stance of unknown identity. Available indirect evidence would suggest that the MSS is not ACTH[1] because (1) ACTH is only about $^{1-2}/_{100}$ as active as MSH in stimulating melanin dispersion[9,10], (2) corticoids do not influence background adaptation although they are known to inhibit ACTH secretion[11] and (3) it is difficult to understand the survival advantage of activating the adrenals each time the frog is required to dark adapt, and vice versa. Furthermore, our results suggest an inhibitory regulation of the secretion of the frog MSS, whereas ACTH secretion in the mammal is regulated by a releasing factor. Since the regulation of MSS secretion appears to be inhibitory and similar studies have provided identical evidence for the inhibitory regulation of MSH secretion by the pars intermedia[7,8] it is possible that the melanocyte-stimulating substance of the pars distalis is MSH.

The presence of a MSS in the hypothalamus has been reported previously[7,8,12] and we[7] have suggested that the accumulated MSS is indicative of a feedback control loop in which the circulating MSS regulates the secretion of the inhibitor to MSH secretion. The lack of MSS in the cortical extracts lends further support to this suggestion. The ability of cortical extracts to inhibit MSS secretion could be due to the catecholamines found in the brain[4].

Further study of the neuroendocrine control of frog background adaptation will require the determination of the identity of the MSS of the pars distalis and the identity of the inhibiting substance(s) of the hypothalamus and cortex.

Zusammenfassung. Nachweis, dass die pars distalis der Hypophyse des Leopardfrosches *Rana pipiens* eine Melanozyten stimulierende Substanz enthält, die in vitro abgesondert wird. Wässerige Extrakte aus Hypothalamus und Kortex des Frosches hemmen die Ausscheidung der die Melanozyten stimulierenden Substanz in vitro.

B. B. Bercu and H. J. Brinkley

Department of Zoology,
University of Maryland,
College Park (Maryland 20742, USA), 10 January 1969.

[9] W. O. Reinhardt, I. I. Geschwind, J. O. Porath and C. H. Li, Proc. Soc. exp. Biol. Med. *80*, 439 (1952).
[10] H. B. F. Dixon, Biochem. Biophys. Acta *34*, 251 (1959).
[11] W. D. Odell and G. T. Ross, Endocrinology *73*, 647 (1963).
[12] C. L. Ralph and S. C. Peyton, Gen. comp. Endocr. *7*, 363 (1966).

In Vitro Metabolism of Progesterone by the Adrenals of Spontaneously Hypertensive Rats

Spontaneous hypertension (SH) has been induced in rats by prolonged inbreeding or cross-breeding[1,2]. The incidence of hypertensive disease in these rats was reported originally to be as high as 100%, and according to Laverty and Smirk[3] the levels of blood pressure become progressively higher with successive generations. The etiology of SH is unknown, although is not dependent on high salt intake, unlike other forms of experimentally produced hypertension, such as hypertension induced by high saline intake, by adrenal regeneration, and by administration of deoxycorticosterone (DOC)[4,5]. Bilateral adrenalectomy lowers the blood pressure in both normal and SH rats but does not modify the preoperative pressure difference between the 2 groups[6].

We have compared the conversion of labelled progesterone by incubated adrenal glands from normal and SH rats in order to establish whether the corticosteroid biosynthetic pattern differs in the 2 groups.

Materials and methods. SH rats, Wistar descendants, were generously donated by Dr. C. T. Hansen, NIH. Female first generation descendants from these animals were used, and Wistar female rats of similar weight obtained from a local breeder served as controls. The mean blood pressure of the hypertensive rats was 200 ± 4 mm Hg (SE; $n = 6$). The animals were decapitated after Nembutal anesthesia (5 mg/100 g) and the adrenals quartered, preincubated for 45 min and then incubated for 4 h in 2 ml Krebs-Ringer bicarbonate glucose supplemented medium, under an atmosphere of 95% O_2 – 5% CO_2, in the presence of 4.84×10^5 dpm of 4-^{14}C-progesterone (SA 2 mC/mg, New England Nuclear). Incubations were performed in quadruplicate. The media were extracted 3 times with dichloromethane, the extracts were chromatographed in the toluene-propylene glycol system[7] and the effluent containing DOC and progesterone was developed in the Bush B3 system[8]. The radioactivity present in the steroid fractions corresponding to 18-hydroxycorticosterone (18-OH-B), aldosterone, 18-hydroxydeoxycorticosterone (18-OH-DOC), corticosterone, DOC and unmetabolized progesterone, was measured by liquid scintillation counting, after derivative formation and multiple chromatography. The methods used for steroid purification have been published elsewhere[9,10]. All the reported values have been corrected for procedural losses from the recovery, at the last stage of purification, of non-radioactive standards added immediately after incubation. The recovery of 18-OH-B was assumed to equal that of 18-OH-DOC, since no pure standard was available.

Results and discussion. Table I gives the organ weights of rats of approximately the same body weight (SH 214 ± 4 g, controls 238 ± 2 g, $n = 6$). No significant differences were observed in kidney, thymus or adrenal weight, but the heart weights were increased in SH rats ($P < 0.001$).

Table II presents the per cent radioactivity recovered in 6 steroid fractions, which was remarkably similar for both groups of rats. These results are in marked contrast with previous findings on other forms of experimental

[1] K. Okamoto and K. Aoki, Jap. Circul. J. *27*, 282 (1963).
[2] F. H. Smirk and W. H. Hall, Nature *182*, 727 (1958).
[3] R. Laverty and F. H. Smirk, Circul. Res. *9*, 455 (1961).
[4] W. J. Louis, S. Spector, R. Tabei and A. Sjoerdsma, Lancet *1013* (1968).
[5] A. J. Plummer, in *Antihypertensive Agents* (Ed. E. Schlittler; Acad. Press, New York 1967).
[6] J. Nolla-Panades and F. H. Smirk, Australas. Ann. Med. *13*, 320 (1964).
[7] R. B. Burton, A. Zaffaroni and E. H. Keutmann, J. biol. Chem. *188*, 763 (1951).
[8] I. E. Bush, Biochem. J. *50*, 370 (1952).
[9] A. F. de Nicola, J. T. Oliver and M. K. Birmingham, Endocrinology *83*, 171 (1968).
[10] A. F. de Nicola and M. K. Birmingham, J. clin. Endocr. Metab. *28*, 1380 (1968).

hypertension in rats, i.e., adrenal regeneration hypertension (which showed decreased aldosterone and 18-OH-B production together with increased production of 18-OH-DOC[9,11]) and renal hypertension due to clamping of one renal artery, in which increased formation of aldosterone has been observed[12].

Table I. Body and organ weights of normal and spontaneously hypertensive rats

	Normal rats	SH rats
Kidney (g; $n = 12$)	0.835 ± 0.017	0.871 ± 0.023
Heart (g; $n = 6$)	0.715 ± 0.020	0.965 ± 0.038
Thymus (g; $n = 6$)	0.396 ± 0.045	0.401 ± 0.037
Adrenal (mg; $n = 12$)	26.45 ± 2.28	29.95 ± 1.17

Table II. Metabolism of 4-^{14}C-progesterone by adrenal glands of normal and spontaneously hypertensive rats

	% of ^{14}C-progesterone added	
	Normal glands	SH glands
18-Hydroxycorticosterone	3.91 ± 0.34	3.85 ± 2.32
Aldosterone	2.62 ± 0.44	2.68 ± 0.41
18-Hydroxydeoxycorticosterone	6.26 ± 0.52	6.66 ± 0.53
Corticosterone	17.32 ± 1.65	18.77 ± 1.85
Deoxycorticosterone	3.87 ± 0.68	4.29 ± 1.33
Progesterone[a]	6.70 ± 0.79	6.37 ± 1.44

Results are expressed as Mean $\pm$ S.E.; $n = 4$. [a] Unmetabolized substrate at the end of incubation.

The apparently normal metabolism of progesterone by the adrenal glands of SH rats suggests that the adrenal cortex is not involved in the etiology of this form of hypertension. LOUIS et al.[4] have reported an increased accumulation of ^{3}H-noradrenaline in the heart of the SH rat, accompanied by normal endogenous levels of cardiac noradrenaline, but these changes were regarded as secondary to the hypertension rather than primary events, which remain as yet unknown.

Zusammenfassung. Der Einbau des ^{14}C-Progesterons in Corticosteroide bei Nebennierensegmenten von Wistar-Ratten mit spontaner Hypertonie unterscheidet sich in nichts vom Einbau bei Nebennieren der Kontrollratten, was im Gegensatz zu Befunden mit regenerierten Nebennieren steht. Eine kausale Beziehung zwischen Nebennierenrindenfunktion und spontanem Hochdruck scheint ausgeschlossen zu sein.

A. F. DE NICOLA, J. T. OLIVER and M. K. BIRMINGHAM[13]

Allan Memorial Institute of Psychiatry, McGill University, Montreal and Pharma Research, Ltd., Pointe Claire (Quebec, Canada), 5 May 1969.

[11] P. VECSEI, D. LOMMER, V. KEMENY, K. FARKAS and H. P. WOLFF, Kongress innere Medizin *71*, 366 (1965).
[12] J. MULLER and F. GROSS, Acta Endocr. *60*, 669 (1969).
[13] Dr. M. K. BIRMINGHAM is a Medical Research Associate of the Medical Research Council of Canada. This work was supported by grants from the Medical Research Council of Canada and the 33rd Degree Scottish Rite Committee for Research in Dementia Praecox. The authors thank Dr. R. A. CLEGHORN for his interest in this project.

Evolution of Nucleic Acids and Proteins in the Course of Regeneration in an Oligochaete (*Aulophorus furcatus*)[1]

Although many papers have been published on the histology of regeneration in Oligochaete worms, biochemical data on this phenomenon are scanty[2]. These worms, and especially the Limicola Oligochaete *Aulophorus furcatus* (Oken), a Naididae, present clear advantages for work in regeneration. The Naididae not only easily regenerate a part that has been cut off but show asexual reproduction in which chains of two to four members are formed, each member then separating as a distinct individual. It is thus easy to obtain clones of genetically uniform material. Our work was made on such clones, obtained from a single individual cultured in the laboratory[3]. Regeneration is obtained by cutting with a von Graefe scalpel the head or the tail part of a demi-anaesthetized worm placed in a watchmaker glass, where the worm is left for regeneration[4]. Regeneration takes place in about 2 days for the tail region or in about 4 days for the head region.

Nucleic acids and proteins were determined at regular intervals in worms in the course of regeneration. Nucleic acids were determined according to BRISTOW and DEUCHAR[5]. Lipid extraction was prolonged to 18 hours and DNA (deoxynucleic acid) extraction to 20–30 min[6]. Total proteins were determined by the method of LOWRY et al.[7], modified by FISZER[7]. Individual worms of a same clone placed in a stoppered glass tube were weighed in a torsion balance. Dried weight was determined after dehydration at 110 °C till constant weight. Results are reported to optical density of solutions of known concentration of calf thymus DNA, yeast RNA (ribonucleic acid) and egg albumin, employing a Beckman DK spec-

[1] This work was supported by a grant from Instituto de Alta Cultura, Lisbon, Portugal.
[2] For references see V. KIORTSIS and H. A. L. TRAMPUSCH, *Proceedings on Regeneration in Animals* (North-Holland Publishing Co., Amsterdam 1965).
[3] Methods described in E. MARCUS, Boln Univ. S. Paulo Zool. *7*, (1943) and R. H. FOULKES, Biol. Bull. mar. biol. Lab. Woods Hole *105*, 80 (1953). The worms were from clones obtained from Santarém (Portugal).
[4] H. HERLANT-MEEWIS, Archs Biol. *65*, 73 (1954).
[5] D. A. BRISTOW and E. M. DEUCHAR, Expl Cell. Res. *35*, 580 (1964).
[6] H. N. MUNRO and A. FLECK, *Methods of Biochemical Analysis* (Ed. D. GLICK; Interscience, New York 1966), *14*, p. 113.
[7] O. H. LOWRY, N. J. ROSENBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951); B. FISZER, Bull. Soc. Chim. biol. *46*, 403 (1964).