

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 observed¹².

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 4-¹⁴C-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 ³H-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 ¹⁴C-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.

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¹¹ P. VECSEI, D. LOMMER, V. KEMENY, K. FARKAS and H. P. WOLFF, Kongress innere Medizin 71, 366 (1965).

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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 scanty². 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 laboratory³. 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 regeneration⁴. 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 DEUCHAR⁵. Lipid extraction was prolonged to 18 hours and DNA (deoxynucleic acid) extraction to 20–30 min⁶. Total proteins were determined by the method of LOWRY et

al.⁷, modified by FISZER⁷. 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. ROSENBOUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. 193, 265 (1951); B. FISZER, Bull. Soc. Chim. biol. 46, 403 (1964).

trophotometer. Optical density was determined between 220–300 nm wavelength for nucleic acids and at 750 nm for proteins.

Results obtained are expressed in $\mu\text{g}/\text{mg}$ worm dry weight for DNA, RNA and proteins. During the first day after operation, a small decrease in the contents of DNA is observed (Table and Figure 1) which persists till the 3rd day of regeneration. The DNA value shown before regeneration is then regained slowly. RNA varies more than DNA during regeneration. RNA concentration decreases sharply during the day after the worm was cut, and increases afterwards rather rapidly during the following 2 days and less rapidly during the 4th and 5th days.

Change in protein concentration (Table and Figure 2) parallels that of RNA during the first day after the opera-

ported⁸ that the concentration of acid peptidases increases by about 80% during the first 2 days of regeneration in another Oligochaete, *Tubifex tubifex*.

DNA appears not to take part in the turnover of the other 2 main cell components during regeneration. Probably it is through dilution, because it is being used up in the mitoses that take place during regeneration, that DNA slightly decreases during the second day of the process. It seems likely that the split fractions of proteins and RNA are used to build the cell components that are rapidly being formed in the region in regeneration. The process may be further discussed in connection with cytological findings after more data on fractionation of proteins and nucleic acid components are obtained, which are under way⁹.

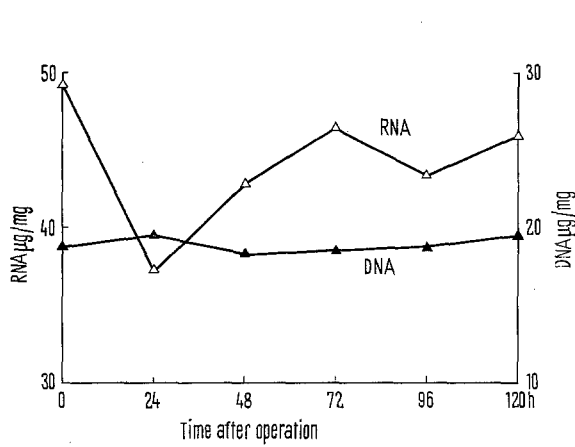


Fig. 1. Variation of DNA and RNA during regeneration.

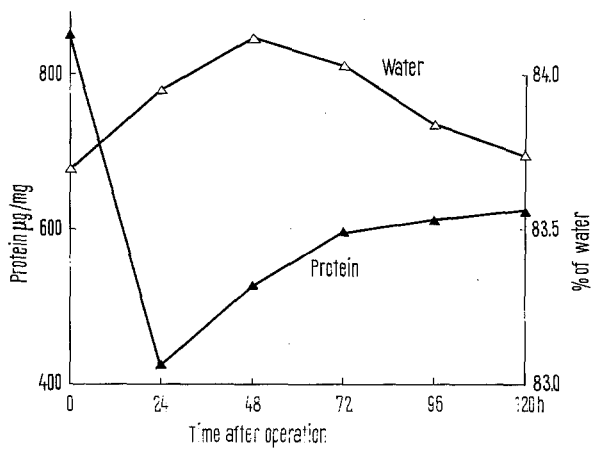


Fig. 2. Changes in protein concentration and percentage of water during regeneration.

Nucleic acids and proteins referred to dry weight, in $\mu\text{g}/\text{mg}$

	Normal	Worms in regeneration				
		24 h	48 h	72 h	96 h	120 h
DNA	19.076 ± 1.08	19.607 ± 0.73	18.45 ± 0.825	18.53 ± 1.145	18.77 ± 0.89	19.80 ± 0.917
RNA	49.45 ± 1.54	37.29 ± 1.58	42.78 ± 1.69	47.40 ± 2.39	43.59 ± 1.34	45.84 ± 1.24
Proteins	849.69 ± 25.95	425.55 ± 17.9	528.96 ± 12.2	599.25 ± 29.7	619.67 ± 31.97	625.0 ± 36.14
% of water	83.70	83.95	84.12	84.03	83.83	83.76

tion. According to determinations at 16 h, this decrease takes place rather slowly. Increase up to normal takes place gradually from the 2nd to the 5th day after the operation. Hydration varies slightly but clearly during regeneration, the maximum being observed at the second day.

Taking into account hydration, RNA concentration is found to decrease after operation to about $\frac{3}{4}$ of the initial RNA value. During the same period proteins decrease to about $\frac{1}{2}$ of their initial value and DNA decreases by only about 3% of its initial value during the 2 days that follow the operation. These results may be interpreted by assuming that in a first phase of the process of regeneration a turnover of proteins and RNA takes place in which these substances are partially split to soluble fractions (small polypeptides or amino acids, and oligonucleotides or nucleotides). In agreement with this interpretation as to the proteins, it has been re-

Résumé. L'étude quantitative des acides nucléiques et des protéines au cours de la régénération du ver Oligochaète *Aulophorus furcatus* montre qu'après 24 h le taux de RNA décroît de 25% et celui des protéines de 50%. Le taux de DNA décroît d'environ 3% en 48 h. Le 5e jour de régénération les valeurs normales sont rétablies.

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⁸ F. CECERE, Acta med. romana 2, 91 (1964).

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