LCM 22 (Supplement—South African Journal of Laboratory and Clinical Medicine)

THE OCCURRENCE OF ENTEROCHROMAFFIN CELLS IN TADPOLES AND JUVENILES OF *XENOPUS LAEVIS*, WITH SPECIAL REFERENCE TO 5-HYDROXYTRYPTAMINE AND ITS POSSIBLE ROLES IN METAMORPHOSIS*

ANN ANDREW, PH.D., *Department of Anatomy, Witwatersrand Medical School, Johannesburg*

The stages at which enterochromaffin (EC) cells become recognizable in embryos have been determined for a number of vertebrates, but no data are available for amphibia. Since estimates of 5-hydroxytryptamine (5-HT) content of *Xenopus laevis* tadpoles have been made,[4] a study of EC cells (which produce 5-HT) in the same species is of interest. It is for this reason that observations are reported here on the time of first appearance of these cells, on the nature of the earliest cells to become recognizable and on their increase in number in relation to the phases of metamorphosis. The study was originally embarked upon as part of an investigation into the possible origin of EC cells from the neural crest.[3] The observations have been amplified by the examination of additional embryos over a more extended period of development.

MATERIAL AND METHOD

A number of batches of fertile eggs of *Xenopus laevis* were obtained by injecting adults with chorionic gonadotropin. Development was slow in batches of tadpoles fed on cultures of unicellular organisms. However, both development and survival were greatly improved by the use of Liquifry No. 2, a commercially available matter containing green vegetable matter and intended for the fry of live-bearing fish. After metamorphosis, juveniles were fed on Tubifex or tiny strips of raw ox liver.

One to three tadpoles or juveniles were killed at stage 27, at 2- or 3-stage intervals from stages 36 to 66 (staging according to Nieuwkoop and Faber),[56] at 2-, 4- and then weekly or bi-weekly intervals after the end of metamorphosis up to 12 weeks and, finally, at 26 weeks. The fixative used was 10% formalin containing 2% calcium acetate. Tadpoles up to stage 49 were fixed whole and sectioned serially at 5μ; the stomach and small and large intestine of older specimens were fixed separately and a number of longitudinal sections of each were mounted.

Some sections from each stage were stained by Hamperl's modification[42] of the Bodian protargol method to demonstrate argentophilia. For older stages exposure to protargol and subsequent reduction were repeated once, as advocated by Dawson and Barnett;[14] a single 24-hour exposure sufficed for younger stages. Other sections were impregnated by the Masson-Fontana method (Pearse,[57] modified by Dry, see Andrew[3]) for argentaffinity. A section of adult Xenopus duodenum previously shown to contain EC cells was mounted on each slide as a control for the success of the silver techniques.

Counts of cells were not made; observations were limited to subjective assessments of increases in numbers.

OBSERVATIONS

Observations made on the occurrence of EC cells in various parts of the gastro-intestinal tract during development are summarized in Table I. The periods into which develop-

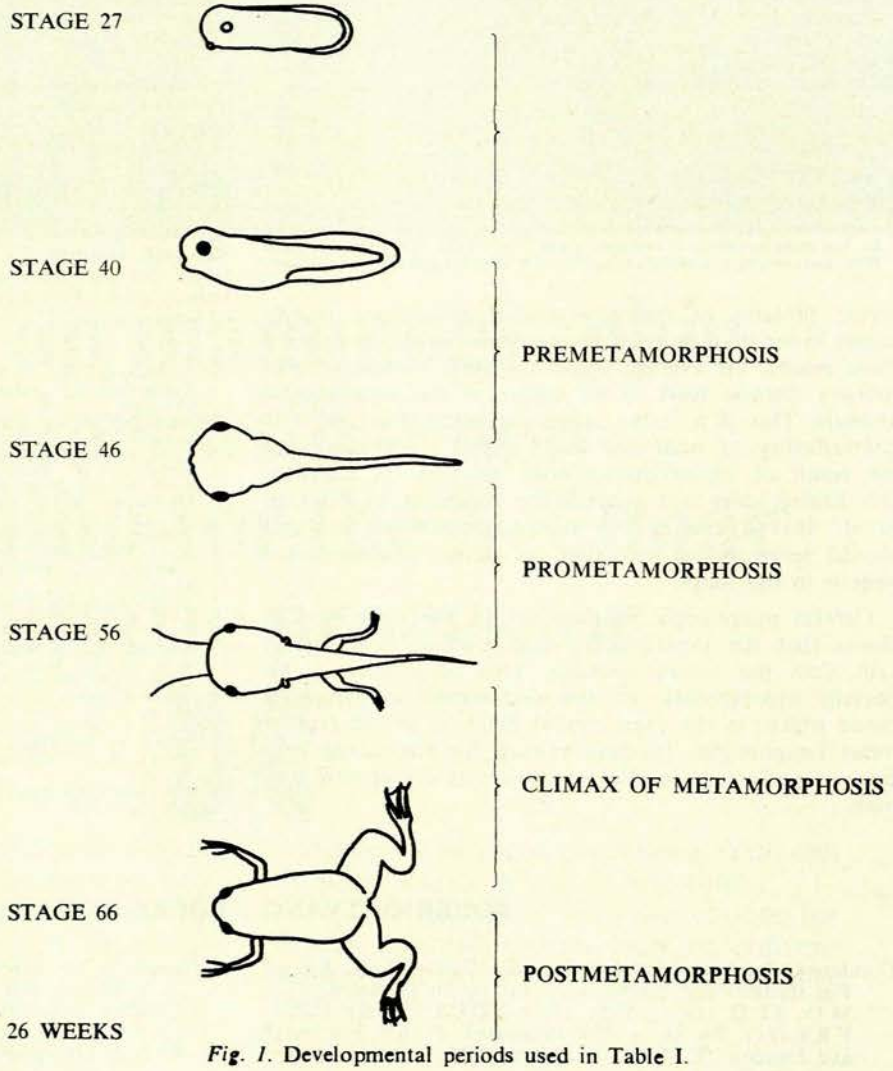


Fig. 1. Developmental periods used in Table I.

*Date received: 21 May 1968.

TABLE I. OCCURRENCE OF ENTEROCHROMAFFIN CELLS IN GASTRO-INTESTINAL TRACT OF XENOPUS TADPOLES AND JUVENILES

	*Stage**	*No. of specimens*	*Part of tract*	*State of epithelium*	*Histolysis starts**	*Epithelium replaced**	*Enterochromaffin cells*	
							Argentophil	*Argentaffin*
	27–39	3	Foregut	Larval			0	0
			Midgut				0	0
			Hindgut				0	0
Premetamorphosis (stages 40–45)	40–43	4	Stomach	Larval			0	0
			Small intestine				0	0
			Large intestine				0	0
	45	2	Stomach				+/++	0
			Small intestine				++	0
			Large intestine				+	0
Prometamorphosis (stages 46–55)	46–47	5	Stomach	Larval			+	0
			Small intestine				++	0
	49–50	2	Stomach-fundus -pylorus				++	++
			Small intestine				++	+
			Large intestine				++	+
	53	2	Stomach-fundus -pylorus				++	+
			Small intestine				+	+
							++	+
							++	+
Climax of metamorphosis (stages 56–66)	56–62	7	Stomach-fundus -pylorus		Stage 60		+/++	+
			Small intestine		Stage 56		+/+++	0/+
			Large intestine		Stage 62		++/+++	+/++
	64–66	8	Stomach-fundus -pylorus				0/+	0
						Stage 64	+++	+
			Small intestine				++	0/+
			Large intestine			Stage 64	+++	+/++
						Stage 66	+	+
Postmetamorphosis		47	Stomach-fundus -pylorus	Adult			++++	++
							++	+
			Small intestine				++++	+++
			Large intestine				++	++

0 none
\+ occasional cell
++ few
+++, ++++ increasing numbers
/ range

* According to Nieuwkoop and Faber.[54]

ment was divided are shown in Fig. 1.

Enterochromaffin cells first become histochemically detectable at or just before stage 45, i.e. at the end of premetamorphosis (Fig. 2). These cells are mostly situated in the pyloric end of the stomach. One or two were found in the terminal portion of the large intestine. In these regions, the epithelial cells contain less yolk than in the rest of the intestine; granules with an affinity for silver apparently do not become evident until a large part of the yolk in the cells has been used up.

These early enterochromaffin cells are argentophil and not argentaffin. Argentaffin cells appear only at or just before stage 49, during the prometamorphic period (Fig. 3). At this time, although very few in number, they may be found in all the subdivisions of the gastro-intestinal tract. It is stressed that in all parts their appearance is preceded by that of cells which are purely argentophil.

During prometamorphosis EC cells are sparse. During the climax period of metamorphosis they increase in number appreciably. One might expect that the increase would follow the epithelial histolysis characteristic of metamorphic climax, and would occur when the epithelium had been replaced. However, this is not so. In all subdivisions of the tract, the increase in EC cells is initiated before the onset of epithelial breakdown. Differentiated EC cells were sometimes identified in the cell groups from which epithelial replacement takes place (Figs. 4 and 5).[56] Such groups form islands in the degenerate epithelium, in which, however, healthy EC cells also occur.

After metamorphosis EC cells gradually increase in number, though even in the adult frog they are sparser than in full-grown birds and mammals.

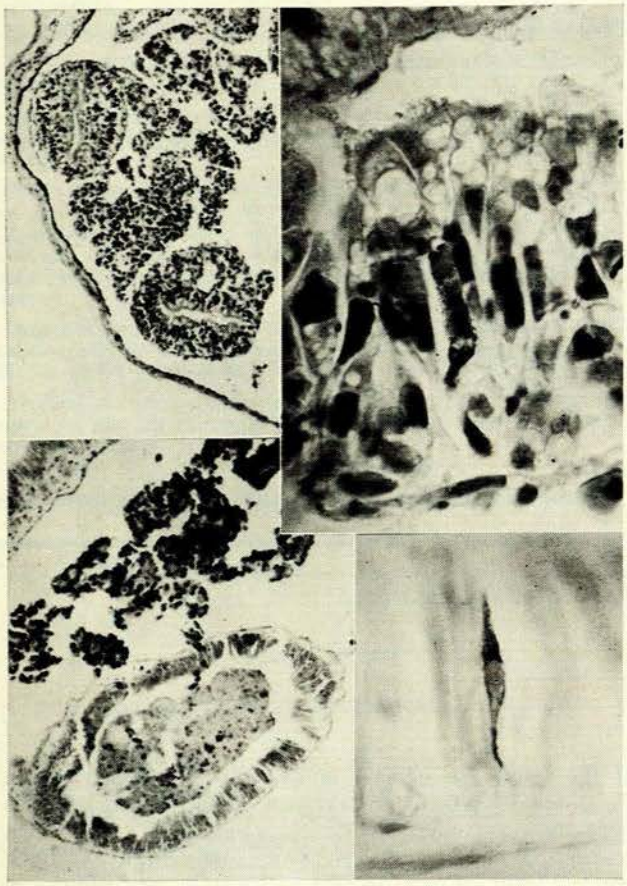
DISCUSSION

It has been shown that argentophil, non-argentaffin cells appear in the gastro-intestinal tract of *Xenopus laevis* before argentaffin cells. The same order of events has been reported in certain amniote embryos.[53,60,75] These findings support Erspamer's concept of a secretory cycle of enterochromaffin cells, in which a purely argentophil phase precedes a phase in which the cells are both argentophil and argentaffin.[23] Such observations are not as easily explained by the contention that the purely argentophil phase follows, rather than precedes, the discharge of secretion by argentaffin cells.[58,61]

Since there are some instances among vertebrates in which argentophil cells are never followed or preceded by an argentaffin phase (teleost and cyclostome alimentary tracts;[70,74] bird gizzard and proventriculus[18]), it is likely that argentophil non-argentaffin cells, at least in these cases, are able to secrete, as suggested by Dawson.[72] It may well be that where an argentaffin phase follows, as in Xenopus tadpoles, the purely argentophil cells merely store their product. However, if they do secrete, it would be of interest to know the nature of their secretion. It has been suggested that it may be 5-hydroxytryptophan (5-HTP), the immediate precursor of 5-hydroxytryptamine (5-HT).[7] A number of workers agree that purely argentophil cells do not contain 5-HT itself.[22,23,41,61] It is thus likely that the gastro-intestinal tract of Xenopus tadpoles does not produce 5-HT much before the middle of prometamorphosis, when argentaffin cells first appear. It might produce a little 5-HTP before this, while there are only argentophil cells present. Since argentaffin cells are sparse until metamorphic climax has set in, an appreciable amount of gastro-intestinal 5-HT would not be expected before the climax. That the appearance and increase in EC cells and gut 5-HT occur concurrently in calf embryo intestine has been shown.[34]

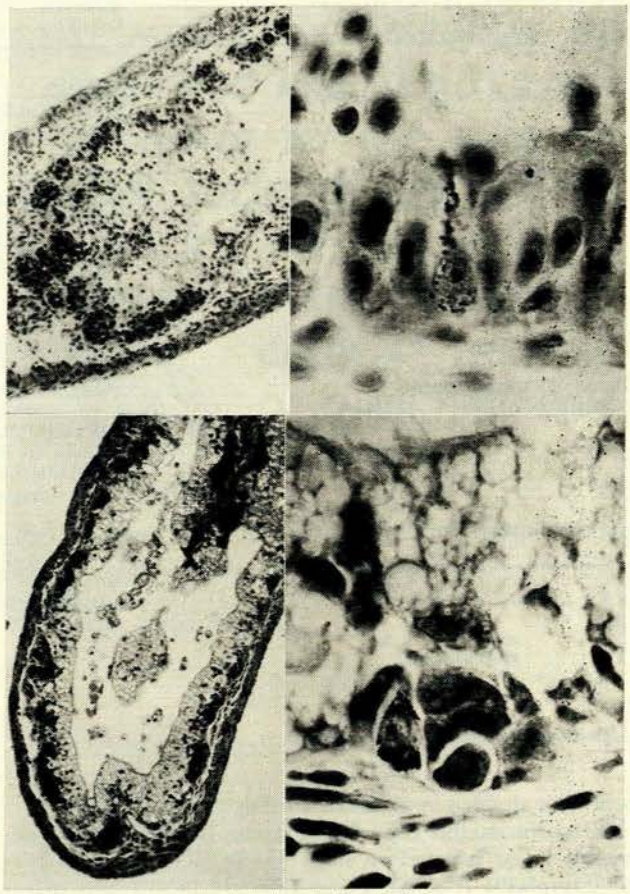
It is therefore unexpected to discover that Baker[4] detected a significant increase in 5-HT in the torso of Xenopus tadpoles between stages 25 and 47—before argentaffin cells differentiate. He separated the torso from the head in order to distinguish gastro-intestinal from brain 5-HT. The concurrent rise in head 5-HT is undoubtedly due to synthesis of 5-HT in the brain: Baker mentions the presence of 5-hydroxytryptophan decarboxylase (5-HTPD), which is an enzyme for 5-HT synthesis, in the tadpoles. Both 5-HT and 5-HTPD have been identified in the brains of other embryos.[39,63,66]

5-HTP can pass the blood-brain barrier[69] but it is not necessary to postulate that any gastro-intestinal 5-HTP does so in Xenopus tadpoles, as 5-HT is present in the brain before 5-HTP could be produced in the gut. 5-HT



Above: Fig. 2(a) and (b)
Below: Fig. 3(a) and (b)

Fig. 2. Duodenum of tadpole, stage 45 (late premetamorphosis). Bodian. *2(a)*. Low-power view of section. *2(b)*. Argentophil enterochromaffin cell in the epithelium (oil immersion).
Fig. 3. Duodenum of tadpole, stage 49 (prometamorphosis). Fontana. *3(a)*. Low-power view of section. *3(b)*. Argentaffin enterochromaffin cell in the epithelium (oil immersion).



Above: Fig. 4(a) and (b)
Below: Fig. 5(a) and (b)

Fig. 4. Small intestine at stage 62 during climax of metamorphosis. Bodian. *4(a)*. Dark-staining cell groups in histolytic epithelium—low-power view. *4(b)*. Argentophil enterochromaffin cell in regenerative cell group (oil immersion).
Fig. 5. Small intestine of same individual as Fig. 4. Fontana. *5(a)*. Low-power view of section. *5(b)*. Argentaffin enterochromaffin cell in regenerative cell group, surrounded by histolytic epithelium (oil immersion).

does not easily pass the blood-brain barrier,[35] though perhaps the barrier may be less efficient in embryos (or tadpoles). It thus seems highly unlikely that 5-HT synthesized in the brain of Xenopus tadpoles would contribute substantially, if at all, to the 5-HT found in the torso before the differentiation of argentaffin cells. Other possible sources must therefore be considered.

It is known that 5-HT occurs in platelets and in the spleen of various vertebrates, but these structures lack 5-HTPD;[15,34,37] their 5-HT is synthesized in EC cells.[6,29] Kidney, liver and lungs contain 5-HT and 5-HTPD,[36,37,59,68] but the parenchyma of none of these organs has differentiated in Xenopus before stage 40,[56] nor has that of the pancreas, and the bladder has not started to develop by then. Both pancreas and bladder contain EC cells.[27] The granular cutaneous glands of amphibia[31] including those of *Xenopus laevis*[73] contain 5-HT, but little if any 5-HT is detectable in the glands before the onset of metamorphic climax.[72] 5-HTPD activity rises in the glands in advance of 5-HT formation.

The only component of the torso which possibly may contain 5-HT before the appearance of argentaffin cells, seems to be the spinal cord. Baker did not consider this possibility, but there is evidence for the presence of 5-HT in the spinal cord of various animals[3,2,10,11] including the frog, Rana.[1] It is more than likely that this 5-HT is synthesized in the central nervous system. I am unaware of any reports of the presence of the necessary enzymes in adult spinal cord, but results with the mouse and Rana do suggest that synthesis of 5-HT occurs in the spinal cord on stimulation of descending tracts.[1]

The significance of 5-HT produced in the central nervous system and by the gastro-intestinal tract is not immediately apparent, since the roles of 5-HT in the organism appear to be manifold.[18,26,29,30,40,46,51,52,62,65,68] A discussion of some of the possibilities may be of heuristic value in promoting research in this field.

The role of 5-HT synthesized in the central nervous system is becoming accepted to be that of a neurohumoral transmitter substance (Douglas).[18] Perhaps brain 5-HT could be concerned in the regulation of thyroid hormone secretion which suddenly rises during metamorphic climax (Etkin).[32] The spurt depends on the neurosecretory activity of the hypothalamus in the production of TSH.[5,8,32,33,35,67,76] There is some evidence that 5-HT can cause an increase in thyroid activity in rats[64] (but see Zizine[77]) and rabbits.[9] Also, the hypothalamus is one of the regions of the brain in which 5-HT is synthesized (Heller *et al.*).[44] A decrease in neurosecretory substance in certain hypothalamic nuclei has been noted following administration of 5-HT to rats. At the same time the store of neurosecretory substance in the neurohypophysis was depleted. It appeared that the result was an increase in liberation of antidiuretic hormone.[49] Could 5-HT in like manner be involved in the increased production of thyroid hormones during the climax of metamorphosis?

An indication that 5-HT might play a role in the controlling mechanism of metamorphosis was given by the findings of Kehl *et al.*[47,48] On administering reserpine (which, among other effects, liberates 5-HT in the central nervous system and also from EC cells) some time beforehand, they obtained earlier initiation and completion of metamorphosis in Rana. Treatment with 5-HT, however, did not have the same effect, but perhaps a dose intermediate between that which was ineffective and that which was fatal should be tested. On the other hand it has been reported[39] that 'reduced' metamorphosis has been obtained in two amphibian species by means of 5-HT.[55]

It may be, of course, that the boot is on the other foot, and that thyroid hormones stimulate the differentiation of EC cells, in the same way as they are responsible for the development of the 5-HT-producing skin glands during metamorphosis.[72] It has been reported that surgical removal of the thyroid has an effect on the enterochromaffin cell system;[20,21] thus the onset of secretion, by which we recognize differentiation of these cells, may be under the influence of thyroid hormones.

The increase in enterochromaffin cells during metamorphic climax is probably related to a number of functional changes known to occur at that time. For instance, increase in limb movements or the initiation of lung breathing may require an increase in 5-HT, since blood-vessels of skeletal muscle dilate in response to 5-HT, and since the latter stimulates smooth muscle of the respiratory tract (Douglas).[18] It has been proposed that 5-HT is antidiuretic.[16,24,26] Such an action might well be involved in the change from ammonia excretion, which requires the loss of large amounts of water, to urea excretion which does not. Such a change occurs in most anurans during metamorphosis.[5,17,64] However, Xenopus is exceptional in that it remains aquatic after metamorphosis, and is ureotelic only during early metamorphosis, thereafter reverting to ammonia excretion.[54,71] It is thus evident that the antidiuretic role of 5-HT is not as great as it might have seemed in metamorphosis, at least of Xenopus.

In the context of water balance, it is of interest that in Rana 5-HT is not concerned with water exchange occurring through the skin.[25]

The stimulation of intestinal motility is one of the important roles suggested for 5-HT (see above). Presumably peristalsis commences soon after the tadpoles start feeding, which happens in Xenopus at stage 45.[56] Thus, in this species, the first appearance of enterochromaffin cells (thought responsible for the 5-HT stimulating the muscularis, whether directly or via the blood stream) slightly precedes, or is coincident with, the onset of peristalsis. It would be interesting to know whether peristalsis becomes stronger as metamorphosis proceeds (certainly the muscular coat thickens),[45,50] perhaps even in relation to dietary change. Xenopus is herbivorous before metamorphosis and carnivorous thereafter—the breakdown product of gastro-intestinal 5-HT, 5-hydroxyindole acetic acid,[43] has been found in the urine of herbivores only during suckling, whereas it is normally present in the urine of carnivores and omnivores.[28] These observations are consistent with the absence of enterochromaffin cells from Xenopus gut until shortly before metamorphosis.

SUMMARY

Enterochromaffin cells first become histochemically recognizable in the gastro-intestinal tract of *Xenopus laevis* tadpoles just before prometamorphosis. The first cells to appear are argentophil, non-argentaffin cells. Argentaffinity becomes apparent during prometamorphosis. Only few enterochromaffin cells are present before the onset of metamorphic climax. During

the climax, they undergo a fairly marked rise in number and thereafter continue to increase gradually for some time.

The significance of the appearance of argentophil, non-argentaffin cells before any argentaffinity is discussed in relation to the phases in the secretory cycle of enterochromaffin cells, and to the secretory product, if any, of the former category of cells.

Possible sources of the 5-hydroxytryptamine found by Baker in the torso of Xenopus tadpoles long before enterochromaffin cells differentiate, are discussed. The spinal cord seems the most likely depôt of 5-hydroxytryptamine; such 5-hydroxytryptamine would doubtless be synthesized in the central nervous system and not in enterochromaffin cells.

Possible roles of 5-hydroxytryptamine during metamorphosis are discussed, including the possibility that it is indirectly concerned in the regulation of thyroid hormone secretion. Other bodily functions which may be involved in an increased supply of gastro-intestinal 5-hydroxytryptamine are mentioned. These include limb movements, respiration, control of water balance and peristalsis.

I wish to thank Mr R. J. Herman and Mrs P. Morrison for proficient technical assistance.

REFERENCES

1. Andén, N. E., Carlsson, A., Hillarp, N. A. and Magnusson, T. (1964): Life Sci., **3**, 473.
2. Andén, N. E., Jukes, M. G. M. and Lindberg, A. (1964): Nature (Lond.), **202**, 1222.
3. Andrew, A. (1963): J. Embryol. Exp. Morph., **11**, 307.
4. Baker, P. C. (1965): Acta Embryol. Morph. Exp. (Palermo), **8**, 197.
5. Bennett, T. P. and Frieden, E. *in* Florkin, M. and Mason, H. S., eds. (1962): *Comparative Biochemistry*, vol. 4, p. 483. New York: Academic Press.
6. Bertaccini, G. (1960): J. Physiol. (Lond.), **153**, 239.
7. Campbell, A. C. P. *in* Collins, D. H., ed. (1959): *Modern Trends in Pathology*. London: Butterworths.
8. Campbell, H. J. and Eayrs, J. T. (1965): Brit. Med. Bull., **21**, 81.
9. Carlier, J., Lejeune-Ledant, G. and Keil, C. (1960): J. Physiol. (Paris), **52**, 42.
10. Carlsson, A., Falck, B., Fuxe, K. and Hillarp, N. A. (1964): Acta physiol. scand., **60**, 112.
11. Carlsson, A., Magnusson, T. and Rosengren, E. (1963): Experientia (Basel), **19**, 359.
12. Czerny, K. and Misiuna, P. (1967): Z. mikr.-anat. Forsch., **77**, 571.
13. Dawson, A. B. (1948): Anat. Rec., **100**, 319.
14. Dawson, A. B. and Barnett, J. (1944): Stain Technol., **19**, 115.
15. Dawson, A. B. and Meyer, S. L. (1948): Anat. Rec., **100**, 493.
16. Del Greco, F., Masson, G. M. C. and Corcoran, A. C. (1956): Amer. J. Physiol., **187**, 509.
17. Deyrup, I. J. *in* Moore, J. A., ed. (1964): *Physiology of the Amphibia*, p. 251. New York: Academic Press.
18. Douglas, W. W. *in* Goodman, L. S. and Gilman, A., eds. (1965): *The Pharmacological Basis of Therapeutics*, p. 644. New York: Macmillan.
19. Eiduson, S. (1966): J. Neurochem., **13**, 923.
20. Eros, G. (1928): *Op. cit.*[32]
21. *Idem* (1930): *Ibid.*, **40**, 155.
22. Erspamer, V. (1938): Anat. Anz., **86**, 379.
23. *Idem* (1939): Z. Anat. Entwickl.-Gesch., **109**, 586.
24. *Idem* (1953): Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak., **218**, 92.
25. *Idem* (1954): Acta pharmacol. (Kobenhavn), **10**, 1.
26. *Idem* (1954): *Ciba Foundation Symposium on Hypertension*, p. 78. London: Churchill.
27. *Idem* (1954): Pharmacol. Rev., **6**, 425.
28. *Idem* (1955): J. Physiol. (Lond.), **127**, 118.
29. *Idem* (1959): Paper presented at the XXIst International Congress of Physiological Science, Buenos Aires, August.
30. *Idem* (1961): Fortschr. Arzneimittel-Forsch., **3**, 151.
31. Erspamer, V. and Asero, B. (1952): Nature (Lond.), **169**, 800.
32. Etkin, W. (1963): Science, **139**, 810.
33. Etkin, W. B. *in* Moore, J. A., ed. (1964): *Physiology of the Amphibia*, p. 427. New York: Academic Press.
34. Faustini, R. (1955): Amer. J. Vet. Res., **16**, 397.
35. Fox, H. and Turner, S. C. (1967): Arch. Biol., **78**, 61.
36. Freedland, R. A., Wadzinski, I. M. and Waisman, H. A. (1961): Biochem. Biophys. Res. Commun., **6**, 227.
37. Gaddum, J. H. and Giarman, N. J. (1956): Brit. J. Pharmacol., **11**, 88.
38. Gal, E. M., Poczik, M. and Marshall, F. D. (1963): Biochem. Biophys. Res. Commun., **12**, 39.
39. Garattini, S. and Valzelli, L. (1965): *Serotonin*. Amsterdam: Elsevier.
40. Gershon, M. D., Drakontides, A. B. and Ross, L. L. (1965): Science, **149**, 197.
41. Ghidini, G. M. (1939): Anat. Anz., **89**, 197.
42. Hamperl, H. (1952): Virchows Arch. path. Anat., **321**, 482.
43. Haverback, B. J. and Davidson, J. D. (1958): Gastroenterology, **35**, 570.
44. Heller, A., Seiden, L. S., Porcher, W. and Moore, R. Y. (1966): J. Neurochem., **13**, 967.
45. Janes, R. G. (1934): J. Exp. Zool., **67**, 73.
46. Johansson, H. (1966): Acta chir. scand., suppl. 359.
47. Kehl, R., Dumont, L. and Czyba, J. C. (1961): C.R. Soc. Biol. (Paris), **115**, 1667.
48. Kehl, R., Dumont, L., Czyba, J. C. and Germain, A. (1960): *Ibid.*, **154**, 1791.
49. Kivalo, E., Marjanen, P. and Rinne, U. (1958): Acta endocr. (Kbh.), **28**, 553.
50. Kuntz, A. (1924): J. Morph., **38**, 581.
51. Lembeck, F. (1958): Pflügers Arch. ges. Physiol., **265**, 567.
52. Lewis, G. P., ed. (1958): *5-Hydroxytryptamine*. London: Pergamon Press.
53. Monesi, V. (1960): Acta anat. (Basel), **41**, 97.
54. Munro, A. F. (1953): Biochem. J., **54**, 29.
55. Niaussat, P. and Niaussat, M. (1960): *Op. cit.*[39]
56. Nieuwkoop, P. D. and Faber, J. (1956): *Normal Table of Xenopus laevis (Daud.)*, 1st ed. Amsterdam: North Holland Publishing Company.
57. Pearse, A. G. E. (1960): *Histochemistry: Theoretical and Applied*, 2nd ed., p. 919. London: Churchill.
58. Ratzenhofer, M. (1966): Klin. Wschr., **44**, 109.
59. Sharman, D. F. (1965): Brit. Med. Bull., **21**, 62.
60. Singh, I. (1963): J. Anat. (Lond.), **97**, 377.
61. *Idem* (1965): Z. Zellforsch., **67**, 338.
62. Smith, A. N. (1960): J. Roy. Coll. Surg. Edinb., **6**, 34.
63. Smith, S. E., Stacey, R. S. and Young, I. M. (1962): Proceedings of the 1st International Pharmacological Meeting, vol. 8, p. 101.
64. Sodeberg, U. (1958): Acta physiol. scand., **42**, suppl. 147.
65. Sollmann, T. (1957): *A Manual of Pharmacology and its Applications to Therapeutics and Toxicology*. Philadelphia: W. B. Saunders.
66. Tissari, A. (1966): Acta physiol. scand., **67**, suppl. 265.
67. Turner, C. D. (1966): *General Endocrinology*, p. 167. Philadelphia: W. B. Saunders.
68. Udenfriend, S., Shore, P. A., Bogdanski, D. F., Weissbach, H. and Brodie, B. B. (1957): Progress in Hormone Research, **13**, 1.
69. Udenfriend, S., Weissbach, H. and Bogdanski, D. F. (1957): J. Biol. Chem., **224**, 803.
70. Uggeri, B. (1938): Z. Zellforsch., **28**, 648.
71. Underhay, E. and Baldwin, E. (1955): Biochem. J., **61**, 544.
72. Vanable, J. W. (1964): Develop. Biol., **10**, 331.
73. Van de Veerdonk, F. C. G. (1960): Nature (Lond.), **187**, 948.
74. Vialli, M. and Erspamer, V. (1933): Z. Zellforsch., **19**, 743.
75. *Idem* (1936): Arch. ital. Anat. Embriol., **37**, 411.
76. Voitkevich, A. A. (1962): Gen. Comp. Endocr., suppl. 1, 133.
77. Zizine, L. (1959): C.R. Soc. Biol. (Paris), **153**, 1156.