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CONTRIBUTION A LA MORPHOLOGIE COMPAREE DES ERYTHROCYTES
CHEZ LES REPTILES

par

MARIE-CHARLOTTE SAINT GIRONS et HUBERT SAINT GIRONS

Muséum National d'Histoire Naturelle Brunoy (Essonne) France

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D'une façon générale, la taille des cellules constituant les différents tissus diminue nettement des Amphibiens aux Mammifères et aux Oiseaux, les Reptiles étant en position intermédiaire. Il résulte d'un certain nombre de travaux récents portant sur l'histologie comparée des glandes endocrines des Reptiles (voir Saint Girons, H., 1967, pour la bibliographie) que la taille et l'aspect des différentes cellules de ces organes présentent des variations assez constantes et significatives d'une famille à l'autre. Toutefois, des résultats comparatifs, rigoureux et chiffrés, n'ont pu être fournis, du fait de l'imprécision des mesures sur coupes et surtout parce que les dimensions de ces éléments varient de façon considérable au cours du cycle fonctionnel. Pour des raisons techniques évidentes, les érythrocytes du sang circulant représentent un matériel beaucoup plus favorable à l'étude de ce problème et il nous a semblé opportun de les examiner chez un certain nombre de Reptiles. Wintrobe (1933) a suggéré que la taille des érythrocytes était en rapport avec leur place dans l'échelle évolutive. Les Vertébrés inférieurs et ceux qui représentent une expérience évolutive infructueuse ont des globules de grande taille et nucléés. Les Vertébrés supérieurs ont, au contraire, des globules rouges de petite taille et anucléés.

Les premières données comparatives sur la morphologie des érythrocytes de Reptiles sont dues à Mandl (1839) et à Gulliver. Après un travail concernant les Crocodiliens (1840), ce dernier auteur a publié une étude comparative des érythrocytes chez 3 Reptiles de la faune anglaise: *Anguis fragilis*, *Natrix torquata* (= *N. natrix*) et *Vipera berus* (1842); enfin, en 1875, il fait le point des données rassemblées chez 37 espèces (13 Ophidiens, 16 Sauriens et Crocodiliens, 8 Cheloniens). Il peut déjà mettre en évidence la taille relativement grande des érythrocytes chez les Crocodiles et les Tortues et souligne les petites dimensions trouvées chez les Lacertidae de petite taille ainsi que chez les Teidae et les Varanidae. Depuis cette époque, d'autres espèces ont été étudiées, soit isolément, soit dans des travaux comparatifs. Parmi les plus importants, il convient de citer: Milne-Edwards (1856-1857), Hayem (1879), Werzberg (1910), Babudieri (1930), Jordan (1938), Ryerson (1949), Villiers Pienaar (1962)⁽¹⁾. Dans l'ensemble, ces études d'hématologie comparée ont complété et confirmé les observations de Gulliver. La position de *Sphenodon punctatus* n'était pas à notre connaissance, étudiée de ce point de vue. Les publications de Komocki (1926-1936-1938) concernent seulement les leucocytes et la formation des érythrocytes chez cette espèce. Il était important d'inclure l'ordre des Rynchocephales dans une étude comparée. Certains de nos résultats ont déjà été publiés; ils concernent, dans ce cas, uniquement les espèces de la faune française (Saint Girons, M. C. et Duguy, 1963).

(1)—Une étude de Salgues (1937) sur l'hématologie comparée des Reptiles de la faune française comporte des résultats assez différents de ceux généralement observés, en particulier chez *Anguis fragilis*, nous avons préféré ne pas en tenir compte.

MATERIEL ET TECHNIQUES

Le travail a porté sur 76 espèces, appartenant à 29 familles (voir Tableau I). Dans la plupart des cas, seuls un ou deux individus de chaque espèce ont été examinés. Cependant, dans une étude préliminaire, l'importance des variations individuelles a été recherchée par des mesures portant sur 8 ou 10 spécimens de chacune des

espèces suivantes: *Testudo graeca*, *Lacerta muralis*, *Anguis fragilis*, *Natrix maura*, *Natrix natrix*, *Vipera aspis* et *Vipera berus*. Nous avons indiqué dans la figure 1 les dimensions moyennes des érythrocytes de chacun des individus examinés, chez 4 de ces espèces.

Les frottis ont été effectués en priorité au moment de l'autopsie, le sang fixé sur lames par simple séchage, puis coloré par la méthode de May-Grünwald-Giemsa. Les mesures, faites à l'aide d'un micromètre oculaire sous un grossissement de 800 diamètres, ont porté sur 100 érythrocytes par animal. Les surfaces furent calculées en considérant que les hématies et leurs noyaux avaient la forme d'une ellipse, ce qui ne correspond évidemment qu'à une approximation, notamment en ce qui concerne les noyaux dont les contours sont souvent irréguliers. La majorité des individus ont été autopsiés très peu de temps après leur capture, souvent dans les minutes qui suivent. Dans quelques cas, faute d'autre spécimen, nous avons été obligés d'utiliser des animaux captifs depuis plusieurs semaines ou même plusieurs mois. Dans ce cas, les animaux sont souvent très parasités.

Seuls, les érythrocytes mûrs ont été mesurés. On peut trouver dans le sang circulant des éléments jeunes de la série érythrocytaire, caractérisés par une forme plus arrondie, un cytoplasme coloré en bleu et un gros noyau moins chromophile que dans la cellule mûre. Ce sont les normoblastes basophiles et polychromatophiles⁽²⁾. Ils sont particulièrement fréquents chez les jeunes et les animaux en mue (Saint Girons, M. C., 1961). Les formes séniles des globules rouges sont des cellules plus grandes que l'érythrocyte normal. Le cytoplasme est peu coloré, le noyau est souvent de forme irrégulière. Au dernier stade, le cytoplasme disparaît et seul le noyau, pyknotique, est visible sur les frottis. Les formes jeunes aussi bien que les formes séniles ont été éliminées de cette étude et les érythrocytes mûrs seuls retenus.

De même, nous avons, chaque fois que cela était possible, éliminé les animaux dont le sang était parasité et, en tout cas, jamais mesuré les érythrocytes contenant des sporozoaires. D'une façon générale, la présence de parasites endoglobulaires peut en effet changer un peu la forme et la taille des érythrocytes. L'infestation des globules rouges de *Tarentola mauritanica* par *Pyrohemocytus tarentolai* (Sporozoa, Pyroplasmose) rend les hématies plus arrondies, ainsi que les noyaux (Wood, 1935; Reichenbach-Klinke, 1963). Une étude effectuée chez des Lézards du Mexique et de la Floride a montré que des espèces du genre *Plasmodium* n'affectent que très légèrement les dimensions des cellules. On constate seulement une faible augmentation de taille (Thomson et Huff, 1944). Des spécimens de *Lacerta muralis* parasités par des hémogregarines (*Karyolius lacertae*) ont généralement des globules plus grands que les érythrocytes sains. En comparant les dimensions des érythrocytes sains et parasités chez quelques Serpents de l'est de Java, Bergmann (1957) a trouvé chez ces derniers des dimensions plutôt grandes.

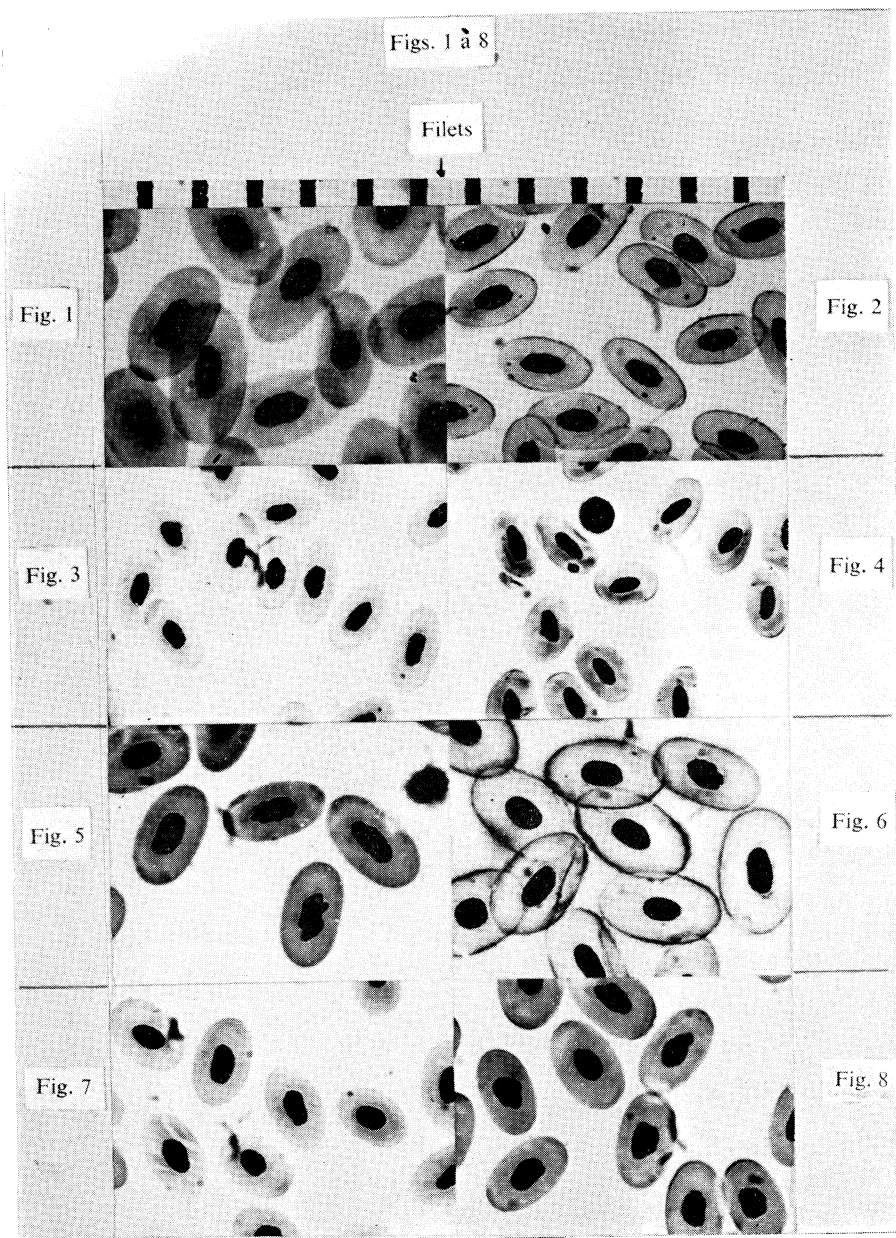
(2) Ils représentent sans doute la deuxième catégorie d'érythrocytes trouvée dans le sang de *Testudo graeca* (Girod et Lefranc, 1958).

Nous avons également vérifié s'il existait des variations sexuelles des dimensions des érythrocytes. Le tableau II montre que, dans les différents ordres, il n'y a pas de différences significatives dans les dimensions des globules ni des noyaux. Il a donc été possible d'utiliser indifféremment des animaux de l'un ou l'autre sexe.

Les normoblastes azurophiles ou polychromatophiles étant plus nombreux chez les jeunes, nous avons étudié uniquement des adultes sauf chez les Crocodiliens. Chez les deux espèces examinées ici, nous avons été particulièrement attentifs à utiliser seulement des globules mûrs. Les dimensions des érythrocytes mûrs sont en effet spécifiques et ne varient pas avec l'âge de l'animal (Villiers Pienaar, 1962).

RESULTATS

Les érythrocytes ou globules rouges du sang circulant sont des cellules nucléées, de forme ovale, arrondies à leur extrémité. Le noyau est également ovale, plus ou moins régulier, central et son grand axe est orienté dans le même sens que celui



de la cellule (à l'exception d'un Léopard, *Acantodactylus erythrurus*, où plus de 50% des noyaux sont déviés par rapport à cet axe chez l'unique individu que nous avons examiné). Sur les frottis de sang colorés suivant la méthode classique de May-Grünwald-Giemsa, le cytoplasme de couleur jaunâtre apparaît le plus souvent hyalin et homogène. Le noyau d'un érythrocyte mûr est chromophile. Les mottes de chromatine sont plus ou moins visibles, suivant l'âge de la cellule. Des colorations plus fines, mais que nous n'avons pas utilisées, montrent l'existence de granulations intracitoplasmiques (Hirschler, 1928; Ryerson, 1949) et d'un appareil de Golgi (Bhattacharya et Brambell, 1924).

Tous les Chéloniens pleurodirés étudiés ici sont pourvus d'érythrocytes d'assez grande taille, le grand diamètre étant compris entre 18,5 et 19 μ , le petit diamètre entre 10,6 et 11,7 μ . Les noyaux, de forme régulière, sont presque ronds sur frottis. Le rapport nucléo-cytoplasmique est légèrement plus faible que la moyenne (0,116 à 0,151). L'unique individu du sous-ordre des Cryptodirés que nous ayons autopsié, *Pelomedusa subrufa*, diffère profondément de ce point de vue des autres Chéloniens. Si les érythrocytes sont assez comparables aux précédents, bien qu'un peu plus allongés (le petit diamètre n'atteignant que 10,2 μ), les noyaux presque sphériques, sont exceptionnellement petits et le rapport nucléo-cytoplasmique s'élève à 0,079, le chiffre le plus bas que nous ayons trouvé chez un Reptile.

Les érythrocytes de *Sphenodon punctatus* diffèrent de ceux de tous les autres Reptiles par leur très grande taille qui permet de reconnaître l'espèce, sans risque d'erreur, sur un simple frottis de sang. A cela près, ils ne présentent aucune particularité saillante. Le rapport nucléo-cytoplasmique est égal à 0,147, et seuls les noyaux, de forme régulière, sont un peu plus arrondis que la moyenne, le rapport GP/PD étant de 1,46.

Comme pour d'autres caractères, les Sauriens font preuve d'une grande diversité en ce qui concerne la morphologie des cellules sanguines. Chez les Gekkota, les érythrocytes des 5 espèces étudiées ici sont de forme variée (le rapport GD/PD des hématies varie entre 1,49 et 2), sans qu'on puisse différencier les Gekkoniidae des Pygopodidae. Mais, dans l'ensemble, la taille des cellules est grande. Le rapport nucléo-cytoplasmique, assez constant, est plutôt inférieur à la moyenne (entre 0,120 et 0,151). Les noyaux sont assez allongés, réguliers, de forme aigüe et non arrondie aux extrémités.

En ce qui concerne la taille des érythrocytes, les Iguania (Iguanidae, Agamidae, Chamaeleonidae) examinés au cours de ce travail se répartissent en deux groupes bien séparés. Dans le premier, constitué par 3 Iguanidae et 2 Agamidae, les hématies sont de petite taille, leur grand diamètre étant inférieur à 15,3 μ . Le second groupe, réunissant 4 Iguanidae, 1 Agamidae et 1 Chamaeleonidae, est caractérisé par des érythrocytes nettement plus gros dont le grand diamètre est compris entre 17,6 et 18,9 μ . Ceci mis à part, les cellules présentent un aspect comparable chez toutes les espèces. Elles sont, dans l'ensemble, assez allongées, ce caractère étant particulièrement accusé chez *Chamaeleo basiliscus* où le rapport GD/PD=2,26. Les noyaux sont de forme régulière, sauf chez *Dipsosaurus dorsalis* où la plupart d'entre eux ont des bords plus ou moins échancrés. Le rapport nucléo-cytoplasmique est voisin de la moyenne ou un peu plus élevé.

Les Lacertidae et Teiidae, représentés par 8 espèces, forment un groupe remarquablement homogène. Les érythrocytes sont très petits (le grand diamètre est compris entre 13,6 et 15,8 μ), pourvus de noyaux allongés, de forme régulière. Le rapport GD/PD et le rapport nucléo-cytoplasmique ne présentent pas de particularité notable. Les Cordylidae étudiés ici diffèrent nettement des deux familles précédentes. Les hématies sont beaucoup plus grandes, avec un grand diamètre de 17,2 et 17,3 μ ; d'autre part, le rapport nucléo-cytoplasmique est élevé du fait de la taille des noyaux, proportionnellement plus gros et arrondis que chez la plupart des autres Sauriens.

Les érythrocytes des Scincoidea sont de taille et de forme assez variées. Petits chez *Chalcides mionecton*, *Lygosoma taeniolata*, *Lygosoma* sp. et *Scincus officinalis*, ils atteignent un grand diamètre de 16,3 μ chez *Egernia* sp. et de plus de 17 μ chez

Eumeces algeriensis et *Tiliqua scincoides*. Le rapport nucléo-cytoplasmique est également très variable. Remarquons, toutefois, que les hématies de *Feylinia currori* sont morphologiquement très semblables à celles de *Chalcides mionecton* ou de *Lygosoma taeniolata* et *Lygosoma* sp.

Les Anguioidea sont caractérisés par des érythrocytes de grande taille, souvent allongés, à grands noyaux aux extrémités arrondies. Le rapport nucléo-cytoplasmique, assez peu variable, est voisin de la moyenne ou un peu inférieur. *Heloderma horridum* se détache nettement du groupe, avec des hématies si grandes que leurs dimensions (21,4 x 13,5 μ) les rapprochent de celles de Sphénodon. Les cellules, comme les noyaux, sont également plus arrondies. Au contraire, *Heloderma suspectum* ne diffère apparemment pas des Anguinae et Anniellidae. Il convient toutefois de signaler que le sang de l'unique animal examiné, captif depuis longtemps, était fortement parasité. Seule la rareté de l'espèce nous a conduit à inclure ces données dans ce travail, mais il n'est nullement certain que la morphologie des érythrocytes des deux espèces du genre *Heloderma* diffère autant qu'il ne le semble d'après le tableau. *Varanus griseus* se distingue nettement des autres Anguimorpha par la petite taille des érythrocytes dont le grand diamètre n'est que de 15,6 μ . Les noyaux sont, proportionnellement, un peu plus volumineux que la moyenne puisque le rapport nucléo-cytoplasmique est de 0,160.

Les Amphisbénieniens se situent à un niveau moyen, tant en ce qui concerne la taille des érythrocytes que celle des noyaux; ces derniers sont un peu plus étroits chez *Blanus cinereus* que chez *Trogonophis wiegmanni*. Il y a, de ce fait, un rapport nucléo-cytoplasmique plus faible chez la première espèce (0,134 au lieu de 0,170).

Les érythrocytes des deux espèces de *Typhlops* étudiés ici diffèrent nettement. Ceux de *Typhlops punctatus*, de taille moyenne, ne présentent pas d'autres particularités qu'un rapport nucléo-cytoplasmique assez faible (0,116) alors que les hématies de *Typhlops vermicularis* sont petites, allongées, avec un noyau proportionnellement très volumineux, le rapport nucléo-cytoplasmique étant particulièrement élevé (0,218). *Leptotyphlops dulcis* est caractérisé par des érythrocytes allongés (rapport GD/PD = 1,97), de petite taille et pourvus, comme ceux de *Typhlops vermicularis*, de gros noyaux, avec un rapport nucléo-cytoplasmique de 0,179.

Les autres Serpents forment un groupe relativement homogène, notamment en ce qui concerne la taille des érythrocytes dont le grand diamètre est compris entre 16 et 19 μ , quelle que soit la famille. Toutefois, il semble que les hématies et leurs noyaux soient légèrement plus arrondis, avec une surface un peu plus grande chez les Viperidae. Les noyaux sont le plus souvent de forme irrégulière chez les Viperidae et les Elapidae. Ce fait est particulièrement net chez *Naja naja*. D'autre part, *Laticauda colubrina* se distingue de l'ensemble par la taille légèrement supérieure de ses érythrocytes. Enfin, un Boidae, *Morelia spilotes*, possède des noyaux particulièrement petits, presque sphériques, avec un rapport nucléo-cytoplasmique égal à 0,091, presque aussi faible que celui de *Pelomedusa subrufa*.

Chez les Crocodiliens examinés, les érythrocytes sont de taille plutôt faible avec de petits noyaux presque sphériques et un rapport nucléo-cytoplasmique un peu inférieur à la moyenne (0,135 et 0,123).

DISCUSSION

L'importance des variations individuelles qui ont été constatées lors de l'étude de 8 à 10 spécimens, chez 7 espèces de Tortues, Lézards et Serpents, doit rendre prudent dans l'interprétation des résultats portant, le plus souvent, sur un seul animal par espèce. Sauf cas particuliers, il ne convient pas d'attacher trop d'importance à des exceptions uniques et seules des tendances générales peuvent être retenues. Il s'en dégage d'ailleurs un certain nombre.

Variations individuelles.

Seul le contrôle de la variabilité individuelle permet d'apprécier le degré de confiance qu'il convient d'accorder à des résultats portant, le plus souvent, sur un

seul individu par espèce. L'expérience a été tentée sur 8 *Testudo graeca* et 10 spécimens de 6 autres espèces: *Lacerta muralis*, *Anguis fragilis*, *Natrix maura*, *Natrix natrix*, *Vipera berus* et *Vipera aspis*. La taille des érythrocytes varie de façon notable chez les 7 espèces étudiées ici (Fig. 9), l'ampleur de la dispersion étant du même ordre (*Testudo*, *Lacerta*, *Anguis*, *Vipera*) ou un peu moindre (*Natrix*) que celle des différentes espèces d'une même famille. Cependant, les résultats confirment l'existence de différences spécifiques très nettes, même entre représentants du même genre. Il est hors de doute que si, sur les figures 10 et 11, nous avons pu faire figurer les dimensions des érythrocytes de 10 individus par espèce, la zone occupée par chacun des différents groupes systématiques se serait notablement agrandie. Mas, vraisemblablement, les positions respectives n'eussent pas été très modifiées. Des constatations identiques peuvent être faites en ce qui concerne les dimensions des noyaux (Fig. 12).

Il n'en est pas de même pour le rapport nucléo-cytoplasmique. Assez constant chez *Lacerta muralis*, ce rapport présente, chez les autres espèces, une variation de même ampleur et parfois supérieure à celle que l'on constate au niveau de la famille correspondante. Ce phénomène n'a rien de surprenant puisque c'est le rapport nucléo-cytoplasmique qui varie le plus au cours de la vie de la cellule.

Forme et taille des érythrocytes.

Le rapport GD/PD des érythrocytes est très généralement compris entre 1,5 et 2 et ses variations entre espèces apparentées, voire même entre individus d'une même espèce, sont notables. Tout au plus, peut-on noter que ce rapport est fréquemment élevé chez les Iguania, notamment *Chamaeleo basiliscus*, chez *Leptotyphlops dulcis*, les Anguinae et les Anniellidae.

La taille des érythrocytes varie presque du simple au double et, comme le montrent les figures 10 et 11, il existe souvent un rapport entre celle-ci et la position systématique. Les Lacertidae et Teiidae forment un groupe homogène, caractérisé par la petite taille des hématies. Les autres Sauriens présentent des variations plus grandes, mais on distingue cependant, de façon nette, deux groupes différents: les Scincoidea et les Iguania d'une part, les Gekkota et les Anguioidea (Helodermatidea inclus) d'autre part. La longueur des érythrocytes est comprise entre 15,5 et 18 μ chez la grande majorité des espèces du premier groupe, entre 17 et 21,5 μ chez toutes les espèces du second. Les hématies de *Varanus griseus* sont plutôt petites. Gulliver (1875) a également noté cette particularité chez *Varanus niloticus*. Les deux espèces de *Cordylus* se détachent nettement des Lacertoidea et, comme les Amphisbénieniens, occupent une position moyenne. Nos résultats sont très proches de ceux de Villiers-Pienaar (1962) concernant *Cordylus vittifer*, *C. giganteus* et *C. jonesii*. Dans un des premiers travaux d'hématologie comparée des Reptiles, Gulliver (1842) a déjà noté que, chez *Anguis fragilis*, le grand diamètre de l'érythrocyte mesure plus du double du petit diamètre. Chez les Serpents, le rapport GD/PD est également très variable mais la longueur des érythrocytes est toujours comprise entre 16 et 19 μ . La seule exception est constituée par *Typhlops vermicularis* qui se situe très loin de *Typhlops punctatus*. Les 4 espèces de Tortues étudiées forment un groupe très homogène et possèdent des hématies relativement grandes, ce qui correspond aux résultats de divers auteurs ayant étudié les érythrocytes des Chéloniens. Quant aux Crocodiliens, nos résultats diffèrent quelque peu de ceux d'autres auteurs. Chez *Alligator mississippiensis*, Wintrobe (1933) trouve au contraire des dimensions fortes pour les érythrocytes (23,2 x 12,1 μ) Tandis que, d'après Reese, (1917) les érythrocytes seraient de taille moyenne chez cette espèce (18,7 x 10,85 μ). Chez *Crocodilus niloticus*, Villiers-Pienaar (1962) trouve des dimensions d'érythrocytes très voisines des nôtres et note que, chez les Crocodiles examinés de ce point de vue par d'autres auteurs, les dimensions des érythrocytes sont nettement plus grandes. Rappelons que nos exemplaires étaient jeunes, avec une assez forte proportion de normoblastes dans le sang circulant. Enfin, *Sphenodon punctatus* se différencie de tous les autres Reptiles par la très grande taille de ses érythrocytes. Seul, *Heloderma horridum* s'en rapproche quelque peu ainsi que *Heloderma suspectum* d'après les données de Ryerson (1949).

Forme et taille des noyaux des érythrocytes.

Alors que les érythrocytes présentent toujours la forme d'une ellipse régulière, simplement plus ou moins allongée, leurs noyaux ont souvent des bords irréguliers, même chez les cellules mûres. Le rapport GD/PD varie nettement plus que pour les hématies; toujours inférieur à 1,5 chez les Tortues, les Crocodiles et le Sphénodon, il est généralement compris entre 1,5 et 2 chez les Serpents, à l'exception de quelques Colubridae et Elapidae, de *Typhlops vermicularis* et de *Leptotyphlops dulcis* où il est nettement supérieur à 2. Dans l'ensemble, le rapport GD/PD des noyaux est un peu moins élevé chez les Lézards que chez les Serpents, bien qu'il soit légèrement inférieur à 1,5 chez quelques Scincoidea et chez *Cordylus cordylus*, ainsi d'ailleurs que chez *Sphenodon punctatus*.

La taille des noyaux varie plus, entre les groupes systématiques ou entre les individus d'une même espèce, que celle des érythrocytes, ce qui était prévisible puisque ce sont eux qui se modifient le plus au cours de la vie de la cellule. Les variations de taille sont très grandes au sein des Scincoidea, des Iguania et des Serpents. Les Lacertidae et Teidae forment un groupe homogène, caractérisé par de petits noyaux allongés, les Cordylidae étant nettement différents. Chez les Gekkota et les Anguioidea, les noyaux sont, dans l'ensemble, d'assez grande taille et ils sont particulièrement volumineux chez *Sphenodon punctatus*.

Rapport nucléo-cytoplasmique—Surfaces respectives des érythrocytes et des noyaux.

Le rapport nucléo-cytoplasmique qui reflète assez fidèlement le cycle fonctionnel des cellules glandulaires, varie également au cours de la vie des érythrocytes. Dans les conditions techniques de ce travail, le rapport nucléo-cytoplasmique est très généralement compris entre 0,1 et 0,2, avec un maximum très net au voisinage de 0,150; 25% des espèces ont un rapport compris entre 0,145 et 0,155. A de rares exceptions près, ce rapport est assez indépendant de la position systématique. Toutefois, un rapport très faible chez *Pelomedusa subrufa* et chez *Morelia spilotes*, dont les noyaux sont particulièrement petits et presque sphériques, est peut-être significatif. De même, le rapport nucléo-cytoplasmique faible de *Leptotyphlops dulcis* correspond sans doute à une tendance de l'espèce, sinon de la famille, car un phénomène encore plus accentué a été constaté en ce qui concerne les cellules adénohypophysaires.

La comparaison de la surface respective des érythrocytes et de leurs noyaux (fig. 13) aboutit à une représentation graphique qui rappelle étrangement celle obtenue par la comparaison du grand et du petit diamètre des hématies (fig. 10 et 11). C'est là une confirmation du fait qu'en général le rapport nucléo-cytoplasmique ne présente pas de signification taxonomique. Les exceptions notables concernent uniquement les 3 espèces déjà signalées au paragraphe précédent.

Taxonomie et dimensions des érythrocytes.

Les rapports que existent entre la morphologie des érythrocytes et la position systématique ne sont pas toujours évidents et il est hors de doute que, dans les conditions techniques de ce travail, l'ampleur des variations individuelles risque de masquer ou, ce qui est pire, de fausser certains résultats. Pourtant, diverses constatations peuvent être faites sans grand risque d'erreurs.

La première et la plus évidente est la position particulière qu'occupe *Sphenodon punctatus* parmi tous les Reptiles, en raison de la très grande taille des érythrocytes.

Inversement, les Lacertidae et les Teidae sont caractérisés par des hématies particulièrement petites, à noyaux réguliers. Il convient d'insister sur le fait que les Cordylidae se séparent totalement de ce groupe particulièrement homogène.

Les données concernant les autres Sauriens sont loin d'être aussi évidentes. Cependant, on peut distinguer, d'une part, les Lacertoidea et les Iguania, dont les érythrocytes sont d'une taille petite ou moyenne, d'autre part les Gekkota et les Anguioidea (Helodermatidae inclus) pourvus d'hématies de taille moyenne ou grande. Dans les limites de l'échantillonnage réalisé ici, il n'apparaît pas de différences significatives entre les familles des quatre grands groupes cités ci-dessus. Remarquons

toutefois la très grande taille des érythrocytes de *Heloderma horridum*, seul Reptile qui se rapproche quelque peu de ce point de vue de *Sphenodon punctatus*. En réalité, les différences entre cette espèce et *Heloderma suspectum* ne sont pas aussi grandes que celles que nous avons trouvées. Ryerson (1949) a noté des dimensions supérieures aux nôtres chez *Heloderma suspectum*: 17,7 à 22,2 x 10,8 à 13,6 μ . Signalons que notre exemplaire était très fortement parasité. En dernière analyse, la morphologie des érythrocytes rapproche davantage les Helodermatidae des Anguidae que des Varanidae dont les hématies sont petites.

Il y a peu à dire des Typhlopidae, sinon pour signaler la différence, peut-être accidentelle, qui existe entre les deux espèces étudiées ici; *Typhlops punctatus* ne diffère guère des autres Serpents, alors que *Typhlops vermicularis* s'en écarte nettement, tant par la taille des hématies que par le rapport nucléo-cytoplasmique. Au contraire, la morphologie des érythrocytes de *Leptotyphlops dulcis* semble caractéristique de l'espèce, sinon de la famille, puisque la tendance à un rapport nucléo-cytoplasmique particulièrement élevé a été constatée pour les cellules d'autres tissus (adénohypophyse).

Les autres Serpents forment un groupe assez homogène. L'étude des variations individuelles montre que, malgré l'ampleur de ces dernières, il existe des différences spécifiques significatives (Fig. 9). Toutefois, l'ampleur de ces différences est assez faible et, comme pour d'autres caractères, c'est l'homogénéité de l'ensemble Boidea-Colubroidea qui, jusqu'à plus ample informé, doit être retenue.

La taille et la forme des érythrocytes sont très semblables chez les 4 Tortues étudiées ici, mais *Pelomedusa subrufa* se différencie nettement des autres espèces par ses noyaux arrondis et un rapport nucléo-cytoplasmique exceptionnellement faible. Il est possible que ce très faible rapport caractérise les Chéloniens cryptodires. Chez *Pelomedusa galeata*, Villiers Pienaar (1962) note également l'existence de noyaux arrondis. Chez *Pseudemys scripta*, le noyau est également rond (Taylor et Kaplan, 1961) et le rapport nucléo-cytoplasmique faible (0,122, d'après les données de ces auteurs).

Il résulte de travaux portant sur la morphologie comparée de l'hypophyse des Reptiles (Saint Girons, H., 1967) que la taille des cellules adénohypophysaires, compte tenu des dimensions relatives des différentes catégories cellulaires et de leur état fonctionnel, varie de façon assez constante selon les groupes systématiques. Ces éléments sont de très grande taille chez *Sphenodon punctatus* et, à un moindre degré, chez les Anguidae, les Helodermatidae et *Tiliqua scincoides* parmi les Scincidae. Ils sont assez grands chez les autres Scincidae, les Varanidae, les Boidae et toutes les familles de Colubroidea, de taille moyenne chez les Gekkota (Gekkonidae, Pygopodidae), les Chamaeleonidae, les Tortues, les Crocodiles et les Typhlopidae, de petite taille chez les Agamidae et les Amphisbeniens, de très petite taille chez les Iguania, les Lacertoidea et les Leptotyphlopidae. Un simple coup d'oeil sur les figures 10 et 11 montre que les observations faites à propos des érythrocytes confirment, dans l'ensemble, ces constatations, particulièrement en ce qui concerne la grande taille des cellules chez *Sphenodon punctatus*, les Helodermatidae, les Anguidae et *Tiliqua scincoides*, ainsi que leur petite taille chez les Lacertidae, Teidae et Leptotyphlopidae. Il existe pourtant quelques différences, les plus saillantes étant le niveau moins élevé qu'occupent, en ce qui concerne la taille des érythrocytes, les Varanidae et, au contraire, le niveau plus élevé qui se trouve attribué aux Gekkota, aux Tortues et aux Cordylidae. Enfin, chez les Iguania, les variations de la taille des hématies entre les différentes espèces sont beaucoup plus fortes chez les représentants de cette famille.

CONCLUSIONS

On peut donc tenir pour certain que, dans une large mesure, la taille des érythrocytes, comme celle d'autres cellules du corps, est en relation avec la position systématique. Reste à savoir si l'interprétation peut être poussée plus loin et s'il est

possible d'établir une corrélation entre la taille des hématies dans une famille et le degré d'évolution de celle-ci. Encore faudrait-il s'entendre sur le sens des termes "primitifs" ou "évolués", attribués à des espèces toutes contemporaines par définition. Nous admettons que les espèces "primitives" sont restées sans modifications morphologiques importantes depuis longtemps (très longtemps même chez les Tortues et le Sphénodon), contrairement aux espèces "évoluées" dont l'évolution s'est poursuivie plus ou moins régulièrement et qui, en règle générale, sont ou ont été récemment en expansion. Le premier adjectif est attribué unanimement aux Rhynchocephales, aux Tortues et, à un moindre degré, aux Crocodiles et aux Anguimorpha. La position des Gekkota et des Boidae est encore discutée. Au contraire, les Iguania, les Lacertoidea et les Colubroidea sont considérés comme des groupes "évolués". On admet généralement que les Amphisbénien, les Typhlopidae et les Leptotyphlopidae présentent un mélange de caractères primitifs et très spécialisés. Une longue discussion sur l'évolution des Reptiles sortirait du cadre de cet article et serait hors de proportion avec l'apport des données originales. Notons seulement que, si l'on considère la grande taille des érythrocytes comme un caractère primitif, les Anguimorpha et les Gekkota seraient les Squamates les plus "primitifs", ou tout au moins ceux dont l'évolution a été la moins rapide. Inversement, les Lacertidae et les Teidae représenteraient les Sauriens les plus "évolués" et, effectivement, ces deux familles font preuve d'un grand dynamisme évolutif. Toutefois, la grande variation de taille des érythrocytes que l'on constate chez les Iguanidae, les Agamidae, les Scincidae et peut-être les Typhlopidae est difficilement interprétable dans cette optique. Il ne semble pas, non plus, que ces différences puissent être rapportées au genre de vie ni — dans la très faible mesure où l'on possède des informations en la matière — à une particularité physiologique quelconque.

RESUME

Les érythrocytes de 76 espèces de Reptiles appartenant à 29 familles des 5 ordres de Reptiles ont été mesurés. La taille des érythrocytes, comme celle des autres cellules du corps probablement, est en relation avec la position systématique de l'espèce considérée et probablement son degré d'évolution. La surface peut varier du simple au triple. Les plus grands érythrocytes sont ceux de *Sphenodon punctatus* (grand diamètre = 23,3 μ). Les Chelonia, les Crocodilia et les Serpents forment des groupes homogènes. Il n'en est pas de même des Lézards. On trouve les plus petits érythrocytes chez les Lacertidae et les Teidae (G.D. = 13,8 μ chez *Psammotromus algirus*). La taille des érythrocytes des Anguidae (G.D. = 18,4 μ chez *Anguis fragilis*) et surtout des Helodermatidae (G.D. = 21,4 μ chez *Heloderma horridum*) se rapproche de celle observée chez *Sphenodon punctatus*. Les érythrocytes sont plutôt petits chez les Varanidae, de taille très variable chez les Iguanidae et le Scincidae. Les noyaux sont fusiformes sauf chez les Tortues. *Pelomedusa subrufa*, en particulier à de petits noyaux presque sphériques. En dehors de ce dernier cas, le rapport nucléocytoplasmique ne varie pas de façon significative entre les différentes familles.

SUMMARY

The erythrocytes of 76 species of Reptiles belonging to 29 families of the 5 orders of Reptiles have been measured. Size of the erythrocytes, probably like that of the other cells of the body, bears a relationship to the systematic position of these species and probably to its degree of evolution. The surface area can vary in proportions of 1 to 3, the largest erythrocytes are those of *Sphenodon punctatus* (largest diameter = 23,30 μ). The Chelonia, Crocodilia and Serpents constitute homogeneous groups. This is not the case with Lizards. The smallest erythrocytes are found in Lacertidae and in Teidae (L.D. = 13,80 μ in *Psammotromus algirus*). The size of erythrocytes of the Anguidae (L.D. = 18,40 μ in *Anguis fragilis*) and particularly of the Helodermatidae (L.D. = 21,40 μ in *Heloderma horridum*) is close to that of *Sphenodon punctatus*. The erythrocytes are rather small in the Varanidae, and of very variable size in the Iguanidae and Scincidae. The nuclei are spindle shaped in

all except Chelonia. In particular those of *Pelomedusa subrufa* have small almost round nuclei. With the exception of the latter the nucleo-cytoplasmic ratio does not vary significantly in the different families.

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TABLEAU I—LISTE DES ESPECES ETUDIEES ET DONNEES NUMERIQUES DES ERYTHROCYTES

	Erythrocytes				Noyaux				Rapport N/C
	GD	PD	GD/ PD	S	GD	PD	GD/ PD	S	
	(μ)	(μ)	(μ^2)	(μ)	(μ)	(μ^2)	(μ)	(μ^2)	
CHELONIENS									
TESTUDINIDAE									
<i>Testudo graeca</i> L.	18,5	10,6	1,75	153,8	6,1	4,3	1,42	20,6	0,134
EMYDIDAE									
<i>Clemmys leprosa</i> Schweigger	19,0	10,9	1,74	162,5	6,5	4,8	1,35	24,5	0,151
<i>Emys orbicularis</i> L.	19,9	11,7	1,70	182,8	6,0	4,5	1,33	21,2	0,116
PELOMEDUSIDAE									
<i>Pelomedusa subrufa</i> Lacépède	19,0	10,2	1,86	150,0	5,1	4,3	1,19	12,1	0,079
RHYNCHOCEPHALES									
<i>Sphenodon punctatus</i> Gray	23,3	13,9	1,67	252,0	8,3	5,7	1,46	37,0	0,147
SAURIENS									
GEMMIDAE									
<i>Coleonyx variegatus</i> Baird	18,9	9,6	1,97	142,5	7,3	3,7	1,97	21,5	0,151
<i>Gehyra variegatus</i> (Duméril et Bibron)	17,2	11,5	1,49	139,5	6,3	3,8	1,66	18,9	0,135
<i>Heteronota binoei</i> Gray	21,4	10,7	2,00	179,9	8,1	3,4	2,38	21,6	0,120
PYGOPODIDAE									
<i>Delma fraseri</i> (Gray)	17,0	9,7	1,75	129,8	6,7	3,1	2,16	18,9	0,146
<i>Lialis burtonis</i> Gray	19,9	12,5	1,59	193,5	7,7	4,3	1,79	25,9	0,134
IGUANIDAE									
<i>Anolis carolinensis</i> Voigt	15,3	8,9	1,72	106,9	6,1	3,6	1,69	17,2	0,161
<i>Crotaphytus collaris</i> Say	17,8	8,6	2,07	120,0	7,2	3,6	2,00	20,7	0,172
<i>Dipsosaurus dorsalis</i> Baird et Girard	18,0	10,3	1,75	145,8	8,2	3,8	2,16	24,5	0,168
<i>Iguana iguana</i> (L.)	15,3	7,8	1,97	93,8	5,1	3,6	1,42	15,2	0,160
<i>Phrynosoma m'Calli</i> Hallowell	18,9	10,1	1,87	149,8	7,1	3,6	1,97	20,1	0,134
<i>Uma inornata</i> Cope	17,8	9,2	1,93	128,5	7,2	3,2	2,25	18,6	0,145
<i>Uta graciosa</i> Hallowell	14,7	9,0	1,74	103,6	6,0	3,2	1,87	15,7	0,151
AGAMIDAE									
<i>Agama bibroni</i> Duméril	14,9	8,2	1,82	96,1	5,9	3,1	1,90	14,3	0,148
<i>Amphibolurus reticulatus</i> Gray	14,7	7,9	1,86	92,4	6,3	2,6	1,86	12,8	0,138
<i>Diporiphora bilineata</i> Gray	17,6	9,0	1,96	124,6	6,5	3,6	1,81	18,4	0,148
CHAMAELONIDAE									
<i>Chamaeleo basiliscus</i> Cope	17,6	7,8	2,26	107,8	6,8	3,6	1,89	19,3	0,178
LACERTIDAE									
<i>Xanthodactylus erythrus</i> Gray	13,6	8,6	1,58	91,9	6,2	2,5	2,48	12,2	0,133
<i>Lacerta agilis</i> L.	14,0	8,5	1,65	93,6	5,8	2,8	2,07	12,8	0,137
<i>Lacerta muralis</i> (Laurenti)	13,8	8,0	1,72	86,9	5,4	3,0	1,80	16,2	0,186
<i>Lacerta viridis</i> (Laurenti)	15,5	8,3	1,86	125,0	6,0	3,1	1,94	14,6	0,117
<i>Lacerta vivipara</i> Jacquin	14,1	8,7	1,62	96,3	5,6	3,2	1,75	14,1	0,146

	Erythrocytes				Noyaux				Rapport N/C
	GD	PD	GD/S	S	GD	PD	GD/S	S	
	(μ)	(μ)	(μ) ²	(μ) ²	(μ)	(μ)	(μ) ²	(μ) ²	
<i>Psammotromus algirus</i> (L.)	13,8	7,8	1,77	84,3	5,4	2,8	1,93	11,9	0,141
<i>Cnemidophorus tigris</i> Baird et Girard	15,8	8,3	1,90	102,8	5,9	2,4	2,46	11,1	0,108
CORDYLIDAE									
<i>Cordylus cordylus</i> (L.)	17,3	9,8	1,77	133,4	6,3	4,5	1,40	22,2	0,166
<i>Cordylus vittifer</i> (Reichenow)	17,2	9,5	1,81	128,0	7,1	4,6	1,54	25,6	0,200
SCINCIDAE									
<i>Egernia</i> sp.	16,3	10,4	1,57	129,9	6,1	4,2	1,45	20,2	0,155
<i>Chalcides mionecton</i> Boettger	14,4	8,1	1,77	91,6	5,7	3,8	1,50	17,2	0,177
<i>Eumeces algeriensis</i> Peters	17,8	11,1	1,61	154,8	8,4	4,0	2,10	26,4	0,170
<i>Lygosoma (Sphaenomorphus)</i> sp.	14,6	8,2	1,78	89,7	5,7	3,1	1,84	13,4	0,149
<i>Lygosoma taeniolata</i> Shaw	14,3	9,4	1,52	108,7	5,4	2,8	1,90	11,9	0,109
<i>Scincus officinalis</i> (Shaw)	15,3	7,4	2,07	89,0	6,6	2,7	2,44	19,1	0,215
<i>Tiliqua scincoides</i> (Shaw)	17,3	9,6	1,80	130,7	7,5	4,3	1,74	25,2	0,193
FEYLINIDAE									
<i>Feylinia currori</i> Gray	14,8	8,1	1,83	94,0	5,8	4,1	1,41	18,7	0,199
ANGUIDAE									
<i>Anguis fragilis</i> L.	18,4	9,8	1,88	143,9	6,8	4,2	1,62	22,5	0,149
<i>Gerrhonotus multicarinatus</i> Blainville	18,8	9,1	2,07	134,5	7,7	3,4	2,26	20,5	0,152
<i>Ophisaurus koellikeri</i> Guenther	17,0	8,7	1,95	121,3	6,3	3,9	1,62	17,8	0,147
ANNIELLIDAE									
<i>Anniella pulchra</i> Gray	19,1	9,1	2,10	136,5	6,4	3,6	1,78	18,1	0,133
HELODERMATIDAE									
<i>Heloderma horridum</i> Wiegmann	21,4	13,5	1,58	227,0	7,9	4,6	1,72	28,5	0,125
<i>Heloderma suspectum</i> Cope	17,3	10,0	1,73	134,2	6,6	4,5	1,47	27,4	0,165
VARANIDAE									
<i>Varanus griseus</i> Daudin	15,6	8,9	1,75	109,2	6,2	3,6	1,72	17,5	0,160
TEIDAE									
<i>Ameiva ameiva</i> (L.)	13,8	7,6	1,82	82,3	5,8	2,7	2,15	12,3	0,149
AMPHISBAENIENS									
AMPHISBAENIDAE									
<i>Blanus cinereus</i> (Vandelli)	17,6	9,5	1,85	131,5	6,6	3,4	1,94	17,6	0,134
TROGONOPHIDAE									
<i>Trogonophis wiegmanni</i> Kaup	16,6	9,5	1,75	123,8	6,7	4,0	1,67	21,1	0,170
OPHIDIENS									
TYPHLOPIDAE									
<i>Typhlops punctatus</i> Leach	17,0	10,1	1,68	134,9	6,6	3,0	2,20	15,6	0,116
<i>Typhlops vermicularis</i> Merrem	15,2	8,3	1,84	98,5	7,2	3,8	1,89	21,5	0,218
LEPTOTYPHLOPIDAE									
<i>Leptotyphlops dulcis</i> Baird et Girard	16,6	8,4	1,97	109,5	8,1	3,1	2,61	19,7	0,179
BOIDAE									
<i>Eryx jaculus</i> L.	16,1	9,5	1,61	120,0	7,0	3,4	2,06	18,7	0,156
<i>Lichanura roseafusca</i> Cope	17,1	9,6	1,78	129,0	6,1	4,0	1,52	19,2	0,149
<i>Morelia spilotes</i> (Lacépède)	18,0	10,2	1,76	144,1	4,9	3,4	1,44	13,1	0,091
COLUBRIDAE									
<i>Coronella austriaca</i> Laurenti	17,5	9,7	1,80	140,0	7,3	3,6	2,03	20,6	0,147
<i>Coluber viridiflavus</i> Lacépède	16,1	9,9	1,63	124,8	8,2	3,3	2,48	21,2	0,169
<i>Elaphe longissima</i> (Laurenti)	18,3	10,6	1,73	152,1	7,1	4,1	1,73	22,7	0,149
<i>Elaphe scalaris</i> (Schinz)	18,8	8,7	2,16	115,8	6,8	4,2	1,57	22,4	0,192
<i>Lycodryas</i> sp.	16,6	9,0	1,84	117,5	5,9	3,5	1,69	16,2	0,138
<i>Macroprotodon cucullatus</i> Geoffroy	16,4	8,7	1,88	112,4	6,0	3,7	1,73	17,5	0,156

	Erythrocytes				Noyaux				Rapport N/C
	GD	PD	GD/S	S	GD	PD	GD/S	S	
	(μ)	(μ)	(μ) ²	(μ) ²	(μ)	(μ)	(μ) ²	(μ) ²	
<i>Natrix maura</i> (L.)	18,1	10,6	1,71	150,4	6,8	4,2	1,62	22,4	0,149
<i>Natrix natrix</i> (L.)	17,3	10,1	1,71	137,5	7,3	3,3	2,21	18,9	0,130
ELAPIDAE									
<i>Bungarus fasciatus</i> Schneider	17,9	9,5	1,88	133,4	6,9	3,2	2,16	17,2	0,129
<i>Denisonia suta</i> (Peters)	16,8	10,4	1,62	137,3	7,4	3,9	1,62	22,7	0,165
<i>Naja naja</i> (L.)	16,2	9,0	1,80	114,5	6,6	4,1	1,61	21,2	0,185
<i>Oxyuranus scutellatus</i> (Peters)	16,6	9,1	1,82	118,7	6,5	3,7	1,76	18,9	0,159
<i>Pseudechis australis</i> (Gray)	18,4	11,2	1,64	162,0	6,4	3,4	1,88	17,8	0,110
<i>Pseudechis porphyriacus</i> (Shaw)	17,0	11,0	1,55	146,8	7,5	3,8	1,97	22,3	0,152
HYDROPHIDAE									
<i>Laticauda colubrina</i> (Schneider)	19,2	11,2	1,71	169,8	7,7	3,4	2,28	20,6	0,121
VIPERIDAE									
<i>Atractaspis</i> sp.	18,5	10,5	1,76	152,5	8,2	3,9	2,10	25,1	0,165
<i>Cerastes cerastes</i> L.	18,2	9,4	1,94	134,1	6,4	4,0	1,59	20,2	0,151
<i>Vipera aspis</i> (L.)	17,3	11,2	1,52	154,5	7,5	4,0	1,87	23,6	0,153
<i>Vipera berus</i> (L.)	16,1	10,5	1,53	132,8	7,3	4,2	1,74	24,1	0,181
CROTALIDAE									
<i>Crotalus viridis</i> Rafinesque	17,9	11,1	1,61	164,6	6,9	4,3	1,60	23,4	0,141
CROCODILIENS									
CROCODILIDAE									
<i>Caiman crocodylus</i> Cuvier	16,9	9,9	1,71	131,5	5,7	4,2	1,36	18,8	0,135
<i>Crocodylus niloticus</i> Laurenti	16,4	9,0	1,82	115,9	5,0	3,8	1,32	14,9	0,129

TABLEAU II—Valeurs moyennes (en μ) du grand diamètre des érythrocytes et de leurs noyaux chez les mâles et les femelles de quelques espèces de Reptiles. On a calculé la valeur moyenne de 100 globules chez un nombre de spécimens égal au chiffre entre parenthèses.

	Erythrocytes		Noyaux	
	mâles	femelles	mâles	femelles
<i>Sphenodon punctatus</i>	22,88 (1)	23,65 (1)	8,41	8,29
<i>Lacerta muralis</i>	13,75 (4)	13,90 (6)	5,59	5,40
<i>Anguis fragilis</i>	18,49 (4)	18,29 (6)	6,81	6,74
<i>Coluber viridiflavus</i>	16,30 (5)	15,89 (4)	8,45	7,97
<i>Vipera aspis</i>	17,35 (5)	17,41 (5)	7,52	7,27
<i>Testudo graeca</i>	18,33 (3)	18,62 (5)	6,08	6,08

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LÉGENDE DES FIGURES

- Fig. 1 à 8. Aspect des érythrocytes chez différentes espèces de Reptiles. May-Grünwald-Giemsa, écran vert, grossissement = 950 diamètres.
- Fig. 1. *Sphenodon punctatus* (Sphenodontidae).
- Fig. 2. *Coleonyx variegatus* (Gekkonidae).
- Fig. 3. *Amphibolurus reticulatus* (Agamidae).
- Fig. 4. *Acanthodactylus erythrurus* (Lacertidae).
- Fig. 5. *Gerrhonotus multicarinatus* (Anguidae).
- Fig. 6. *Heloderma horridum* (Hélodermatidae).
- Fig. 7. *Varanus griseus* (Varanidae).

- Fig. 8. *Crotalus viridis* (Crotalidae).
- Fig. 9. Variations individuelles de la taille des hématies chez *Lacerta muralis* (Lacertidae), *Anguis fragilis* (Anguidae), *Natrix maura* (Colubridae) et *Vipera berus* (Viperidae). Chaque signe correspond à la moyenne des résultats obtenus chez un individu.
- Fig. 10. Taille des érythrocytes chez le Sphénodon, les Sauriens et les Amphibéniens. Les traits entourent la surface occupée par les différentes espèces appartenant au groupe systématique considéré. V = *Varanus griseus*. C = *Cordylus*.
- Fig. 11. Taille des érythrocytes chez les Ophidiens, les Chéloniens et les Crocodiliens. Même légende que Fig. 10. Lc = *Laticauda colubrina*. Tp. = *Typhlops punctatus*. Tv = *Typhlops vermicularis*. Ld. = *Leptotyphlops dulcis*.
- Fig. 12. Taille des noyaux des érythrocytes chez différents Reptiles. Même légende que Fig. 10. P = *Pelomedusa subrufa*. V = *Varanus griseus*.
- Fig. 13. Surface comparée des érythrocytes et de leurs noyaux chez différents Reptiles. Même légende que Fig. 10. A = Amphibéniens. V = *Varanus griseus*.

..... Sphenodon	----- Amphibéniens
----- Gekkota	----- Boïdae
----- Iguania	----- Colubridae - Elapidae
----- Lacertidae - Teïdae	----- Viperidae
----- Cordylus	----- Chéloniens
----- Scincoïdea	----- Crocodiliens
----- Anguioïdea	

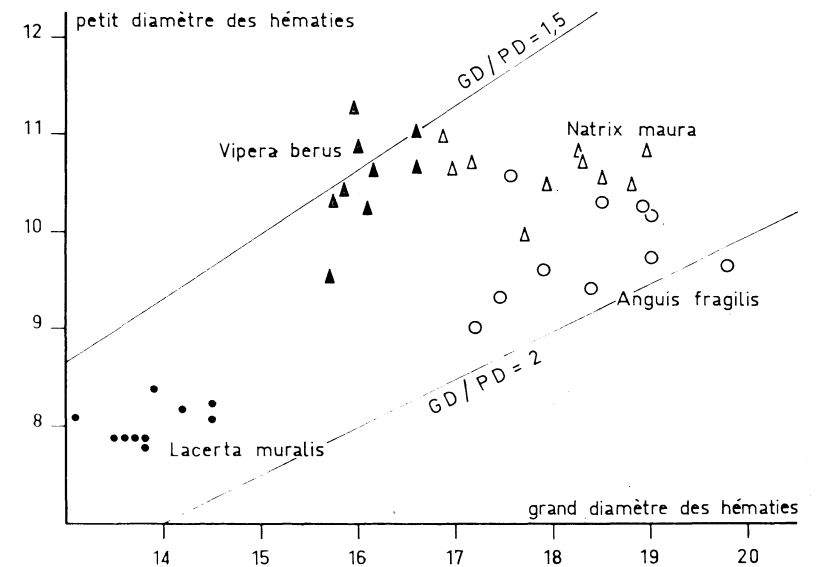


Fig. 9

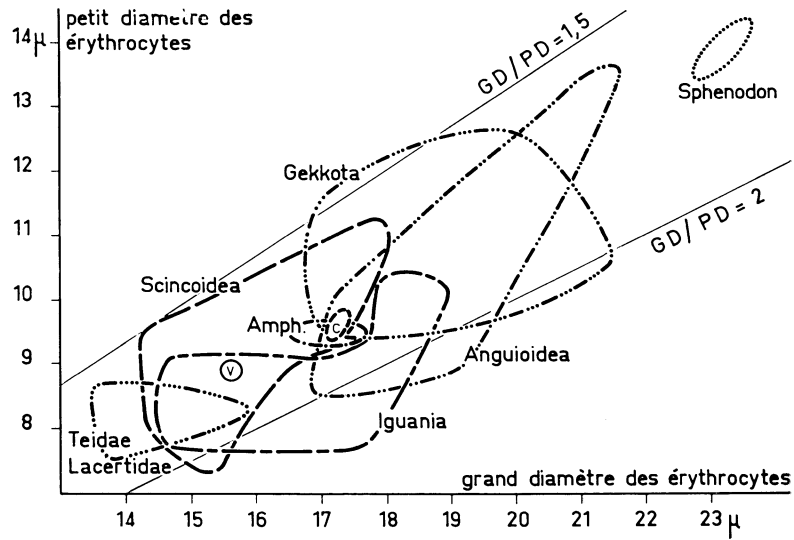


Fig. 10

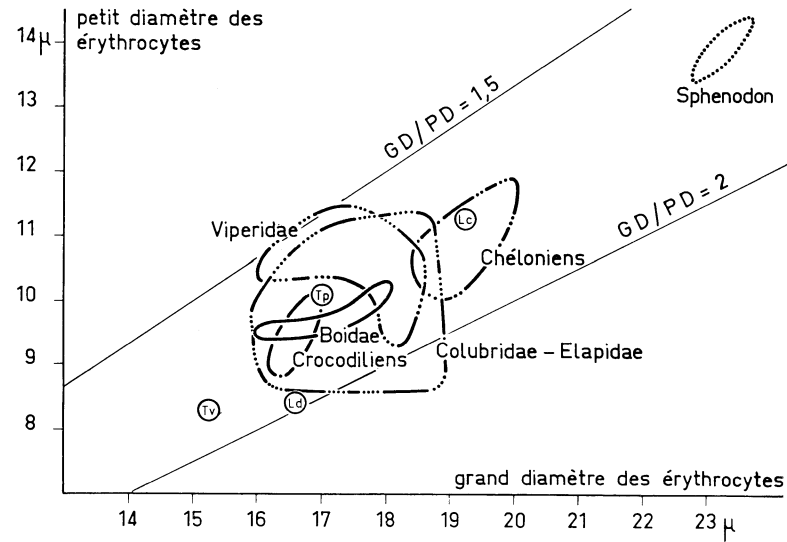


Fig. 11

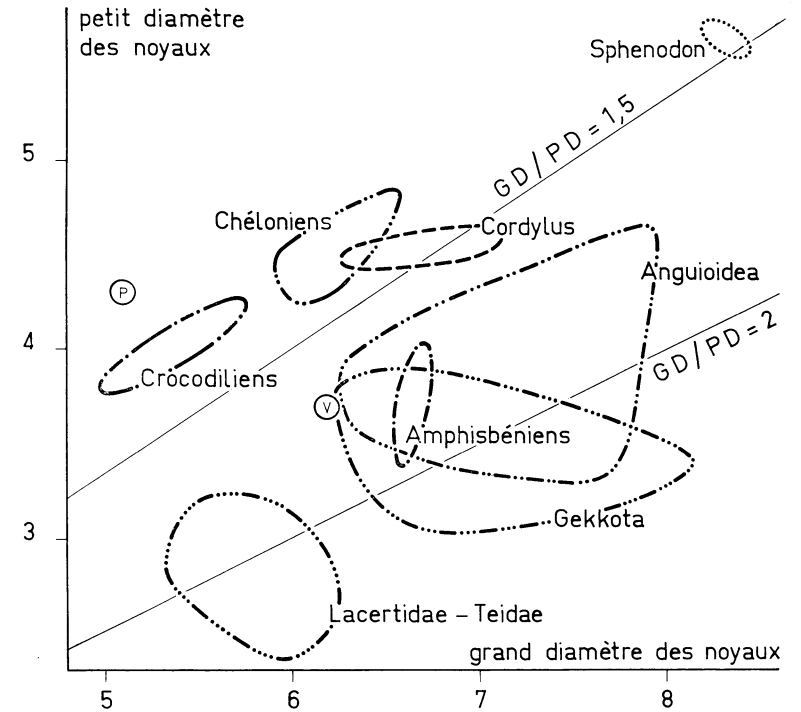


Fig. 12

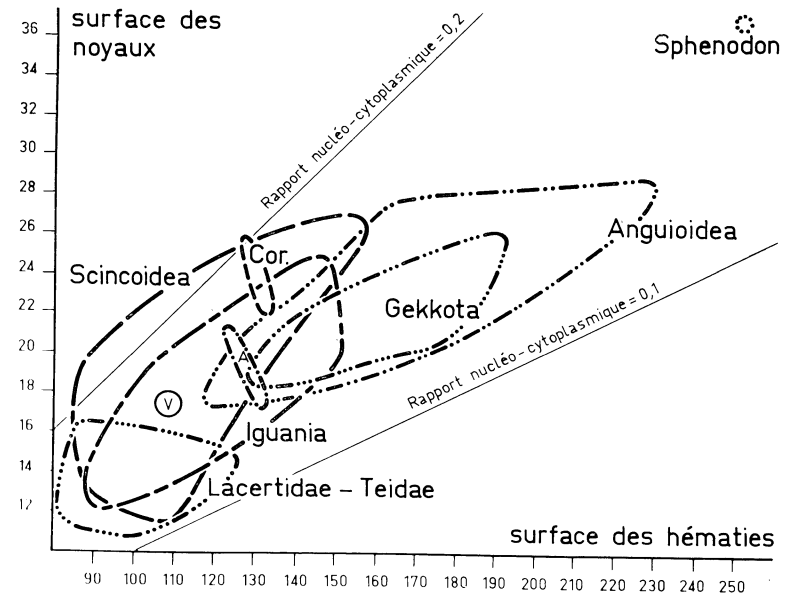


Fig. 13

AMPHIBIAN METAMORPHOSIS

By

S. C. TURNER

Department of Biological Sciences, College of Technology, Portsmouth

(Received 10/8/68)

Most adult anurans are adapted terrestrially while their aquatic larvae retain features of fish ancestry. The transition from the larval to the adult form, or metamorphosis, in anurans involves most of the organ systems with concomitant changes in physiological and biochemical processes. Some structures—the tail, gills and pronephros—regress, while others, such as the limbs and mesonephros, undergo growth and development.

Experiments by Gudernatsch (1912) and Allen (1918) first indicated that such changes depended upon the presence of an iodine containing hormone from the thyroid gland. The former worker induced precocious metamorphosis in anuran tadpoles by feeding them on powdered sheep thyroid. Other tissues used failed to accelerate metamorphosis.

Fresh thyroid tissue provides the protein thyroglobin from which can be separated the active iodine compounds tri-iodothyronine and thyroxine, found throughout the vertebrates (Pitt-Rivers and Tata, 1959). The action of tri-iodothyronine is three to five times that of thyroxine (Wilkins, 1960).

The changes required to convert an aquatic anuran larva into a terrestrial adult begin as the thyroid gland starts to differentiate. The early developed solid strand of cells which constitutes the thyroid rudiment soon forms vesicles filled with colloid and stores thyroglobin. This process occurs in *Xenopus laevis* at stage 47 (Nieuwkoop and Faber; Fox and Turner, 1967) and at stage 41 in *R. temporaria* (Cambar and Marrot, 1954; Fox, 1966).

Different tissues react to the thyroid hormone in different ways. This is well illustrated by the structural and physiological changes involved when the mesonephros replaces the pronephros as the organ of excretion and osmoregulation (Fox, 1962, 1963, 1965). Just before the metamorphic climax the pronephros begins to degenerate and by the end of the climax it has disappeared. Simultaneously the mesonephros increases in size. In parallel with these changes an elaborate endocrinal system differentiates, including the production of the hormones oxytocin and arginine vasopressin of the pars nervosa, which act on the skin, mesonephros, and urinary bladder to facilitate water conservation (Gorbman, 1964). The venous system meanwhile undergoes an extensive reorganisation. Much of the posterior cardinal venous system which drains the pronephros, regresses and the interior vena cava, which serves the mesonephros, develops and grows (Kellicott, 1912). Simultaneously there is a change in nitrogen metabolism; the liver cells begin to synthesise Krebs's ornithine-urea cycle enzymes which convert ammonia (the nitrogenous excretory product of the larva), to urea. Excretion of urea and water conservation are the more important functions of the mesonephros. It is of interest that in the permanently aquatic *Xenopus* the main excretory product is still ammonia (Frieden, 1961). None of these changes occur in thyroidectomised animals (see Fox, 1967).

Other important changes in anuran metamorphosis include tail degeneration and modification of the alimentary canal. Tail degeneration begins with a reduction of the fin, followed by atrophy of muscles and notochord (Shaffer, 1963). Degeneration of tail muscle initially is manifested by a loss of striation and breakdown of sarcoplasm and mitochondria, probably occurring by some little understood autolytic process. There is a sharp increase in the number of macrophages which can arise from the connective tissue of the tail (Weber, 1957); these are phagocytically involved in the process of degeneration.

Changes in the alimentary canal involve profound changes in the pharynx: gill clefts close and branchial cartilagenous arches and their associated blood vessels disappear. The coiled intestine shortens considerably. Moreover the larval stomach is modified into an adult organ capable of peptic digestion (Reeder, 1964).

Etkin (1964) has described the metamorphic morphological sequence of events for *Rana pipiens* at about 20°C. which may be divided into three phases—

- (a) premetamorphosis (lasting about 40 days) up to the formation of the hind limb bud; not dependent upon thyroid hormone.
- (b) prometamorphosis (lasting about 20 days) during which time the most obvious external change comprises growth and development of the hind limb; the comparable phase for *X. laevis* takes about 39 days at 18°C. (Fox and Turner, 1967). The end of this period is marked by the appearance of a clear area of epidermis through which the fore-limb eventually emerges.
- (c) metamorphic climax which lasts about one week, is a period of drastic anatomical change. It includes among other things further development and extrusion of the fore-limbs, regression of the tail, pronephros, and parts of the pharyngeal system of cartilage and vessels, and modification of the alimentary canal.

Normal metamorphosis in thyroidectomised animals is only possible if exogenous thyroxine is supplied in carefully regulated doses (Kollros, 1961). The circulatory thyroxine concentration normally rises throughout prometamorphosis until at the time of climax its level rises abruptly and remains high until tail regression is complete. At termination of metamorphosis the level is markedly reduced (Etkin, 1964).

Histological (D'Angelo and Charipper, 1939) and chemical (Kaye, 1961) studies confirm that there is an increase in the activity of the thyroid gland up to the time of metamorphic climax, at which time there is a discharge of colloid. In *R. temporaria* the thyroid gland enlarges by cellular proliferation and vesicular expansion reaching a maximum size just before the beginning of climax (Fox, 1966).

It has long been known that the thyroid is controlled by the pituitary gland (Etkin, 1964). Removal of the anterior lobe (pars anterior) of the latter (hypophysectomy) in the frog tadpole retards growth of the thyroid and prevents the accumulation of colloid within it. If the operation is performed early enough a thyroid gland does not develop at all (Adler, 1914; Allen, 1932).

The pituitary influences the thyroid via the circulation by producing thyrotropin, a thyroid stimulating hormone (T S H), from special thyrotroph cells in the anterior lobe (Etkin, 1964). These thyrotroph cells are distinguished by the possession of granules which stain with Alcian Blue and P A S (Cordier, 1953; Kerr, 1966). During prometamorphosis thyrotrophs of *Xenopus* larvae increase in nuclear size and accumulate granules in their cytoplasm (Figs. 1, 2).

I^{131} , when fed to these larvae, is incorporated in quantity by the thyroid gland. At climax the granules of the thyrotrophs are discharged into the circulation, and the thyroid vesicles collapse to release colloid with incorporated I^{131} to raise the level of circulatory protein bound I^{131} (Saxen, et al., 1957).

It is now generally believed that the relationship between the thyroid and pituitary is a feed-back mechanism (Etkin, 1964). This is well illustrated when the level of circulatory thyroxine is lowered by the use of antithyroid compounds such as phenylthiourea (PTU) or thiourea (TU). During the time anuran larvae are immersed in P T U synthesis of thyroxine is inhibited. Under these circumstances the thyroid often grows to an enormous size and becomes goitrous. Conversely when the level of circulatory thyroxine is artificially raised by immersing the tadpole in a dilute solution of thyroxine the rate of metamorphosis is increased but growth of the thyroid ceases (Fox and Turner, 1967) (Table 1, Figs. 3, 4, 5).

TABLE 1

Measurements of the volume of the thyroid of *Xenopus* larvae
In controls and experimental specimens

(Compiled from results of Fox and Turner (1967) including new data)

Treatment	Stage	No. of specimens	Mean and standard error of thyroid gland (mm ³ x 10 ⁻³)
Controls in Tapwater	57	8	97.87 ± 3.96
Specimens in 0.0075% phenylthiourea (28 days)	57	6	1631.4 ± 296.86
Control in tapwater Immersed in thyroxine	58	2	86.70
Specimens in (1 : 10 ⁷ thyroxine) at stage 47, until stage 58	58	2	0.472

Cytological studies of TSH cells in goitrogenically and in surgically thyroidectomised animals support the view that a reduced level of thyroxine elicits the release of thyrotrophin. The amount of stainable material in the cytoplasm of TSH cells is reduced in fish grown in solutions of thiourea (Sage, 1955). Degranulation of thyrotrophs is also caused by surgical thyroidectomy in *Xenopus* larvae (Kerr, 1966).

The sequence and spacing of metamorphic events is strongly influenced by changes in the level of circulatory thyroxine. According to the stoichiometric view (Etkin, 1964) each tissue involved in metamorphosis reacts when it has accumulated a specific quantity of thyroxine; the hind limbs are sensitive to a comparatively low amount in contrast to the tail which requires a greater total quantity. This situation is attained slowly when thyroidectomised tadpoles are immersed in very low concentrations of thyroxine (2 µg/litre), or more quickly when immersed in higher concentrations.

According to the threshold view however, thyroidectomised tadpoles, immersed in thyroxine solution and brought to a particular point in metamorphosis, remain unchanged in form however long they are immersed unless the concentration of thyroxine is increased (Kollros, 1961). Hypophysectomised tadpoles of *Rena pipiens* immersed in 0.001 µg/litre of thyroxine failed to develop beyond stage V. However, similar tadpoles in thyroxine concentrations of 0.002 µg/litre developed from stage V to IX (for description of stages see Kollros and Taylor, 1946; Kollros, 1961). This concentration would appear to be a true threshold.

At stage IX the hind-limb has three separate toes, an increase in thyroxine concentration to 0.008 µg/litre is required before development advances to the five toed stage (Kollros, 1961). According to the threshold theory each of the morphological (and probably physiological and biochemical) changes of metamorphosis reacts to an individual threshold concentration of hormone.

Nevertheless in stoichiometric or threshold action the increase in level of circulatory thyroxine can only occur if the sensitivity of the thyrotrophs to feed back inhibition undergoes a reduction which must be small during prometamorphosis and large during climax. The large climactic change in sensitivity is dependent on an influence from the brain; if the rudiment of the hypophysis, before it has developed its connection with the brain, is autotransplanted to the tail, a late and slower metamorphosis proceeds only to the end of prometamorphosis. The transplanted cells are otherwise functioning as evidenced by the fact that growth under the influence of the anterior lobe still takes place (Etkin, 1938; Etkin and Lehrer, 1960).

In adult tetrapods the link between the hypothalamus and hypophysis consists of a neurosecretory system defined as the hypothalamo-hypophyseal tract. This tract is made up of modified neurons, the cell bodies of which secrete neurohormones (=neurosecretion) which can be differentially stained by procedures such as modified Gomori's stain and Paget's aldehydethionin technique (Bargmann, 1960; Wingstrand, 1959).

In adult amphibia the tract begins in front of the optic chiasma as a group of cell bodies forming the preoptic nucleus. The axons arising from these cell bodies lead to the pars nervosa or to the median eminence which abuts against the pars anterior. Those neurons, which terminate in the median eminence, discharge their secretion into a system of short blood vessels (hypothalamo-hypophyseal portal system) which enter the pars anterior (Green and Maxwell, 1959). Neurosecretion reaching the pars anterior by this pathway controls the production of adrenocorticotrophic hormone in toads (Jorgensen and Larsen, 1960). Thyrotrophin may also be under the control of a neurosecretion acting as a thyrotrophin releasing factor (TRF).

Metamorphic climax in larvae of *R. temporaria*, *R. ridibunda*, and *R. chensinensis* is dependent upon the preoptic nucleus; tadpoles in which this hypothalamic centre is removed fail to metamorphose (Ivanova, 1948, 1952). During metamorphosis of *Bufo viridis*, *R. esculenta*, *Bombina bombina*, and *Pelobates fuscus*, the number of neurons in the preoptic nucleus which stain for neurosecretory granules increases as climax approaches. At the same time the capillary network of the median eminence develops. Furthermore, the amount of neurosecretion is related to the duration of metamorphic climax; *P. fuscus* for example which has a climax of the greatest duration has the least neurosecretion (Voitkevich, 1962).

Experimental evidence confirms that the capillaries of the median eminence are an essential part of the neurosecretory pathway. If a physical barrier is placed between the pituitary and hypothalamus, climax is only completed in those larvae in which the portal vessels regenerate and grow round the barrier (Etkin and Sussman, 1961). It has been concluded from this experimental work that hypothalamic control is necessary for climax but not for prometamorphosis, although the latter proceeds more slowly when the hypophysis is separated from the hypothalamus (Etkin, 1964).

Etkin (1964, 1966) has suggested that the facts of hypothalamic neurosecretory and endocrinal control of metamorphosis may be linked in the following way:—

During premetamorphosis the thyrotrophs are extremely sensitive to thyroxine feed-back; that is their activity is inhibited by a low threshold of thyroxine, and the level of circulatory thyroxine is very low.

It is known that the thyro-pituitary feed-back mechanism is established early in larval development (Fox and Turner, 1967), probably during premetamorphosis because at this stage goitrogens cause hyperplasia of the thyroid gland (Gordon et al., 1945).

Growth and differentiation of the median eminence begin at the onset of prometamorphosis and are dependent upon a functional thyroid gland; the median eminence fails to develop in thyroidectomised tadpoles unless they are given exogenous thyroxine (Etkin, 1963).

The neurosecretion (TRF, or thyroid releasing factor) from the hypothalamus desensitises the thyrotrophs to thyroxine feed-back and stimulates their further differentiation and production of TSH. The level of circulatory thyroxine therefore rises, and in a positive feed-back stimulates the hypothalamus to further development especially the median eminence. Thus a self accelerating system progresses with thyroxine acting on the hypothalamus, which further stimulates the pituitary to influence the thyroid and resulting thyroid hormone production. At the end of climax the hypothalamus loses its sensitivity to thyroxine and TRF is inhibited; the amount of TSH is thence reduced and thyroid secretion falls to a low level again.

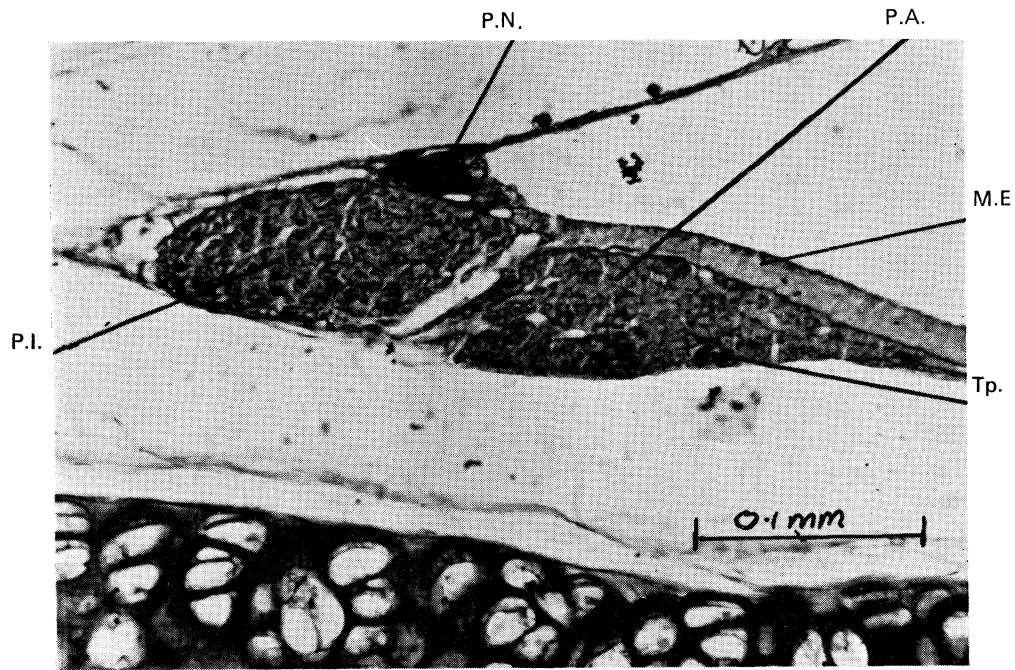


Fig. 1

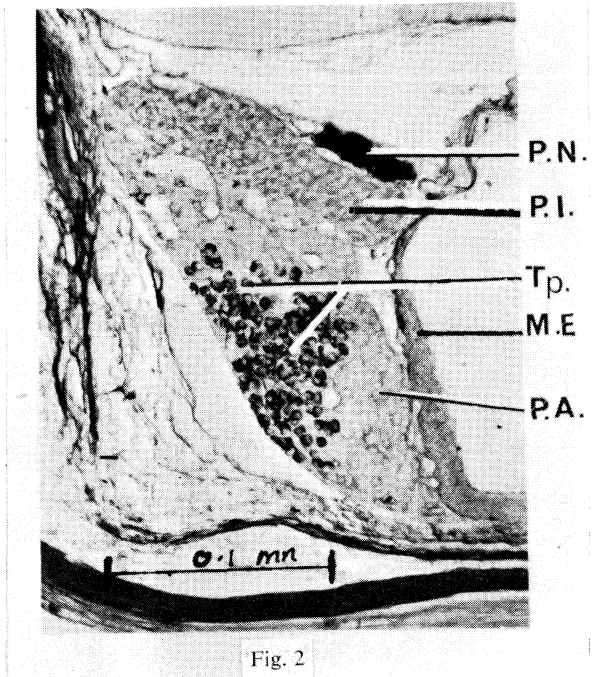


Fig. 2

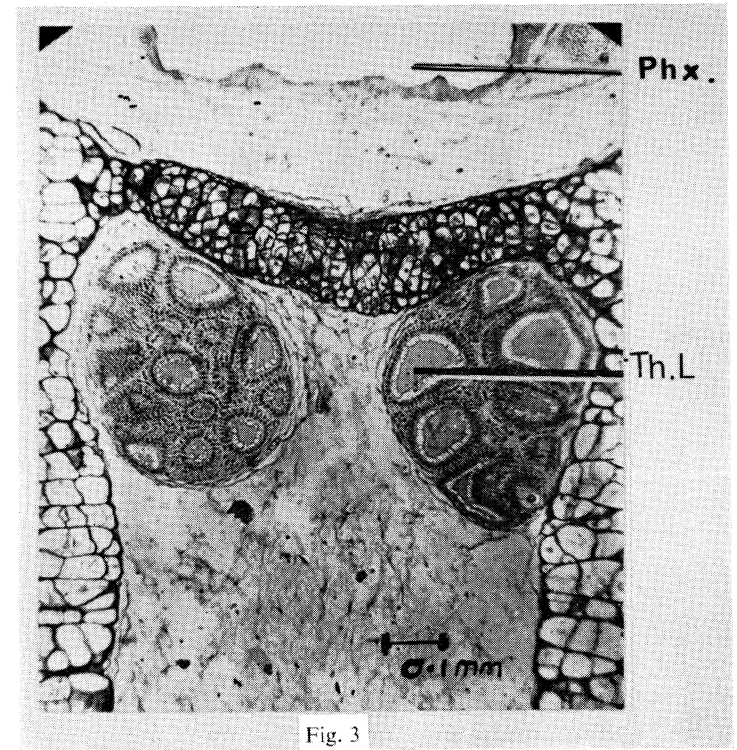


Fig. 3

INDICATIONS OF THE DECLINE OF BREEDING AMPHIBIANS AT AN ISOLATED POND IN MARGINAL LAND, 1954 - 1967

By

C. SIMMS

The Yorkshire Museum, York

(Received 5/3/68)

INTRODUCTION

The artificial pond at Little Scugdale in the North Yorkshire parish of Guisborough (NZ 589165) provided water for cattle-rearing. It was constructed around 1928, of three concrete walls about eighteen inches deep enclosing a rectangular concrete floor approximately twenty feet by ten feet. A water level of approximately twelve inches was maintained by an overflow pipe to a small area of marshy ground to the south, and the pond is supplied by field drains from the west and natural run-off and ground drainage from pasture slopes, with rough grazing and afforestation higher up, to the north. The altitude of the pond is about 400 feet OD. The north side of the pond has no retaining wall, thus providing easy access for amphibians and cattle alike; its water is acid and supports a poor algal flora. Rapid summer growth of the algae has occasioned periodic clearance of algae and of silt brought in by the field drains and by trampling on the north side.

There is no other permanent amphibian breeding-place within over a mile radius of the pond. Before it was built, and during most of the ensuing period of local drainage improvement, Little Scugdale was very badly drained. Local farmers who remember the area in the 'twenties and earlier' speak of several small ponds and a number of minor streams. These ponds presumably served the ancestors of the modern fauna. Little Scugdale has a long history of wet conditions; it is clearly a 'spillway' valley which carried proglacial or subglacial meltwaters south into the Guisborough basin during periglacial or glacial times, or both. Today the valley carries a small 'misfit' stream, whose originally-meandering course has been straightened in the interests of improved drainage. The study pond was completely drained when adjacent fields were ploughed, for the first time in living memory, in March 1967. This may mark the close of the history of the pond as a focus for amphibian breeding in the area; it has therefore been considered an appropriate time to review results so far achieved.

METHODS AND RESULTS

The pond was examined and netted with a coarse-mesh pond net on one or two dates in March or April of each year, according to prevailing weather conditions. The object was to see every adult amphibian in the pond on dates when each species would be present for breeding. The higher figures obtained for both sexes of the various species are shown in Table 1. The data merely offer indications of numbers and not either maxima for the respective seasons or estimates of animals actually breeding. Figures for *Triturus vulgaris* and *T. helveticus* must be regarded as minimal because a small proportion of *Triturus* females could not be specifically determined with any confidence. These individuals are included in the Table under 'Incertain sedis.' Spawn and larvae were examined in order to establish whether there had been any particularly early breeding of any species. Life-cycle diagrams in Smith (1964) indicate the desirability of excluding this potential source of error. Early breeding was not a feature at this rather exposed pond; late visits in some seasons indicated that the development of ova and larvae were prolonged and the breeding season itself of *Triturus helveticus* was more protracted than expected.

TABLE 1

NUMBERS OF ADULT AMPHIBIANS AT THE STUDY POND, 1954 - 1967

	<i>T. cristatus</i>		<i>T. vulgaris</i>		<i>Incertain sedis</i>	<i>T. helveticus</i>		<i>B. bufo</i>		<i>R. temporaria</i>		Clearances	
	♂	♀	♂	♀	♀	♀	♂	♂	♀	♂	♀		
1954	5	7	6	4	↔ 1	↔	20	21	2	2	1	1	
1955	5	3	2	0	↔ 2	↔	19	30	3	2	5	6	
1956	5	5	0	0	↔ 2	↔	21	28	3	3	5	4	July
1957	4	3	4	8	↔ 3	↔	16	18	3	4	2	1	
1958	5	2	4	12	↔ 4	↔	20	30	0	0	3	1	March, July
1959	2	2	7	11	↔ 2	↔	14	16	0	0	9	8	
1960	6	2	12	7	0		10	8	0	0	7	6	June
1961	10	4	11	11	↔ 1	↔	11	12	0	0	1	1	May
1962	11	7	5	7	↔ 1	↔	8	8	0	0	0	0	June
1963	7	4	4	4	0		6	6	0	0	0	1	July
1964	6	5	6	4	0		5	6	2	0	0	0	
1965	7	5	6	2	↔ 1	↔	3	2	0	0	0	0	
1966	0	0	2	1	0		0	1	0	0	0	0	March, July
1967	1	0	0	0	0		0	0	0	0	0	0	

ANNOTATED CHECKLIST OF THE HERPETOFAUNA OF LITTLE SCUGDALE, 1954 - 1967 (following Mertens and Wermuth, 1960).

AMPHIBIA: Caudata

Triturus cristatus (Laurentus 1768) Great Crested Newt, Warty Newt.

Adults were very aquatic and found at all seasons. Larvae from the previous season were often found during netting visits, indicating that some individuals needed more than one season before metamorphosis. Males were noted in autumn displaying the secondary sexual characters associated with breeding conditions of the gonads, and a female captured in 1965 bred in both April and November of the following year in vivaria at the Yorkshire Museum. (Simms, in press.)

Triturus vulgaris (Linnaeus 1758) Common Newt, Smooth Newt.

After the breeding season adults and immature specimens were found thinly distributed along the valley bottom within half a mile of the pond and where there was suitable cover.

Triturus helveticus (Razoumowsky 1789) Palmate Newt.

The terrestrial range of this species was evidently wide, including slopes of rough grazing land with rocks and drystone walls up to over half a mile from the pond. This is the dominant species of *Triturus* in upland North East Yorkshire; the other species are usually found in association, in lowland ponds and ditches.

Salienta:

Bufo bufo (Linnaeus 1758) Common Toad.

Bufo bufo Linnaeus 1758 Common Toad.

There was no evidence of successful metamorphosis in the Little Scugdale pond in any season, although some spawn was deposited in some seasons when adults were recorded there. Possibly predation by *Triturus cristatus* accounted for many larvae in a small pond lacking in cover. The numbers recorded in the early seasons of this survey represent the smallest breeding concentration of Common Toads known to the author in more than a hundred breeding ponds.

Rana temporaria Linnaeus 1758 Common Frog.

A very wide terrestrial range appeared to support a very small breeding population; possibly some frogs were using temporary pools elsewhere. This is the most widespread and conspicuous upland amphibian in North East Yorkshire, particularly in the vicinity of 'spillway' channels and other boggy terrain.

REPTILIA: Sauria

Lacerta vivipara Jacquin 1787 Common lizard, Viviparous lizard.

Present on steeper slopes above the pond and with a southerly aspect, prior to their afforestation. At the present time either very much reduced in numbers or absent; none could be found during repeated visits in 1965 and 1966.

Vipera berus Linnaeus 1758 Viper, Adder.

Present in small numbers in the valley bottom prior to 1963; since then a female, found in July 1966, represents the only record. This is, of course, a potential predator of other herpetofauna in the district.

DISCUSSION

Against the current background of growing concern by herpetologists in this country over the apparent decline of amphibians, and in particular the Common Frog, the present study of the status of five species at a single pond in marginal land cannot offer conclusions generally applicable elsewhere. In particular, no comparison can be made with areas of intensive agriculture, because it would appear that modern seed-dressings and other insecticides have not been used within the limited catchment area of the study pond's water supply. Such chemicals may have toxic effects on amphibians and other organisms. Considerable changes in the habitat have occurred within the study period and these might be listed, although a casual connection with the recorded decline of the fauna cannot be demonstrated.

a) Drainage of the valley bottom north of the pond by the improvement of the 'misfit' stream it carries. This has greatly reduced the area of marshy ground which provided shelter for amphibians, and in particular the immature Common Newts and newly-metamorphosed Common Frogs which previously abounded there.

b) Part of the floor and sides of the spillway channel have been cleared, ploughed, and planted with young conifers. The clearance involved the removal of fallen logs and boulders which had offered shelter, and possibly hibernation sites, to many amphibians. Ploughing and planting represent a further serious disturbance to animals restricted to the ground.

c) Some previously 'permanent' pasture has been ploughed for arable purposes during the past few years. Although the original old hedgelines remain to offer some cover for herpetofauna it is likely that the amount of cover (and possibly also of food) available to Common Frogs which used to frequent the area was seriously reduced.

d) The aquatic environment at the pond itself appears to have altered little over the study period, but an impression of overall stability masks quite violent changes *within* some seasons, resulting from periodic wholesale clearances of algal growth and silt. These activities must also have removed spawn or larvae of amphibians, particularly those of the Caudata, whose eggs are laid in aquatic vegetation and whose larvae are not strong swimmers. The Table records the minimum number of clearances within the study period; some might have passed unrecorded.

SUMMARY

Annual counts of urodelan and anuran amphibians, in a pond at the junction of arable with hill grazing land in North East Yorkshire, have revealed a striking

decline in the number of species and of individuals recorded. Changes in land-use in the district have been considerable over the period of the study and it is suggested that changes in the habitat, rather than intensive collecting or the use of toxic insecticides or other agricultural chemicals, has been a major cause of decline.

ACKNOWLEDGMENTS

Farmers and forestry workers have been both patient and helpful throughout the survey. Help in the field from Mrs. B. Kendall, the late D. Pearson and J. T. Todd, is gratefully acknowledged. Work at the pond during recent seasons has been pursued in the course of fieldwork from the Yorkshire Museum, York.

REFERENCES

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A STUDY OF A BREEDING COLONY OF FROGS AT THE CANON SLADE GRAMMAR SCHOOL, NEAR BOLTON, LANCS.

The Canon Slade Grammar School occupies the site of a previous Hall and estate, which includes a depression in the field near the School itself, and borders the original field hedge supported by a wall of large irregular pieces of gritstone; beyond the hedge is the Oaks Lane. An old farm drain of rubble and a subsequent school drain of earthenware pipes lead into the depression which became a pond. Its base is of clay so that the water level varies with rainfall and is very low in the summer; this pond has been used successfully for breeding by both frogs and newts (Fig. 1).

February 1965 was unusually dry (monthly total 0.43in.) and so were the first thirteen days of March (total 0.15in.). For the first time the pond was completely dry showing the clay bottom of deep mud and stones with the usual rubbish of an old kettle, bottles, drainpipes, etc., so that the reawakening of the frogs was awaited with interest. The first signs of movement occurred in the forenoon on 5th March during an interval of sunlight which, however, failed to melt the ice on the herbage. The mud literally came alive with frogs (153 were counted)—obviously here was a chance too good to miss for a colony survey. These were immediately collected and marked by clipping the tip of the last *phalanx* of one toe. The subsequent code was:

1965 left	pes,	digit	2
1966	3
1967 right	2
1968	3

As a precaution 1.0% mercurochrome was painted onto the amputation wound against possible infection, especially *Monilia*. Regeneration proved negligible and all the 'clips' subsequently healed neatly and were entirely trouble free.

RESULTS

1965—The initial marking on 5th March was of 20 mature females, 119 mature males and 14 immatures. The dry bitterly cold weather, though bright, continued and there was no further sign of population movement until the ground frost cleared for the first time on 14th March. There was a sudden rise of temperature from 10°F. to 38°F. with the screen maximum at 54°F. and minimum at 45°F. with slight rain, following 8 hours of bright sunshine on both the 12th and 13th; the total rainfall for the first thirteen days of March was 0.15in. with 65.7 hours of bright sunshine. On Monday the 15th of March the entire mud bottom of the pond seemed to be

leaving an endless numbers of frogs staggered out, the colour of the mud itself. On turning over some of the large stones, groups of up to 25 frogs, tightly packed together, were seen huddled (some still torpid) in small tunnels. A few frogs not so protected were still partly frozen solid to the earth itself; they were almost black in colour, absolutely hard to the touch and their skin glistened with ice particles. They were left to thaw loose naturally to avoid physical damage—a truly remarkable sight. This day's collection included 368 males, 42 females and 6 immatures. By the 17th all these frogs had been sexed, measured and clipped and then returned to the pond without further delay. By the end of the month 652 males, 106 females and 35 immatures had been clipped; very probably the total number of frogs marked comprised all those resident at the pond. In addition 82 dead frogs (38 male, 44 female), apparently traffic casualties, were retrieved in Oaks Lane for *post mortem*.

On 18th March croaking was intermittent, the few frogs in amplexus were searching for water and some spawn was found in the only available water; other frogs were frequenting holes in the stone wall and banking. Snow, sleet and high winds then commenced and by the 22nd the water was covered by a layer of slush. No frogs were visible. Water temperature was 37°F. and 33 clumps of spawn had been laid. Two frogs were seen lying at full stretch near the pond (female 81mm., male 65mm.) completely torpid.

All the spawn had been deposited at the school end of the pond. After the rainy period the frogs remained in the pond and on the 26th 216 heads above water were counted. A 'spot check' resulted in 98 being caught and of these 34 males and 2 females had not been previously marked. On the 29th a further 30 unmarked frogs were caught. It was thus likely that frogs were invading the pond since the earlier marking operation had been complete. The total figures for 1965 were 673 males and 106 female mature frogs. Immature frogs are not included in any figures.

1966—On 2nd March three pairs of frogs were seen in amplexus. Nine other males were also seen of which 6 had previously been labelled in 1965. Having received 6.92in. of rainfall during February, the pond was completely full of water and the mud bottom with its varied collection of oddments made collection of frogs difficult. The 7th of March was the special day of emergence and by the 9th most of the spawn appeared to have been laid and the frogs were very wary. The spawning site had been changed to the opposite side of the pond where the field drain entered but as some spawn was situated too near the edge to remain undisturbed (by local children in the evenings) 153 clumps of it were thrown further into the centre of the pond. All frogs were returned to the pond on the 14th of March; 420 had been checked, 38% of them marked in 1965.

Actually a tragedy happened during the night of 10th March, 1966, when 99 frogs awaiting check died. Of these 45 males and 2 females had been marked in 1965 and two had suffered amputation of the left leg; these frogs have been omitted from the 1966 figures.

1967—March 1967 came in with a cold, dry wind roughing the pond surface. The water temperature was 42°F. at a depth of 4in. near the edge of the pond and on the 2nd, one solitary female, marked in 1965, was disturbed there. Two days later the wind dropped, the day became warm and sunny and the pond was quiet. At 10.30 a.m. 30 frogs had appeared; on the next day some were pairing and these were left undisturbed. On the 6th of March (water temp. 50°F.) 412 males, 42 pairs in amplexus and 15 other females (totalling 511) were scored. By the end of the month 493 males and 92 females had been examined of which all but 188 males and 62 females had been marked. All the frogs were released on the 9th, a cold, wet, windy day although a bright sunny half-hour occurred at noon. Small numbers of frogs were caught on subsequent days which eventually produced the full 1967 total of 609 frogs inspected (total 57% previously marked in 1965 and 1966); obviously many of the late-comers were once again new invaders.

1968—The first frogs were seen in the pond on 31st January, but by the beginning of March the pond was almost dry. Between 6th February and 13th March only 0.36in. of rain fell and frost and ice had been almost continuous. On 2nd March the frogs emerged from the mud and were seen moving under the ice surface, some being already paired. Other pairs began immediate spawning on the drier patches of mud around the edges of the pond. They were photographed next day in sunshine when very active. Collection and checking commenced on the 5th, when 83 males and 14 females (all in amplexus) were examined. Some of the small unmarked males complete with nuptial pads presumably were breeding for the first time. On 13th March the drought was followed by sleet, snow showers and torrential rain which by the 20th filled the pond (March rainfall was 4.00in.). 376 frogs were handled of which 58% had been previously marked in 1965-7.

Thus between 1965 and the end of April 1968 2,184 frogs were handled, which number included 1,163 males and 298 females (total 1,461) actually marked once or several times.

Discussion: A large number of frogs, mostly males, hibernated in the mud at the bottom of the pond using hollows under stones, drains, etc., for additional protection. About 70% of the females come to the pond from elsewhere. Loose stone walls are much used for habitation, possibly because local gritstone maintains a warm and rather humid atmosphere. Frogs appear to inhabit these walls during periods of drought for on 3rd June 1965 the water level of the pond was low and many frogs had returned to the stones of the wall. Again on 2nd May 1967, although 22 frogs were recognized in the pond, many were seen hopping and crawling over the now extensive mud remaining after the water had receded from the bottom of the wall; all were moving in a direct line towards the wall to enter the crevices among the stones. Of 9 males inspected at random all had previously been marked but only 2 of the 6 females were marked, proving previous occupancy of the area.

Despite the variable weather and water conditions the frogs emerged from hibernation apparently according to a set temporal pattern. Temperature seemed to be of little consequence but light intensity may be important for irrespective of day temperature, if the day be bright, out they come. Although the odd frog has been observed in the pond even in January, the time of emergence is fairly constant, e.g. 5th, 7th, 6th and 6th March in 1965 - 8 respectively.

Year	Total Marked	Marked for 1st Time	Previously Marked		
			1967	1966	1965
1968	376 {♂ 295 ♀ 81	103 35% 54 67%	137 46% 22 27%	65 22% 10 13%	50 17% 10 13%
1967	609 {♂ 497 ♀ 112	188 38% 77 69%	—	228 46% 26 23%	202 41% 22 19%
1966	420 {♂ 349 ♀ 71	199 57% 61 86%	—	—	150 43% 10 14%
1965	779 {♂ 673 ♀ 106	673 100% 106 100%	—	—	—
Grand Totals	2,184 {♂ 1,814 ♀ 370				

Table 1 shows the separate percentage for the sexes marked as a breeding colony of adult frogs from 1965 - 1968. It is of special interest to note that in 1968 35% of the males were invaders, and 17% males and 13% females had been marked when breeding in this pond in 1965, i.e. they are at least eight years old, and residents.

N.B. The 16 males and 1 female frog seen in 1968 whose amputations interfered with any previous marking they may have had been included only in the 1968 total figure; they could not be marked in 1968.

Frogs were active immediately they emerged from hibernation but there is delay in commencing amplexus which may be related in some way to the time taken for ova to reach the oviducts.

The first frogs recognized in the water were those which had merely surfaced after hibernation. The first to pair were the largest (males 71-79mm., females 89-91mm.). There appeared to be an invasion of frogs soon afterwards and among these the sexes were in approximately equal numbers—the only times a vast preponderance of males was not recorded. This invasion was first noticed on the 5th of March, 1965, when some specimens heading in our direction were found squashed, probably run over by cars at night, on Oaks Lane opposite to the pond. These were 11 females and 14 males followed by a further 21 males and 22 females discovered on the 8th. *Post mortem* examination of the remains of the 33 females and 35 males revealed that their fat reserves were almost completely exhausted and four specimens, although fully grown, were not sexually in breeding condition. On 8th March, 1968, at the exit of the old field drain, where it enters the pond, 22 male and 17 female frogs were found packed tightly together. Six pairs were in amplexus and already spawning, and only five of the total had been marked. It is possible that the frogs moved around from place to place in the field drains. Activity has been noted in this vicinity on previous occasions and in 1966 all the spawning was there.

In the breeding season sensitive dermal papillae arise mainly on the back and sides of the females and are presumed to be of assistance to the male in recognizing the female.

At this time the skin of the first and second digits of the male *manus* thickens and blackens to form the well known nuptial pads. The epidermal cells each develop into a cone-shaped horny prominence jointly producing the rough pad. After breeding the *stratum corneum* is shed, in pieces, which drop to the bottom of the pond. The nuptial pads are then shed, separately, and soon disintegrate.

Many of the breeding males were in a 'lymphoid' state, the width of their bellies varying between 52-60mm. This was due to the *stratum compactum* becoming somewhat gelatinous so that the underlying subcutaneous lymph spaces were filled with fluid. In some specimens great skin sacs overhung the body both at the sides and even on the top of the head; when the position of the animal changed, these sacs also changed their position.

On five different occasions during this survey different females were found in amplexus grasped by a male in the normal position and a second male 'in amplexus' from the ventral side. In one particular instance the latter's snout was pressed tightly into the tip of the female's buccal floor causing her external nares to be closed. She was almost dead from suffocation and severe pressure marks of the first male's nuptial pads were seen on her ventral anterior body wall. One female, a resident marked every year (1965-8) was found on 5th March, 1968, in the death-grip of a male who held her until the afternoon of the 9th. The clasped hands were low and the *xiphisternum* was pushed deeply inwards where it must have interfered with the functions of both heart and lungs.

Spawn is deposited within a day or two of amplexus whatever the aquatic conditions of the breeding locality. If the prevailing water is limited, spawn is deposited on the exposed mud. Even though at a later date the pond fills with water, the spawn may fail to surface and no hatching takes place. Indeed spawn apparently laid normally in water often fails to rise from the bottom and no tadpoles develop. Probably, however, much of it is infertile.

Although April and May 1965 were both wet months with total rainfall of 3.11in. and 3.93in. respectively, so that there was no shortage of water for the

young tadpoles, by 11th June conditions were extremely dry and numerous large tadpoles, many already dead, inhabited the last available pool, approximately 18in. in diameter. On the 15th light rain occurred during the night but only sufficient to dampen the surface of the pond.

Although a few frogs tend to linger in the pond, by June adults are to be found in the grass in the immediate neighbourhood. They do not travel far from the pond during summer but it seems likely that some do so during periods of rain when they were seen to be most active. During the survey there was a steady increase in the number of frogs which suffered amputations of legs, feet or digits, no doubt injured by the school mowing machine. All wounds healed successfully and no regeneration occurred. Most of our frogs remained at the pond side of the field although in June the ditch below the tennis courts is popular (Fig. 1). Immatures are seldom found in company with mature frogs; they spend their time in nearby damp ground among the rushes thus avoiding competition with them.

In each year during August frogs travel farther afield, but towards the end of September and early in October they were seen moving closer to the pond to return to their regular hibernation quarters. Such appears to be the annual cycle of the School frogs. Perhaps neglect of field drains and certainly lack of rain during late February and early March are contributory factors to the decrease in this particular frog population up to and including 1966.

There was great variation in colour and pattern; almost no two frogs were alike. A resident male had large black blotches which tended to unite so that colouration from snout to shoulders, and hind limbs to *urostyle* region, was almost entirely black leaving a body band of unbroken copper-orange. Another striking marking is one in which there is a small white spot in the centre of each black spot. Only three frogs could be designated red and although very pretty were not particularly outstanding. Colour change occurred swiftly in most cases—within minutes only—whilst in other specimens it was much more protracted.

All mature frogs handled between 1965 and 1967 (total 1,808) were measured from snout to cloaca (SV). The average SV length of 547 male frogs in 1965 was 65.7mm. and of 90 females 73.2mm. The largest male and female were 80mm. and 89mm. respectively. It is interesting to note that the average lengths of these frogs were

In amplexus (40 pairs) ♂ 70mm., ♀ 77mm.

Not in amplexus ♂ 64mm., ♀ 63mm.

The SV of the largest male recorded was 84mm. and that of the largest female 94mm. The largest measurement so far reported in the British Isles* was from Cornwall (male 73mm., female 85mm.) and in 1893 from N. Scotland (male 80mm., two females 93mm. and 95mm.).

The thinnest frog we saw was a female on 22nd. September, 1967, SV 70mm., weight 30gm. Three pairs of frogs in amplexus on 22nd. September, 1966, measured:

♂ 72mm. with a ♀ 91mm. (135gm.)

♂ 79mm. ,, ,, ♀ 90mm. (125gm.)

♂ 71mm. ,, ,, ♀ 89mm. (125gm.)

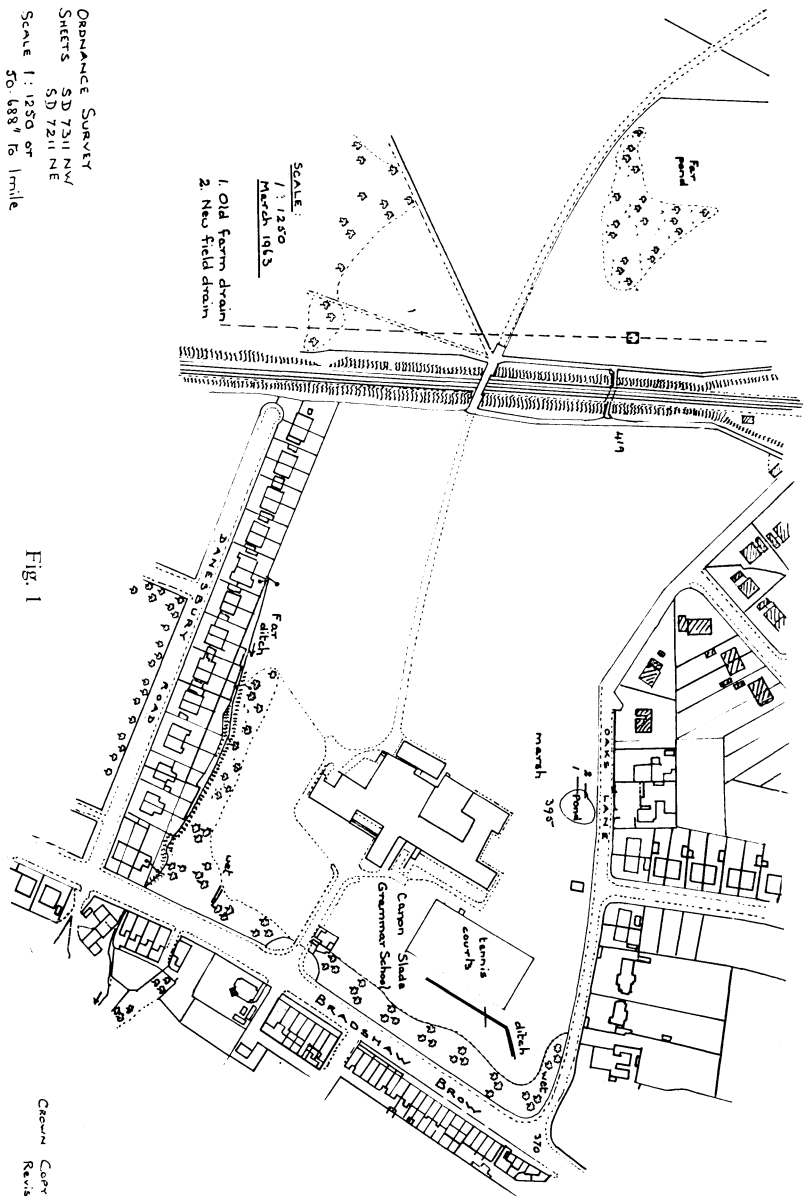
A lone male of 69mm. weighed but 55gm. Various males before hibernation had the following measurements: 64mm. (35gm.), 75mm. (60gm.), 71mm. (49gm.), 62mm. (35gm.), 59mm. (46gm.), 82mm. (42gm.) and two females 72mm. (50gm.), and 75mm. (60gm.). On 9th October two frogs immediately prior to hibernation weighed 51gm. (♂) and 75gm. (♀).

SUMMARY

1. A continuous breeding population of *Rana temporaria* located in a school pond near Bolton, Lancs., was studied from 1965-1968.

ORDNANCE SURVEY
SHEETS SD 7211 NW
SD 7211 NE
SCALE 1:1250 or
50:1889 to 1 mile

Fig. 1



CREW CONYBEARE 1965
Revised June 1966

2. Yearly markings showed that approximately 70% of the females and 40% of the males were newcomers; if the 'invaders' arrive in time to breed, the sexes are approximately equal in numbers and in many cases already paired. Common Gulls have been seen to take a few frogs as in 1968 when the frogs had just emerged from hibernation and were stranded on wet mud.

3. A description of frog sizes, structure, habits and appearance has been given.

ACKNOWLEDGMENTS

I am delighted to record the immense enthusiasm with which so many younger as well as senior pupils of the School volunteered to assist in the various activities associated with this survey. Mr. Eric Brett supplied a fine photographic record for 1967 - 8 for which I record my thanks.

REFERENCE

*BOULENGER, *Tailless Batrachians of Europe*, Ray Society, 1897.

ILLUSTRATION

1. The Canon Slade Grammar School showing the location of the Pond with (1) the old and (2) the new drains.

ELLEN HAZELWOOD

44, Rigby Lane, Bradshaw, Bolton, Lancs.

Received 26/9/68

Raw Data from which Table 1 was prepared:

1965 Total Marking:

Frogs marked for the first time ♂ 673 and ♀ 106 = 779 total.

1966 Total Marking:

Frogs marked in 1965	♂ 150 and ♀ 10 = 160
.. .. first time	♂ 199 .. ♀ 61 = 260

349 71 = 420 total

1967 Total Marking:

Frogs marked in 1965 and 1966	♂ 121 and ♀ 13 = 134
.. .. 1965 only	♂ 81 .. ♀ 9 = 90
.. .. 1966 only	♂ 107 .. ♀ 13 = 120
.. .. first time	♂ 188 .. ♀ 77 = 265

497 112 = 609 total

1968 Total Marking:

Frogs marked in 1965 only	♂	7	and	♀	2	=	9
" " " 1965 and 1966	♂	5	"	♀	2	=	7
" " " 1966 only	♂	3	"	♀	0	=	3
" " " 1965, 1967	♂	24	"	♀	0	=	24
" " " 1967 only	♂	80	"	♀	14	=	94
" " " 1966 and 1967 only	♂	19	"	♀	2	=	21
" " " 1965, 1966 and 1967	♂	38	"	♀	6	=	44
Marking amputated	♂	16	"	♀	1	=	17
Frogs marked first time	♂	103	"	♀	54	=	157
		295			81	=	376 total

Marking Summary:

Frogs marked	♂	673	+	199	+	188	+	103	=	1,163
	♀	106	+	61	+	77	+	54	=	298
										1,461 total
Frogs handled		779	+	420	+	609	+	376	=	2,184 total

ACTIVE ACQUISITION OF STOMACH STONES IN A SPECIMEN OF
ALLIGATOR MISSISSIPPIENSIS DAUDIN

By

M. PEAKER

A.R.C. Institute of Animal Physiology, Babraham, Cambridge

(Received 6/3/68)

Cott (1961) reviewed the occurrence of stones in the stomach of *Crocodilus niloticus* and concluded that these stones may serve a hydrostatic function and are probably deliberately ingested. Kennedy and Brockman (1965) have recorded the presence of a stone in the stomach of a captive specimen of *Alligator mississippiensis*. These authors suggested that in this species a similar function to that proposed by Cott (1961) for *C. niloticus* might prevail. Direct evidence has now been obtained that at least one species of crocodylian deliberately ingests stones.

A 54cm. specimen of *A. mississippiensis* maintained in the writer's collection in England on transfer from a glass tank to a semi-aquatic enclosure was seen to pick up and swallow pebbles (approximately 1.5cm. in diameter) and adhering earth from the floor. Several days later, the reptile was examined and stones could be palpated in the stomach. The animal was feeding normally on raw meat and dead mice, supplemented with vitamins and minerals, at this time.

These observations support Cott's (1961) suggestion that crocodylians deliberately acquire stones rather than ingest them accidentally whilst swallowing food items. Many reptiles do, of course, accidentally pick up non-food items whilst feeding (see Kennedy and Brockman, 1965).

It seems desirable, therefore, to distinguish between those non-food items found in the stomachs of various reptiles that are ingested deliberately, *i.e.* actively acquired and those accidentally or passively ingested during feeding or other activities. Most instances, it appears, fall into the latter category but, in at least some crocodylians, it now appears that the acquisition is an active behavioural process reminiscent of the situation in granivorous birds although the function of the ingested material apparently differs (Cott, 1961). Although further observations are required, it appears that the stimulus for the active acquisition of stones by crocodylians may warrant further investigation.

Since it would be desirable to further substantiate these observations it is requested that any records of a similar nature be sent to the writer.

REFERENCES

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KENNEDY, J. P. and BROCKMAN, H. L. (1965). Stomach stone in the American Alligator, *Alligator mississippiensis* Daudin. *Brit. J. Herpetol.* **3**, 201-203.

LETTERS TO THE EDITOR

The Society may be interested in the fact that on Sunday, 8th September, on Ham Common, near Richmond Park, I was able to get two Common Lizards to take grasshoppers from my fingers. A male took a grasshopper at the first offering, but backed away from the subsequent ones, while a female at first retreated, but later took three grasshoppers from me, on separate occasions, even going so far as to move slowly towards my outstretched hand.

Of course, getting vivarium specimens to feed from the fingers is normally very easy, but this is the first time I have heard of wild lizards doing this in the country.

These two lizards, together with a very young one, were sunning themselves on a dead branch in the middle of a small gorse bush entwined with blackberry, and dropped into the prickly areas if I was too "pushing" with the grasshopper. However, they reappeared on the branch after a very short while.

The quite small area I examined had a higher population of lizards than I can remember for a long while, and they all seemed particularly "tame", by which I mean not nervous and given to running for shelter in the gorse and blackberry bushes. My theory to explain this is that, as it is now the blackberrying season, with their bushes disturbed much more than usual, but by people who did not even know the lizards were there, they have found it necessary to return to their sunning areas much sooner after being disturbed, if they were not to miss most of the sun.

R. G. AUBERTIN,
"Ayron",
9, Manor Road,
Guildford,
Surrey.
26/10/68

ERRATA

Reference: R. J. Clark (1969). A Collection of snakes from Greece. *Brit. J. Herpet.* **4**, 45-8. Table 1, the record of *Malpolon monspessulanus insignitus* is for Poros only. In table 2 the same species and *Coluber najadum dahlii* are again from Poros only.

In table 1 (page 47), beginning of line 2 should read, *E.g. quatuorlineata* ↔ *E. quatuorlineata sauromates*.

BOOK REVIEW

DISEASES OF MAN, ACQUIRED FROM HIS PETS, BY B. BISSERU, (1967). pp. 428. 94 Illustr. 84/. Heineman, London.

Since there are very few animals which have not, sooner or later, been kept as 'pets', a book designed to cover the damage such pets can inflict on their owners, must necessarily be weighty and encyclopaedic. Since, at the same time, both author and editors will want to keep the volume at a reasonable size and price, the text must be very concise. This inherent conflict of aims caused the merits and demerits of this book. It will serve as an introduction but if the reader should want guidance to further, and particularly to recent literature he will get little help from the few references appended to each chapter. Of these, chapter 6, dealing with Reptiles and Amphibians, will interest our readers and they will be reassured to see that it is the shortest of all because nothing but snakebite needs to worry the Herpetologist very much. Or so it seems, but then, the Salmonellosis of ornamental terrapins which has recently attracted so much attention in the medical Press, is not even mentioned. The statement (p.405) that native tribes in Colombia poison their arrows with secretions of the "giant S. American toad" (*Bufo marinus*) may be questioned. As far as the reviewer knows the frog used is a species of *Dendrobates*, neither a *Bufo* nor a giant.

The 94 illustrations have the merit of being well chosen and original. As usual, the absence of colour must be deplored.

The book ends with a Table of Human zoonotic infections and a glossary of unfamiliar terms. An interesting book which deserves considerable expansion in future editions.

E. ELKAN

OBITUARY

HAMPTON WILDMAN PARKER, died 2nd September, 1968, aged 71

CHARLES HENRY MAXWELL KNIGHT, died 24th January, 1968, aged 67

Born in Yorkshire in 1897, elder son of a schoolteacher, Hampton Parker was educated at the Blue Coat School, Christ's Hospital, where he won a scholarship to Cambridge. During World War One he served in France as a lieutenant in the 6th Duke of Wellington's Regiment, and was wounded in the leg. In spite of this, on his return to college, he excelled in athletics, and gained a first in Science (Zoology, Botany and Chemistry).

In 1923 he joined the staff of the British Museum (Natural History) and took over the curatorship of the reptile and amphibian collection from Miss Joan Proctor, who left to go to the London Zoo.

Parker's main love was in the field of Systematics. Unfortunately, his University did not recognise this as a suitable subject for his Doctorate thesis. On the other hand his researches in this field were received with acclaim by the authorities at the University of Leiden in Holland. To this ancient seat of learning he presented his findings on the Snakes of Somaliland, for which he was awarded his Doctor of Science degree.

As Keeper of the Department of Zoology for 10 years before his retirement Dr. Parker had much arduous administrative work to contend with, in spite of which he continued with his taxonomic research until retirement at 60. It is a great misfortune that his gruff and sometimes brusque Yorkshire manner, actually concealing a sensitive nature, did not endear him to his British colleagues. He was perhaps best appreciated by his American contemporaries and by other overseas herpetologists who held him in high esteem. Dr. Parker was hardly known to the public at large, and his unhappy manner of withdrawal must explain the lack of understanding and closeness evident among his immediate associates.

H.W.P. was nevertheless a much respected and admired herpetologist, if only for his contribution to science.

Born in 1900 of a lawyer father Maxwell Knight spent his boyhood in the, then, Surrey village of Mitcham. Since earliest days he showed a passion for animals, both wild and domesticated, keeping many of them as pets during his school years. This included reptiles and amphibians for which he had an abiding love throughout his life. During World War I he became a naval cadet on H.M.S. Worcester, then served as midshipman in trawlers; also on an armed merchantman.

Max's early ambition had been to become a zoo keeper, but with a more mature outlook gained from his sea adventures he desired to take up medicine and become a naval doctor. Lack of family finance prevented this, and he had to keep himself going through writing stories, and for a while, by teaching in a preparatory school.

During these lean years Max discovered a special talent for writing and lecturing which was to bring him fame in later years. After service in World War II, on the General Staff at the War Office, for which he was given the O.B.E., he emerged as a leading British naturalist, becoming known to thousands, if not millions, of his fellow naturalists and country lovers through his numerous books and broadcasts. He served on very many committees and was associated with many leading societies, including the Linnean, R.S.P.B., Council of Nature and World Wildlife Fund.

In his lifelong devotion to wildlife education and conservation, Maxwell's foremost interest and concern was always for the young, to encourage and guide them along the right path of understanding and respect for all wild things. At his memorial service James Fisher spoke of him to a packed church as 'a gentle Knight', which perhaps best sums up his character and regard for life.

Both H.W.P. and Max were founder-members of our British Herpetological Society, and served on the committee. Both helped to foster enthusiasm among our members, and helped to set the B.H.S. along its proper course—an institution for the herpetologists to join and share in, whether as beginner or specialist, amateur or professional.

Although the very opposite in temperament and outlook—the layman and scientist, extrovert and introvert, educationist and research worker, both men shared a devotion and humility for their work. Both were seekers after truth, which in their individual ways they have left behind in print for others to share. Our Society, their friends and families, and the herpetological field at large, are the poorer for their passing.

ALFRED LEUTSCHER