body containing large nuclei is situated on the parietal nerve near the "eye." Could this be a ganglion? Nowikoff (*3*) noted a similar structure in *Anguis*, which he interpreted as an ectopic piece of retina.

We have studied serially sectioned heads of 16 parietalectomized *Sceloporus occidentalis*, a sample from our field study (*1*). The parietal nerve can be identified in specimens sacrificed 7 to 15 months after parietalectomy. Presumably we are observing only sheath cells and perineurium. Nerve stains have not been used. The strand can be traced in association with the anterior pineal artery from the epiphysis to the former site of the parietal eye where the "nerve" frays out on the surface of the artery or in the remnant of the capsule. Parietalectomy involved the destruction of only the retina and lens (*1*). Older specimens, 18 to 21 months after surgery, show progressive degeneration of the "nerve." In some instances remnants can be found in the vicinity of the epiphysis only. In many the strand is intensely pigmented with black granules of various sizes and shapes. Much of the pigment is within large irregular cells which may be macrophages, or melanophores which have migrated from the meninges, or possibly modified sheath cells.

The finding of a parietal nerve from the third-eye of *S. occidentalis* alters the interpretation of the effects of parietalectomy upon the behavior of the lizard. We had postulated (*1*) that these effects might be attributed to the loss of a hormone produced by the "eye." Injury to and degeneration of the neural connection of the parietal eye to the habenular region of the brain must now be considered. The possibility that the eye has an endocrine function, however, is not negated. Further studies are being conducted to elucidate this point (*8*).

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Structural Similarities between Hemoglobins A and F

Abstract. A striking similarity has been found between the composition of peptides obtained from tryptic digestion of normal adult hemoglobin (hemoglobin A) and fetal hemoglobin (hemoglobin F).

Normal adult and fetal hemoglobins have been considered to have entirely different structures because of different physical properties (*1*), different immunological characteristics (*2*, *3*), and significant dissimilarities in amino acid composition (*4*). The synthesis of these two hemoglobins has been considered to be under the control of two separate genes (*5*). It was, therefore, surprising to discover a large degree of similarity between fingerprints of tryptic digestion mixtures of these two hemoglobins, a finding which has been confirmed by qualitative analyses of peptides eluted from the fingerprints.

Samples of hemoglobins A and F were prepared by techniques previously described (*3*). Briefly, purified hemoglobin A was isolated by a modification of Drabkin's technique of fractionation against $(NH_4)_2SO_4$ (*6*). Hemoglobin F was prepared from a specimen of cord blood after preliminary treatment with 0.0833N KOH (*3*). The purified hemoglobins were converted to the CO form and kept as the freeze-dried powder at 20°C until used. Hemoglobin A was

Table 1. Qualitative amino acid analyses of the tryptic peptides from hemoglobins A and F illustrated in Fig. 1. The plus symbol indicates a strongly positive amino acid; *tr* indicates a weakly positive analysis. The results which are marked with an asterisk are those wherein a difference between hemoglobins A and F was detected; no spots corresponding in position to 10A and 19A have been found in hemoglobin F. Abbreviations: *ala*, alanine; *arg*, arginine; *asp*, aspartic or asparagine; *glu*, glutamic or glutamine; *gly*, glycine; *his*, histidine; *ileu*, isoleucine; *leu*, leucine; *lys*, lysine; *met*, methionine; *phe*, phenylalanine; *pro*, proline; *ser*, serine; *thr*, threonine; *tyr*, tyrosine; and *val*, valine.

Peptide	Lys	Arg	His	Asp	Glu	Gly	Ala	Val and Met	Ileu and Leu	Ser	Thr	Tyr	Phe	Pro
1A	+		+			+	tr							
1F	+		+			+	tr							
2A	+		+			+	+							
2F	+		+			+	+							
3A	+													
3F	+													
4A	+							+						
4F	+							+						
5A		+							+					
5F		+							+					
6A	+				+									
6F	+				+									
7A		+			+						+			
7F		+			+						+			
8A	+			+				+			+			
8F	+			+				+			+			
9A	+			+	tr*		+*	+	+	+	+			+
9F	+		+*	+	+*	tr*		+	+	+	+		tr*	+
10A	+*		+*		+*			+*	+*		+*			+*
10F														
11A†		+	+									+		
11F†		+	+									+		
12A	+		+	+		+	+	+*	+					
12F	+		+	+		+	+		+					
13A	tr	+	+	+	+	+	+	+	+			+		
13F	tr	+	+	+	+	+	+	+	+			+		
14A	+		+	+	+	+	+	+	+	+	+*		+	+
14F	+		+	+	+	+	+	+	+	+			+	+
15A	+			+			+	+	+	+				+
15F	+			+			+	+	+	+				+
16A	+					+	+	+*	+	+	+			
16F	+					+	+		+	+	+			
17A		+		+	+	+	+	+	+					
17F		+		+	+	+	+	+	+		+*			
18A	+								+	+	+		+	+
18F	+			+*		+*	+*	+*	+	+	+		+	+
19A	+*			+*	+*	+*	+*	+*	+*	+*	+*		+*	+*
19F														

†These peptides are probably composed of two separate peptides, histidine and arginine-tyrosine.

denatured by heating for 2 minutes in distilled water at 95°C, while hemoglobin F was denatured for 10 minutes at 100°C. Ammonium carbonate was then added to each sample to give a final concentration of 0.2M and a *p*H of 8.4. Crystalline trypsin (Worthington 2x, salt free, No. SF685), 2 percent by weight, was added, and digestion was carried out for 5 hours at 37°C. The insoluble "core" remaining at the end of digestion was removed by centrifugation; this constituted from 10 to 25 percent of the starting material and was not examined further in this study. The soluble peptides were lyophilized to remove the buffer salt and resuspended in distilled water, and 2 to 3 mg of the digest was applied to the fingerprints. Two-dimensional chromatography and electrophoresis was carried out on full 18¼- by 22½-in. sheets of Whatman No. 3 filter paper (*7*). The first dimension, descending chromatography in *n*-butanol-acetic acid-water (4:1:5), was followed by electrophoresis for 1 hour at 2000 v in a buffer of *p*H 3.7 composed of pyridine-acetic acid-water (1:10:289). The resulting chromatograms were stained with 0.5-percent Ninhydrin in absolute ethanol or with a starch iodine stain (*8*) to locate the peptides.

Qualitative analyses were carried out on the stronger of the peptides, which could be located on fingerprints stained lightly with 0.025-percent Ninhydrin. The peptide spots so located were washed with acetone and eluted with constantly boiling HCl, in which they were hydrolyzed at 108°C for 18 hours in sealed tubes. After the tubes had been opened and dried over NaOH, the amino acids were resuspended in water and applied to Whatman No. 1 filter paper and run in *n*-butanol-acetic acid-water (4:1:5) followed by pyridine-water (4:1). The amino acids were identified after staining with a 0.1-percent Ninhydrin-acetic acid-collidine system (15:5:2) (*9*). This system separated all the amino acids with the exception of two pairs which cannot be differentiated: caline and methionine, and leucine and isoleucine. Tryptophan was destroyed by acid hydrolysis, while glutamine and asparagine were deaminated to yield the corresponding dicarboxylic amino acids.

Nineteen peptides from the fingerprints of hemoglobins A and F have been subjected to analysis (Fig. 1). These peptides, when eluted, gave consistently high yields of amino acids after hydrolysis. Other peptides are being examined further. Of the 19 peptides analyzed here, 11 were qualitatively the same, five showed minor differences, and only three (Nos. 10, 18, and 19) appeared grossly different (Table 1). The number of peptides expected from complete tryptic digestion of all the lysyl and arginyl bonds in hemoglobins A and F is 29 and 30, respectively (*4*). Since no cystine-containing peptides have been found in this study, it seems likely that the cysteine-residues of the hemoglobins were present, bound together by disulfide bridges, in the "core." Only 65 percent of the expected number of peptides has been found, and it is possible that some of the different peptides described here were from the same part of the peptide chain, a secondary split accounting for the difference (peptides Nos. 1 and 2, for example).

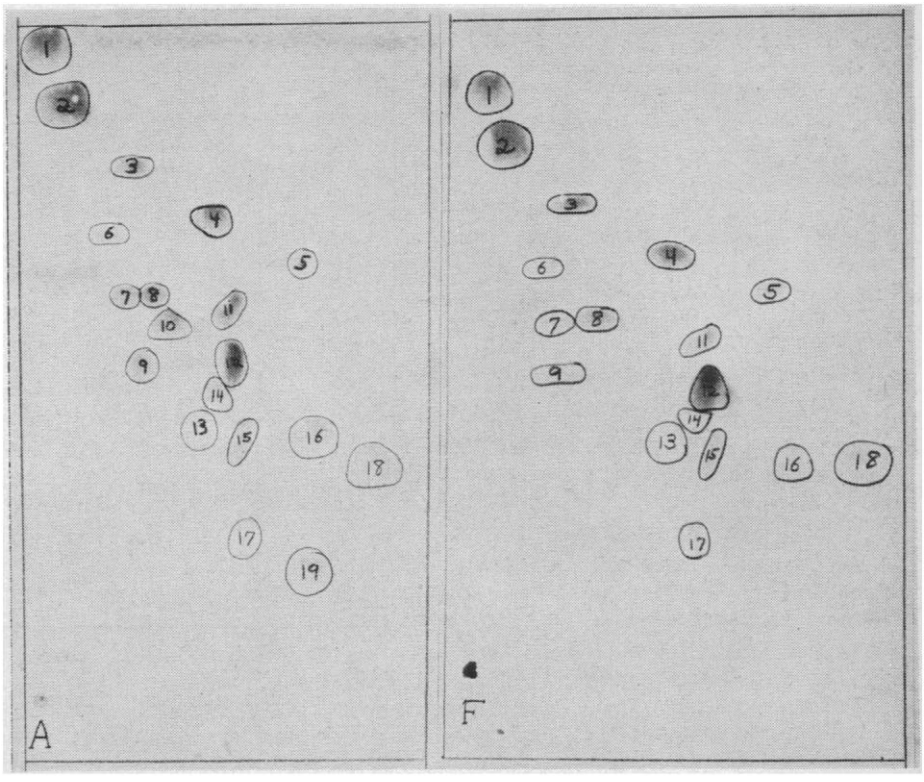

Fig. 1. Fingerprints of hemoglobins A (left) and F (right) stained with 0.5-percent Ninhydrin. The peptide mixture was applied to a spot in the lower left-hand corner of each fingerprint. Chromatography (horizontal) was followed by electrophoresis (vertical), the cathode being at the top of the paper.

The results reported here are consistent with the recent finding that the α-chains of hemoglobins A and F have similar fingerprints and N-terminal amino acids (*10*). While the two chains of hemoglobin have not been separated prior to analysis in the present study, the results show that the qualitative amino acid analyses of most of the tryptic peptides from hemoglobins A and F are identical. In view of the paucity of major differences, it seems likely that the β-chain of these two hemoglobins may be similar, although at least one clear-cut alteration, peptide No. 10, is from the β-chain, since it is the peptide altered in hemoglobin S (*10, 11*). It should also be pointed out that some of the differences noted need not be due to generic differences between hemoglobins A and F but may be due to individual differences between the patients from whom the hemoglobins were obtained (*12*).

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Oxygen Isotope Paleotemperature Determinations of Australian Cainozoic Fossils

Abstract. Fossil marine shells collected in southern Victoria within half a degree of latitude of 38½ °S have been analyzed for O^{18}/O^{16} by mass spectroscopy, and their paleotemperatures have been determined. For the genera *Chlamys, Ostrea,* and *Glycymeris* the temperature rises from early to mid-Tertiary, then falls again to the present.

The method of Urey and his co-workers (*1*) was used to determine paleotemperatures, the experimental accuracy being estimated as about 1°C, made up of instrumental errors and variation in the CO_2 preparations using phosphoric acid. Errors due to alteration of the fossil carbonate cannot be estimated, but a careful selection of shells together with a thorough mechanical cleaning of their surfaces was made to minimize variations. The absolute value of the temperature scale was determined to be within 1°C of the Chicago standard PDB II, but living shells gave values about 2°C lower than averaged sea-water temperatures. Epstein and Lowenstam (*2*) also found a variation between sea-water and shell O^{18}/O^{16} temperatures in Bermuda shoal waters, where pelecypods and gastropods had different growth habits, resulting in different isotope temperatures in the same waters. An *Anadara* shell from Moreton Bay, Queensland, was found by us to have a value 5°C below the sea-water temperature, but this was probably due to its living between tide marks. This mollusk thus lived part of the time at a mud-water interface and part of the time at a mud-air interface.

Mollusks from Melbourne and a limpet from Macquarie Island yielded isotope temperatures about 2°C lower than measured sea-water temperatures. This difference is interpreted as being due to the growth habits of the organisms, to the difference between the mean temperature of the sites where they lived and the sites where the temperatures were measured, or to both. Despite these drawbacks inherent in using specimens from shelf faunas [see also Epstein *et al.* (*1*) and Lowenstam and Epstein (*3*)], a definite picture of the paleotemperature changes can be seen.

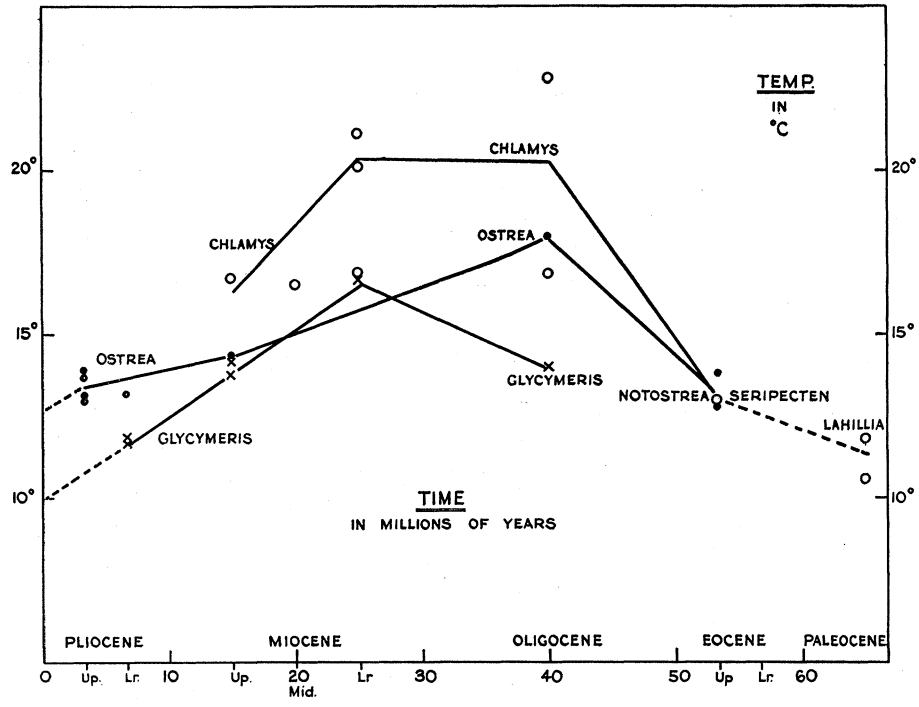

Fig. 1. Results obtained for the Tertiary fossils. The sites which yielded the fossils whose paleotemperatures are given are as follows:

Age	Species	Site
Pliocene		
Lower	*Ostrea*	Grange Burn, near Hamilton
Upper	*Ostrea*	Maretimo member, Whaler's Bluff formation, Portland
Lower	*Glycymeris*	Muddy Creek, near Hamilton
Miocene		
Upper	*Ostrea*	Beaumaris
Middle	*Ostrea*	Bairnsdale
Upper	*Glycymeris*	Beaumaris
Lower	*Glycymeris*	Balcombe Bay
Upper	*Chlamys*	Beaumaris
Middle	*Chlamys*	Bairnsdale
Lower	*Chlamys*	Balcombe Bay
Oligocene	*Ostrea*	Waurn Ponds, near Geelong
Oligocene	*Glycymeris*	Jan Juc formation, Torquay
Oligocene	*Chlamys*	Jan Juc formation, Torquay
Oligocene	*Chlamys*	Waurn Ponds, near Geelong
Eocene		
Upper	*Notostrea*	Brown's Creek, Cape Otway district
Upper	*Notostrea*	Hamilton Creek, Cape Otway district
Upper	*Seripecten*	Hamilton Creek, Cape Otway district
Paleocene	*Lahillia*	Pebble Point, Otway Coast

Figure 1 summarizes the results obtained for the Tertiary fossils. It shows a rise in temperature in the first half of the Tertiary and a fall in the second half. This is in keeping with the biological evidence obtained from the fossils themselves. The Middle Tertiary is characterized by masses of tropical foraminifera such as *Lepidocyclina, Cycloclypeus* and *Carpentaria,* echinoderms such as *Phyllacanthus, Eucidaris* and *Lovenia* and mollusks such as giant cowries and volutes, *Cucullaea* and *Hinnites* (the last two in great numbers). It is not until the Pliocene that the marine fauna is comparable with that living in the same area now. Emiliani (*4*) has also traced this Upper Tertiary fall in temperature for Pacific abyssal waters.

The localities for Pleistocene fossils are the Yarra delta formations (dated in part by radiocarbon), emerged shell beds of the 25-ft eustatic level, and Lower Pleistocene beds (Werrikooian stage) in far western Victoria. The sites which yielded the Tertiary fossils whose paleotemperatures are given in Fig. 1 are tabulated in the legend. Further details are given elsewhere (*5*).

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