

direct action of calciferol on these tissues, since this vitamin also exerts the same inhibitory effect when added *in vitro* to homogenates of appropriate tissues.

The reduction of ATPase activity in tissues of calciferol-intoxicated rats appears to be a result of a biochemical lesion underlying metastatic calcification. This view is supported by the finding of Grant *et al.*⁴ that 2:4-dinitrophenol stimulates the activity of ATPase but depresses the degree of mineralization induced in soft tissues by calciferol. Further evidence in support of this concept is the correlation between the degree of calcification of various tissues in calciferol intoxication and the degree of inhibition of ATPase in these tissues. Grant *et al.*³ showed that the tissues of rats could be arranged in the following order of intensity of calcification: aorta > lung > stomach. The tissues in our experiments could be arranged in the same order for the degree of inhibition of ATPase activity by vitamin D₂ *in vivo*.

It is well known that a large fraction of magnesium-stimulated ATPase activity is involved in oxidative phosphorylation^{7,12}. The inhibition of ATPase activity by vitamin D₂ may possibly be due to impairment of oxidative phosphorylation by large doses of this vitamin. This view is supported by the fact that EDTA exerting stabilizing and coupling effects on oxidative phosphorylation^{13,14} has been found in our experiments to reduce the inhibitory effect of calciferol on ATPase activity (see Table 1).

In good agreement with this view is the recent finding that the administration of large doses of vitamin D₂ could cause uncoupling of oxidative phosphorylation in some rat tissues¹⁵. This effect of vitamin D₂ may be a result of its ability to damage the structural integrity and function of lipoprotein membranes¹⁶ and (or) to a direct interaction with some components of the respiratory chain. Our findings supporting the latter possibility will be published elsewhere.

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- ¹ Vanderveer, H. L., *Arch. Pathol.*, **12**, 941 (1931).
² Hass, G. M., Trueheart, R. E., Taylor, C. B., and Stumpe, M., *Amer. J. Pathol.*, **34**, 395 (1958).
³ Grant, R. A., Gillman, T., and Hathorn, M., *Brit. J. Exp. Pathol.*, **44**, 220 (1963).
⁴ Grant, R. A., Hathorn, M., and Gillman, T., *Biochem. J.*, **81**, 352 (1961).
⁵ Chappell, J. B., and Perry, S. V., *Nature*, **173**, 1094 (1954).
⁶ Myers, D. K., and Slater, E. C., *Biochem. J.*, **67**, 558 (1957).
⁷ Hülsmann, W. C., and Slater, E. C., *Nature*, **180**, 372 (1957).
⁸ Cooper, C., *Biochim. Biophys. Acta*, **30**, 529 (1958).
⁹ Dragneva, L. A., Ysaeva, V. A., Blazhevich, N. V., and Spirichev, V. B., *Prob. Nutrit.* (in the press).
¹⁰ King, E. J., *Biochem. J.*, **26**, 292 (1932).
¹¹ de Duve, C., Wattiaux, R., and Wibo, M., *Proc. Intern. Pharmacol. Meeting*, **5**, 97 (1963).
¹² Ernster, L., Ljunggren, M., and Lindberg, O., *Acta Chem. Scand.*, **8**, 658 (1954).
¹³ Slater, E. C., and Cleland, K. W., *Nature*, **170**, 118 (1952).
¹⁴ Jacobs, E. E., and Sanadi, D. R., *Biochim. Biophys. Acta*, **38**, 12 (1960).
¹⁵ Schipizina, L. P., *Proc. Symp. D-Hypervitaminosis, Moscow*, 3 (1965).
¹⁶ Blazhevich, N. V., and Spirichev, V. B., *Prob. Med. Chem.* (in the press).

Biogenic Amines and Active Polypeptides in the Skin of Australian Amphibians

In the course of an extensive study of biogenic amines and active polypeptides in the skins of amphibians collected throughout the world, extracts of the skin of a number of Australian species were subjected to paper chromatographic and biological screening. Research on the Australian species is still in progress and this report is intended to communicate only some preliminary results.

So far, the dried skins of fourteen amphibian species have been examined for their content of aromatic biogenic amines (indolealkylamines, imidazolealkylamines and phenylalkylamines) and the dried skins of eighteen

species for their content of active polypeptides (bradykinin-like polypeptides, physalaemin-like polypeptides and other polypeptides active on blood pressure and/or on smooth muscle).

Results of analyses of biogenic amines present in the skins of fourteen species are summarized in Table 1. Indolealkylamines, represented not only by 5-hydroxytryptamine (5-HT) but also by its N-methyl derivatives, were found in 9-10 of the species examined. Only *Hyla caerulea* and the related *Hyla infrafrenata* contained important amounts of histamine. Leptodactyline (*m*-hydroxyphenylethyltrimethylammonium) was not detected in any of the species examined.

Table 1. CONTENT OF BIOGENIC AMINES IN SOME AUSTRALIAN AMPHIBIANS (in μg base per g dry skin)

Species	Indolealkylamines			Histamine	Leptodactyline
	5-HT	N-methyl-5-HT	Bufotenine		
<i>Hyla pearsoniana</i>	50	0	750-2,500	0	0
<i>Hyla peroni</i>	80-250	12	10-15	0	0
<i>Hyla lesueuri</i>	180	0	5	0	0
<i>Hyla latopalmata</i>	30	=	=	=	=
<i>Hyla rothi</i>	40	0	0	170	0
<i>Hyla infrafrenata</i>	500	0	0	170	0
<i>Hyla caerulea</i>	210-350	0	0	140-320	0
<i>Adelotus brevis</i>	0	0	0	0	0
<i>Mixophyes fasciolatus</i>	0	0	0	0	0
<i>Limnodynastes peroni</i>	0	0	0	0	0
<i>Limnodynastes fletcheri</i>	0	0	0	0	0
<i>Limnodynastes ornatus</i>	2-3(?)	0	0	0	0
<i>Cyclorana uboguttatus</i>	30-35	175	0	0	0
<i>Lechriodus fletcheri</i>	15	0	0	0	0

0, not detectable (< 1-2 $\mu\text{g}/\text{g}$).
=, not investigated.

The skins of the species listed in Table 1 together with those of four additional species (*Hyla nasuta*, *Hyla gracilentata*, *Hyla rubella* and *Limnodynastes dorsalis*) were submitted to screening for polypeptides. It was found that bradykinin-like and physalaemin-like polypeptides were either completely lacking or present in amounts which were always less than 1 μg and usually less than 0.1 μg per g dry skin. A new polypeptide was discovered, however, in the skin of several species of *Hyla*. This polypeptide possessed, in addition to conspicuous effects on some external secretions, a potent, relatively long-lasting hypotensive action in the dog and other mammals and, of special interest, sometimes marked, spasmogenic actions on extravascular smooth muscle. It was present in large amounts in the skins of *Hyla caerulea* and *Hyla infrafrenata*, in lesser concentrations in the skins of *Hyla rothi*, *Hyla lesueuri*, *Hyla gracilentata*, and was possibly also present in the skins of *Hyla verreauxi* and *Hyla bicolor*—two species which require further investigation.

Investigation of the new polypeptide, for which the name "caerulein" is suggested, is in progress and its amino-acid composition and sequence have almost been established. The polypeptide is quite different, both from a chemical and pharmacological point of view, from all other known polypeptides which have an active effect on smooth muscle.

It is hoped that, when completed, this research will be of interest not only from the point of view of comparative biochemistry and pharmacology but also from that of biochemical taxonomy.

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