

fixant a ruxibosomes, interfère avec la fixation de l'ARN messager sur les ribosomes empêchant ainsi la synthèse des protéines. Cette activité inhibitrice est liée à la structure du chloramphénicol. C'est ainsi que le dérivé L-thréo du chloramphénicol n'inhibe pas la synthèse des protéines¹⁷ alors que le thiophénicol a conservé cette aptitude^{19,20}. Ces deux agents sont toutefois dépourvus d'effets végétalisants chez l'oursin. Il apparaît donc que la configuration D-thréo de la chaîne latérale et la présence du groupe nitro fixé sur le noyau benzénique sont nécessaires à l'activité végétalisante. Ces considérations nous conduisent à suggérer l'hypothèse suivante pour rendre compte de l'activité végétalisante du chloramphénicol: l'inhibition des synthèses protéiques serait nécessaire pour obtenir un effet végétalisant mais cette inhibition serait orientée par le groupe nitro responsable de la fixation de la molécule de chloramphénicol sur un site déterminé des ribosomes ou sur un type particulier de ribosomes. Les ribosomes dont le fonctionnement est ainsi inhibé par la fixation du chloramphénicol seraient responsables de la synthèse des protéines impliquées dans la différenciation des structures ectodermiques²¹.

Summary. The effects of L-threo-chloramphenicol and thiophenicol on the differentiation of sea-urchin eggs are

compared with those exerted by D-threo-chloramphenicol. Only D-threo-chloramphenicol caused vegetalization; L-threo-chloramphenicol inhibited development. These effects resemble those of nitrobenzene. Thiophenicol, differing from D-threo-chloramphenicol by replacing the nitro group with a methylsulphonyl group, slows down development at high concentrations. The effects of chloramphenicol on oxidative phosphorylation and synthesis of proteins are discussed. It is suggested that both the steric configuration type D-threo of the side chain and the presence of a nitro group are necessary for the vegetalizing activity.

R. LALLIER

*Station Zoologique, 06 Villefranche-sur-Mer (France),
30 juin 1966.*

²⁰ A. S. WEISBERGER, S. WOLFE et S. ARMENTROUT, *J. exp. Med.* 120, 161 (1964).

²¹ Remerciements. Je remercie le Dr. G. HAGEMANN des Services Scientifiques des Laboratoires Roussel-Uclaf pour un don de L-thréo-chloramphénicol et les Laboratoires Clin-Comar qui ont mis le Thiophénicol à ma disposition.

Rate of Fall in Choline Acetyltransferase Activity in Denervated Diaphragms as Dependent on the Length of the Degenerating Nerve

The supersensitivity to chemical agents which develops in denervated skeletal muscle, has been found to appear earlier when the motor nerve is cut close to the muscle than when it is severed at some distance from it^{1,2}. Thus in isolated strips of diaphragms of rats, a much higher supersensitivity to acetylcholine could be demonstrated 4 days after denervation when the phrenic nerve of the anaesthetized rat had been cut near its entrance into the muscle than when cut in the cranial part of the thorax². Denervation supersensitivity in cells supplied with cholinergic nerves has been assumed to be due to the loss of some normal action of acetylcholine^{3,4}, and it was therefore suggested that the normal acetylcholine release from the endings of the cut nerve might cease earlier the shorter the degenerating nerve stump².

In the present experiments an attempt has been made to see whether the acetylcholine synthesizing capacity declines more rapidly when the peripheral stump of the transected nerve is short than when it is long. The diaphragm of the rat was chosen for these experiments since comparisons could be made with our previous observations on supersensitivity, and since choline acetyltransferase activity has been demonstrated in the rat diaphragm; section of the phrenic nerve was found by HEBB, KRNJELIĆ and SILVER⁵ to cause a marked fall in the enzyme activity in this tissue.

Male rats weighing between 160 and 360 g were anaesthetized with pentobarbitone (60 mg/kg i.p.). To suppress bronchial secretion, atropine sulphate, 1 mg/kg, was in addition injected by the same route. Artificial respiration was given by a pump through a fine glass tube inserted into a small cut in the trachea. The right phrenic nerve

was divided between 2 ribs either just above the diaphragm or as high as possible within the thorax. After 24, 48 or 72 h the rats were killed with ether, and the diaphragms cut out, cleaned and divided into right and left hemidiaphragms. These were washed in Ringer solution, dried between gauze pads, and weighed. Acetone powder was then prepared from them and used for estimation of the choline acetyltransferase activity according to the method of HEBB (see NORDENFELT⁶). Acetylcholine formed on incubation was assayed in duplicate samples on the frog rectus preparation. The choline acetyltransferase activity was expressed as μ g acetylcholine formed/h per hemidiaphragm. Usually 4 litter mates were used in each experiment; in 2 of them the phrenic nerve was cut proximally, in 2 distally. The 2 hemidiaphragms denervated in the same way were pooled and extracted with acetone together, and the activity of this sample was expressed as a percentage of that of 2 corresponding, normal, left hemidiaphragms. In some experiments, however, the acetone powder was made from one hemidiaphragm only, either because the other one was found not to be denervated or because 1 rat had died before the final experiment. Samples were prepared from altogether 40 rats: 12 one day, 16 two days and 12 three days after denervation. The length of the nerve stump in connection with the diaphragm after proximal denervation was found to vary

¹ J. V. LUCO and C. EYZAGUIRRE, *J. Neurophysiol.* 18, 65 (1955).

² N. EMMELIN and L. MALM, *Q. Jl. exp. Physiol.* 50, 142 (1965).

³ H. H. DALE, *Proc. R. Soc. Med.* 28, 319 (1935).

⁴ N. EMMELIN, *Experientia* 21, 57 (1965).

⁵ C. O. HEBB, K. KRNJELIĆ, and A. SILVER, *J. Physiol.* 171, 504 (1964).

⁶ I. NORDENFELT, *Q. Jl. exp. Physiol.* 48, 67 (1963).

Experiment No.	Right nerve cut	No. of rats	Choline acetyltransferase activity, %		
			Right hemi-diaphragm	Left hemi-diaphragm	Right-left
After 1 day					
1	{ Proximal	2	11.8	21.9	53.9
	{ Distal	2	16.1	29.4	54.8
2	{ Proximal	2	10.9	18.5	58.9
	{ Distal	2	9.0	15.0	60.0
3	{ Proximal	2	6.4	11.1	57.7
	{ Distal	2	7.6	11.7	65.0
Mean:	Proximal 56.8%				
	Distal 59.9%				
After 2 days					
4	{ Proximal	2	5.6	24.7	22.7
	{ Distal	2	4.4	28.1	15.7
5	{ Proximal	1	5.3	22.2	23.9
	{ Distal	2	4.6	24.9	18.5
6	{ Proximal	1	4.0	16.6	24.1
	{ Distal	2	3.4	21.0	16.2
7	{ Proximal	2	4.2	21.4	19.6
	{ Distal	2	2.1	20.0	10.5
8	{ Proximal	1	11.3	35.3	32.0
	{ Distal	1	3.9	24.9	15.7
Mean:	Proximal 24.5%				
	Distal 15.3%*				
After 3 days					
9	{ Proximal	2	1.4	17.4	8.0
	{ Distal	2	1.3	18.6	7.0
10	{ Proximal	2	1.0	11.4	8.8
	{ Distal	2	1.0	11.4	8.8
11	{ Proximal	2	2.9	24.4	11.4
	{ Distal	2	2.4	22.0	10.9
Mean:	Proximal 9.6%				
	Distal 8.9%				

* $P < 0.01$.

between 23 and 34 mm. No decrease in weight of the denervated hemidiaphragm, when compared with the contralateral one, was observed during the 3 days after the denervation.

The Table summarizes the results. It can be seen that the choline acetyltransferase activity quickly decreased after denervation. Already after 3 days it was less than 10% of that of the normally innervated side. 40 days after section of the phrenic nerve HEBB et al.⁵ found it to be 3.2–3.7%. The Table shows that 2 days after denervation there was a significant difference ($p < 0.01$) between hemidiaphragms denervated by proximal, and those denervated by distal section of the nerve. It is obvious that, at this stage, the acetylcholine synthesizing power of the degenerating parts of the phrenic nerve within the diaphragm decreases more rapidly when the nerve stump in connection with the muscle is short than when it is long; whether this means that material in some way required for the synthesis is transported along the axon, even when the nerve has been divided, remains a matter of speculation. It is of interest to note that on the fourth day after denervation the supersensitivity of the muscle cells towards acetylcholine is much more advanced when the peripheral nerve stump is short than when it is long^{2,7}.

Zusammenfassung. An narkotisierten Ratten wurde der rechte Nervus phrenicus im Thorax entweder so hoch wie möglich, oder unmittelbar oberhalb des Diaphragms durchschnitten. Zwei Tage später erwies sich die Cholinacetyltransferase-Aktivität in der rechten Hälfte dann wesentlich geringer, wenn der Nerv distal statt proximal durchschnitten wurde.

N. EMMELIN, I. NORDENFELT,
and C. PEREC

*Institute of Physiology, University of Lund (Sweden),
June 22, 1966.*

⁷ Supported by a grant from the Swedish Medical Research Council.

Translocation of Donor DNA into Nucleolar Associated Chromatin of Recipient Cell in vitro

Previous studies in vivo have shown that labelled deoxyribonucleic acid (DNA) released from transfused donor lymphocytes may be incorporated into the nuclei of the recipient cells¹⁻³. The evidence was derived mainly from the appearance of label in differentiated cells that did not synthesize DNA and further from the uneven distribution of label in the nucleus as well as in the cytoplasm of the recipient cells. The data suggested that some amount of macromolecular DNA of the donor was incorporated into the recipient cells without previous degradation to acid-soluble precursors. On the other hand, the re-utilization of DNA degradation products was definitely demonstrated in vivo by ROBINSON and BRECHER⁴ and by BRYANT⁵. Probably both DNA and its metabolites may be re-utilized simultaneously, depending upon the type of experiment (see LEDOUX⁶ for review). The

exact identification of each type of re-utilization seems to be very difficult under in vivo conditions, especially if the re-utilization is followed in the proliferating cell population. For this reason, a system in vitro has been worked out which we shall describe in this communication.

L cells were cultivated in Roux flasks in a modified Parker 199 synthetic medium supplemented with 10% calf serum. The cells grown in monolayer were pulse-labelled for 1 h in a fresh medium containing 10 μ C ³H-

¹ M. HILL, *Expl Cell Res.* 24, 405 (1961).

² M. HILL, *Expl Cell Res.* 28, 21 (1962).

³ W. O. RIEKE, *J. Cell. Biol.* 13, 205 (1962).

⁴ S. H. ROBINSON and G. BRECHER, *Science* 142, 392 (1963).

⁵ B. J. BRYANT, *Expl Cell Res.* 37, 490 (1965).

⁶ L. LEDOUX, in *Progress in Nucleic Acid Research and Molecular Biology* (Ed. J. N. DAVIDSON and W. E. COHN; Academic Press, New York and London 1965), vol. 4, p. 231.