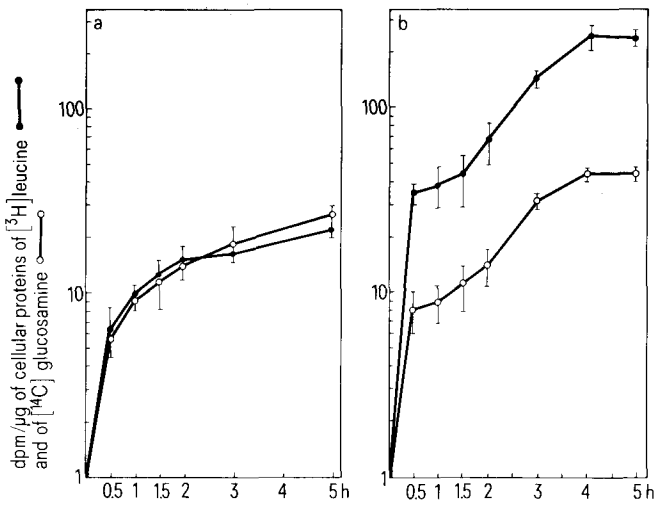
Results and discussion. The cultured fibroblasts, on reaching subconfluency, were elongated and had adhered to the substratum. Trypsin treatment altered these characteristics. Although the cell viability was not affected, the cells were spherical and did not adhere to the substratum immediatly following their treatment by trypsin. After a 30 min culture, 40% of the cells were attached to the substratum. With time this percentage increased, reaching 66 and 73% after a 1.50-h and a 4-h culture respectively (table). It was observed that, as the percentage of cells attached to the substratum increased with the time of culture, there was also a concomitant increase in cell elongation, complete fibroblastic morphology being restored to all cells after a 4-h culture (table).

Cell morphology appeared to be dependent on the adhesion of cells to the substratum. These results are in agreement with those of Maroudas[7] and Martin and Rubin[8], who established that the cell adhesion of chick and mouse fibroblasts affects their morphology and proliferative capacity. Parallel to the adhesion of cells, the incorporation of [^{3}H] leucine and [^{14}C] glucosamine in trypsin-sensitive glycoproteins increased markedly from a 30-min to a 1-h culture. This incorporation was then slight between a 1.50-h and a 4-h culture, almost reaching a plateau (figure, A). These results suggest that the regeneration of trypsin-sensitive glycoproteins was reached within 1.50 h following the trypsin treatment of 8-day fibroblasts. This time appeared to be short in comparison with that observed by other workers[9] using different cells possessing low proliferative capacities. The time involved in the regeneration of cell surface glycoproteins seemed to be dependent on the cell type. However, our results are in agreement with those of Moreau and Bourrillon[10] obtained using Zajdela's tumor cells in primary cultures, suggesting that the proliferative capacity of cells may be associated with the velocity of glycoprotein regeneration. On the other hand, the incorporation of [^{3}H] leucine and [^{14}C] glucosamine in trypsin-treated cells reached a plateau after a 3-h culture (figure, B). This may be due to their incorporation into intracellular glycoproteins and/or cell surface glycoproteins, insensitive to the trypsin action. These results suggest that the regeneration of cell surface glycoproteins following the trypsin-treatment, may be due to their in situ synthesis. Therefore, the restoration of cell adhesivity and the synthesis of the cell surface glycoproteins occured in parallel after culturing for 1.50 h following the treatment of cells with trypsin. In addition, cell morphology appeared to be dependent on these 2 properties, since it was completely restored within 4 h following the trypsin treatment.



Incorporation of [^{3}H] leucine and [^{14}C] glucosamine in *A* The trypsin-sensitive cell surface glycoproteins and *B* in the 8-day chick embryo fibroblasts following trypsin-treatment. Each point represents the value obtained from 4 separate experiments.

1 Acknowledgment. This work was supported by grants from INSERM, ATP No. 43-76-75.
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A promoting action of cyclic GMP on contractions of guinea-pig vas deferens[1]

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Summary. The effect of dbc-GMP has been studied in ductus deferens of the guinea-pig. The nucleotide potentiated the contractions induced by electrical field stimulation and by adrenergic agonists. The site of action was probably in the smooth muscle cell, since the release of excitatory transmittor was not influenced.

Earlier observations have indicated that cholinergic drugs, and other agents which stimulate contraction, also enhance the accumulation of cyclic GMP of isolated smooth muscle preparations[2-5]. It has been suggested that cyclic GMP may be causally involved in the contractile response. In the vas deferens of the rat it was observed that norepinephrine and phenylephrine increased the cyclic GMP level about 2fold after 3 min[6]. Recently Schultz et al.[7] demonstrated that some relaxing agents, such as nitroprusside, also increased the cyclic GMP level of vas deferens. The authors suggested that their results support the hypothesis that cyclic GMP may act as a negative feedback inhibitor of hormonally stimulated calcium influx into cytoplasm. Earlier studies performed on rabbit colon smooth muscle in our laboratory[5,8] are not consistent with this assumption. Our data are more consistent with the suggestion that cyclic GMP may act as a comediator with calcium to promote contraction.

In this work we present some data from vas deferens of guinea-pig according to our earlier suggestions. The dibutyryl derivative of cyclic GMP has been tested on vas deferens of guinea-pigs. For these tests, vasa deferentia were removed and dissected free from adjacent tissue. The

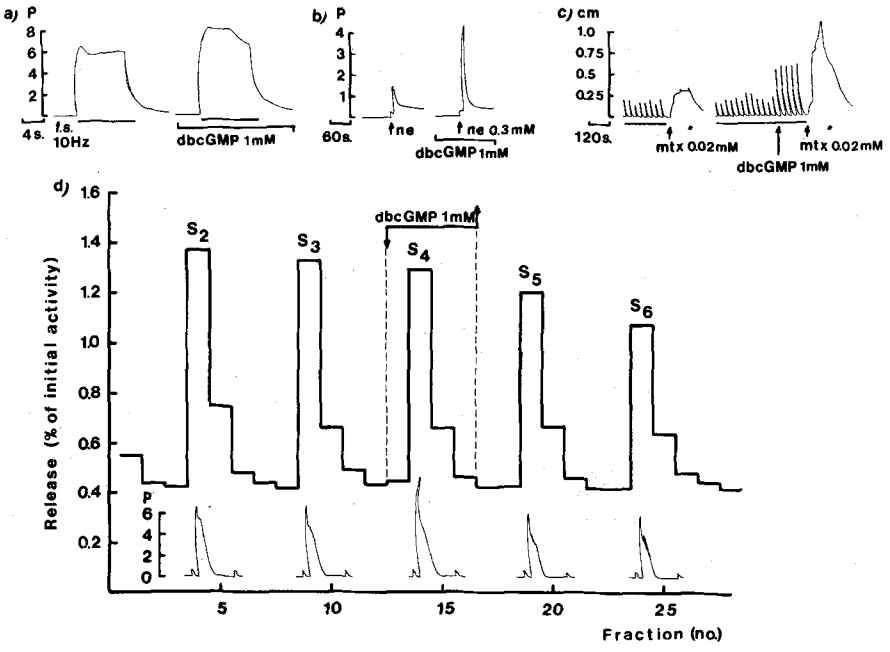

Fig. 1. Isolated guinea-pig vas deferens stimulated electrically or by drugs in the absence or presence of dibutyryl cGMP (dbc-GMP, 1 mM). *a* The response to electrical field stimulation (f.s.) with 100 volleys at 10 Hz (1 ms rectangular pulses at supramaximal current). Isometric registration. *b* The contractile effect of norepinephrine (ne, 0.3 mM added at the arrows). Isometric registration. *c* The response to electrical field stimulation (10 Hz, 5 volleys) at 30 sec intervals and at arrows methoxedrine (mtx, 0.02 mM). Dots indicate washing. Isotonic registration. *d* Isolated, superfused guinea-pig vas deferens, preloaded with ^{3}H-NE. Effect of dibutyryl-cGMP (dbc-GMP, 1 mM) on the response to electrical field stimulation (S) (4 Hz, 400 volleys) during 100 sec. Upper trace release of ^{3}H-NE in % of initial activity calculated to be present in the tissue at the start of the experiment. Lower trace contraction in p. The time elapsed between the washing artifacts present before and after the electrically elicited contractions is 120 sec.

preparations were mounted in organbaths containing Krebs solution oxygenated by 95% O_2+5% CO_2 at 37 °C. The isometric tension was recorded by means of a FT 03 transducer and a Grass polygraph. In some tests, the contractions were recorded isotonically by means of a Brush® isotonic transducer loaded with 0.6 g. Dibutyryl cyclic GMP (dbc-GMP) (1 mM) potentiated the responses to electrical field stimulation (4–10 Hz), norepinephrine (0.03–0.3 mM) and methoxedrine (0.02 mM, figure 1). The potentiation was observed both during isometric and isotonic conditions. As can be seen in figure 1a, dbc-GMP not only caused an increase of peak tension but also a slow-down of the decline of peak tension when 10 Hz stimulation was used. Cyclic GMP (1 mM) was much less effective than dbc-GMP. Sodium butyrate (2 mM) and 5′-GMP (1 mM) were completely ineffective.

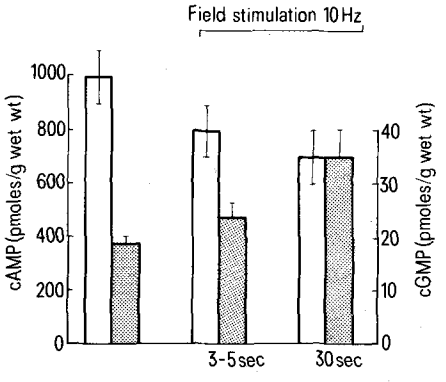

Fig. 2. The effect of electrical field stimulation (10 Hz) on the levels of cyclic AMP (open bars) and cyclic GMP (filled bars) in guinea-pig vas deferens. The preparations were fixed in frigen containing solid CO_2 at −80 °C, homogenized in PCA and chromatographed on AG-1-X8 (200–400 mesh, formate form) columns before the analyses of the cyclic nucleotides[12,13].

In order to test whether the effect of dbc-GMP was of neurogenic or myogenic origin, we investigated the influence of the nucleotide on the release of tritiated norepinephrine (^{3}H-NE) from preparations preloaded with ^{3}H-NE. When the release of the transmitter was studied from vas deferens, the preparation was incubated with 10 µCi ^{3}H-NE (NET-451, 24.4 Ci/mmole, NEN) at a concentration of 0.2 µM for 1 h. After the incubation, the preparation was washed for 1 h with a Krebs solution containing desmethylimipramin (0.6 µM) and normethanephrine (10 µM). 4-ml fractions were collected in 2 min intervals and counted in Instagel® in a liquid scintillation spectrometer. As can be seen in figure 1d, dbc-GMP had no effect on the release of ^{3}H-NE induced by electrical field stimulation (4 Hz, 400 pulses) of vas deferens, although the contractile response was increased. This data is inconsistent with a neurogenic action of dbc-GMP, if it is accepted that the main motor innervation of guinea-pig vas deferens is adrenergic[9]. However, the first part of the contractile response to nerve stimulation, the twitch, does not behave as if it was elicited through an adrenergic mechanism[10]. Therefore an effect on another type of neurotransmitter cannot be excluded. In guinea-pig ileum, e.g., dbc-GMP was believed to stimulate cholinergic neurons[11]. Dibutyryl cGMP, however, potentiated both the initial peak phase and the subsequent plateau phase of a contraction elicited by electrical field stimulation (figure 1, a and d).

In another smooth muscle, rabbit colon, we have presented the theoretical basis for a myogenic action of cyclic GMP[8]. These results prompted us to study the effect of a physiological stimulus on the cyclic nucleotide levels in vas deferens, since in most studies performed on this preparation very high concentrations of hormones and drugs have been used[6,7]. Electrical field stimulation (10 Hz) of the vas

deferens elicited a contraction of the preparation within 1-3 sec. The cyclic nucleotide levels were determined by the methods of Gilman[12] and Steiner et al.[13] in preparations frozen 3-5 sec and 30 sec after electrical field stimulation. The cyclic AMP level was decreased by about 25% after 3-5 sec and remained lowered after 30 sec (figure 2). The cyclic GMP level was slightly increased after 3-5 sec, but after 30 sec the level was increased about 2-fold. We have also studied the cyclic nucleotide levels after norepinephrine stimulation. Norepinephrine (0.3 mM) elicited a contraction which lasted for about 60 sec. 5 sec after the norepinephrine addition, the cyclic AMP level decreased from 0.6±0.1 to 0.4±0.02 nmoles/g wet wt, but 30 sec after the addition the level had increased to 0.8±0.2 nmoles/g wet wt. A corresponding time study of the cyclic GMP level gave no significant changes. This is in accordance with earlier studies on vas deferens of rat[6], where no significant change of cyclic GMP level was demonstrated after 20 sec.

Our present data on guinea-pig vas deferens is not consistent with the recent suggestion[7] that cyclic GMP may act as feedback inhibitor of hormonally stimulated calcium influx. If cyclic GMP is a presumptive modulator of vas deferens contractility, our results agree better with the hypothesis that cyclic GMP may act as a co-mediator with calcium to promote contraction. However, in gallbladder it was recently demonstrated that cholecystokinin produced the same degree of contraction, even if the cyclic GMP production was blocked by indomethacin[14].

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A radioenzymatic method to measure picogram amounts of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) in small samples of brain tissue

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Summary. A radioenzymatic method for simultaneous determination of dopamine and DOPAC in small brain areas is described. By using this assay, 250 pg of dopamine and 150 pg of DOPAC can be estimated. The present method has been applied to compare the effect of different psychotropic drugs on the dopamine and DOPAC levels in the caudate nucleus, substantia nigra and medial basal hypothalamus.

Several radioenzymatic methods for measuring catecholamines in small tissue samples and biological fluids have recently been described[1-4]. These very sensitive methods are based on the estimation of labelled normetanephrine, metanephrine and 3-methoxytyramine (3-MT) formed from norepinephrine, epinephrine and dopamine (DA) respectively in the presence of catechol-O-methyl-transferase (COMT, E.C.2.1.1.6.) and ^{14}C- or ^{3}H-methyl-S-adenosyl-methionine (SAMe) as methyl donor. We described here a radioenzymatic procedure for the simultaneous determination of DA and 3,4-dihydroxyphenylacetic acid (DOPAC) in the same sample.

The present method is based on the O-methylation by COMT of DA and DOPAC to 3-MT and homovanillic acid (HVA), respectively, in the presence of ^{3}H-SAMe of high specific activity. ^{3}H-3-MT is then isolated by means of a small cation exchange chromatographic column of Dowex 50 W×4, while ^{3}H-HVA is isolated by Sephadex G-10, as described by Westerink and Korf[5]. The method described is the combination of 2 different assays: one for DOPAC by Argiolas et al.[6] and the other for DA by Zschaeck and Ramirez[7] with some modifications.

Material and methods. Reagents. All reagents used were 'pro analysis' and obtained from Merck. Dithiothreitol and 3-methoxytyramine-HCl were purchased from Calbiochem; S-adenosyl-methionine-methyl-^{3}H (8 to 12 Ci/mmole) from Amersham. Norepinephrine (NE), 3,4dihydroxymandelic acid (DOMA), vanilmandelic acid (VMA), 3,4-dihydroxyphenylethyleneglycol (DHPG), DOPAC, HVA, DA, L-DOPA from Sigma Chemical Company; Sephadex G-10 from Pharmacia Fine Chemicals; Dowex 50 W×4 from Bio Rad.

Purification of catechol-O-methyl-transferase. COMT was purified from rat liver according to Cuello et al.[3] up to ammonium sulphate back-wash step. Then the enzyme was dialyzed twice against 1000 vol. 0.1 M phosphate buffer pH 7, containing 10^{-4} M dithiothreitol, divided in small volumes and stored at −20 °C in small batches.

Animals and tissue preparation. Rats weighing 150-200 g were obtained from Charles River (Milan, Italy). All rats were housed at 24 °C and were under diurnal lighting conditions with light from 06.00 h to 18.00 h. The animals were killed by decapitation; caudate nucleus, substantia nigra and cerebellum were dissected as described by Spano et al.[8] and medial basal hypothalamus by Brown et al.[9]. The tissues were homogenized with a teflon microhomogenizer in about 50 vol. of ice-cold 0.1 M perchloric acid. Samples were centrifuged at 10,000× g for 10 min at 4 °C. A 50 µl aliquot of the clear supernatant was placed in 15 ml conical centrifuge glass tubes. Tissue blanks were prepared from cerebellum homogenates of normal rats. Standards were prepared by homogenizing the cerebellar tissue of similar weights as the brain area to be analyzed in ice-cold 0.1 M perchloric acid containing known concentrations of DA and DOPAC. These steps were carried out in a ice-cold bath.