner (*1*) as the B protein of lactose synthetase (E.C. 2.4.1.22) which catalyzes the formation of lactose.

$$\text{UDP-galactose} + \text{glucose} \rightarrow \text{lactose} + \text{UDP} \quad (1)$$

In the absence of α-LA, the A protein of lactose synthetase is a galactosyltransferase utilizing *N*-acetylglucosamine or a terminal *N*-acetyl unit on an oligosaccharide moiety of a glycoprotein (*2*)

$$\text{UDP-galactose} + N\text{-acetylglucosamine} \rightarrow N\text{-acetyllactosamine} + \text{UDP} \quad (2)$$

This galactosyltransferase is found predominantly in the mammary gland, but has been isolated from most tissues (*3*). α-Lactalbumin functions as a "specifier" protein, in that it alters the substrate specificity of the A protein, thus regulating an organ-specific reaction, lactose synthesis (*4*).

In our study, α-LA was isolated from whole, human milk by a modification of the procedures published by Nagasawa *et al.* (*5*). Skim milk at 25°C was adjusted to *p*H 4.6 with 0.1*N* HCl, and the precipitated casein was removed by centrifugation at 15,000*g* for 20 minutes. The *p*H of the clear supernatant (whey) was raised to 7.0 with 0.1*N* NaOH, and the solution was concentrated tenfold in an Amicon ultrafiltration cell with a UM-2 membrane. Percolation through a Sephadex G-100 column equilibrated with 0.01*M* imidazole-hydrochloride buffer (*p*H 7.0) resulted in the separation of α-LA from most of the whey proteins (Fig. 1A). After concentration in the Amicon ultrafiltration cell, homogeneous α-LA was eluted from a DEAE-cellulose column with a linear NaCl gradient in 0.01*M* imidazole-hydrochloride buffer, *p*H 7.0 (Fig. 1B). The eluate was concentrated, filtered under pressure against distilled water until free of chlorides, lyophilized, and stored at 20°C.

The isolated α-LA was judged to be homogeneous on the basis of alkaline (*p*H 8.9) disc electrophoresis, 0.2 percent sodium dodecyl sulfate gel electrophoresis (*p*H 7.8), and acid (*p*H 4.3) disc electrophoresis over a 35-fold protein concentration range (7 to 240 μg). In each instance only a single protein band was observed after staining with Coomassie brilliant blue. Acrylamide gel isoelectric focusing (*6*) (*p*H 3 to 10) also yielded a single band. The α-LA failed to react in Ouchterlony double diffusion against goat antiserum to human serum and rabbit antiserum to human casein.

Amino acid analyses were performed on a 6*N* HCl hydrolyzate (Technicon amino acid analyzer) (*7*). The amino acid analyses were in excellent agreement with the recorded values for α-LA, based on the amino acid sequence, indicating freedom from casein contamination (*8*).

Antibodies against human α-LA were produced in male New Zealand white rabbits by immunization with purified α-LA. The α-LA (5 mg) was dissolved in 0.5 ml of

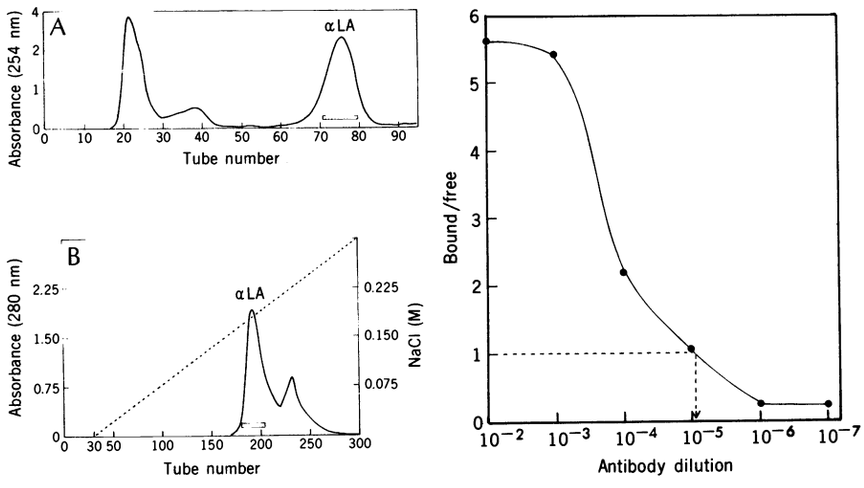

Fig. 1 (left). (A) Separation of α-LA from human whey proteins. Whey proteins (150 mg) were separated on a column (5 by 100 cm) of Sephadex G-100 (fine) equilibrated with 0.01*M* imidazole-hydrochloride buffer, *p*H 7.0. The flow rate was 60 ml/hour; 15-ml fractions were collected. The fractions indicated with the horizontal bar were pooled and concentrated by ultrafiltration. (B) Diethylaminoethyl-cellulose chromatography of α-LA. DEAE-cellulose (0.9 meq/g, lot 11140, Bio-Rad Laboratories) was equilibrated with 0.01*M* imidazole-hydrochloride buffer (*p*H 7.0) and packed into a column (2.5 by 85 cm); 150 mg of α-LA isolated from (A) was placed on the DEAE-cellulose. The column was then washed with starting buffer. The α-LA was eluted with a linear gradient formed from 1000 ml of 0.01*M* imidazole-hydrochloride (*p*H 7.0) and 1000 ml of the same buffer containing 0.30*M* NaCl (*p*H 7.0). The flow rate was 40 ml/hour, and 10-ml fractions were collected. Fig. 2 (right). Titration curve of rabbit antiserum to human α-LA with ^{125}I-labeled α-LA. The diluent for all radioimmunoassay reagents was PBS containing EDTA (1 m*M*), disodium salt, and bovine serum albumin (1 mg/ml). The sodium phosphate buffer was 0.05*M* and NaCl was 0.10*M* with a final *p*H of 7.4. A series of tenfold dilutions ranging from 10^{-2} to 10^{-7} of rabbit antibody to human α-LA was made in diluent buffer. Each serum dilution (50 μl) was reacted with 0.5 ng (10 μl) of ^{125}I-labeled α-LA antigen in the presence of 128 μg of normal rabbit gamma globulins (final volume, 150 μl); incubation proceeded at 37°C for 2 hours in a Dubnoff metabolic shaking incubator and was terminated by the addition of goat antiserum to rabbit gamma globulins; the tubes were then incubated for another hour at 37°C, and 16 hours at 4°C. The immunoprecipitate was centrifuged at 900*g* for 60 minutes, and the radioactivity of a 100-μl sample of supernatant (unbound ^{125}I-labeled α-LA) counted in a liquid scintillation spectrometer, calibrated for ^{125}I (*6*).