l'abaissement de la concentration le développement, toujours ralenti, progresse davantage; le stade blastula avancé est atteint avec 20 μg par ml; la gastrulation est ébauchée chez quelques larves avec 10 μg par ml. Enfin, avec 5 μg par ml, le stade gastrula asymétrique est atteint. Toutes ces larves dégénèrent rapidement. Le report dans l'eau de mer améliore légèrement le développement des larves traitées par des concentrations de 5 et 10 μg par ml.

Les larves traitées au stade blastula mésenchyme par l'actinomycine (40, 30 et 20 μg par ml) cessent de se développer et se lysent en quelques heures. Plusieurs travaux[4,5] ont montré que l'addition d'acide désoxyribonucléique au milieu protège les organismes contre les effets de l'actinomycine D. Chez l'oursin nous avons observé que l'acide désoxyribonucléique ajouté au milieu de culture à raison de 500 μg par ml protège les larves contre les effets inhibiteurs de l'actinomycine (10 μg par ml).

Les oeufs traités simultanément par le chlorure de lithium et l'actinomycine (10 μg par ml) forment, après leur report dans l'eau de mer, des larves plus fortement végétalisées que les larves traitées par le chlorure de lithium seul. En contraste, les effets animalisants des ions zinc et du bleu d'Evans apparaîssent diminués dans les larves traitées simultanément par ces agents et l'actinomycine (10 μg par ml). En effet, l'extension de l'épaississement ectodermique apical et de la touffe ciliée qui le recouvre sont toujours moins importants dans ces larves que dans celles traitées par le chlorure de zinc ou le bleu d'Evans.

Conclusions. Les résultats obtenus montrent que l'actinomycine D, sans influence notable sur la segmentation aux concentrations utilisées, arrête le développement au stade blastula. L'arrêt du développement à ce stade peut être obtenu en faisant agir l'actinomycine directement sur les blastulas. Ces données indiquent que les stades ultérieurs du développement, c'est à dire la gastrulation et la différenciation des pluteus, sont étroitement liés au fonctionnement du noyau ou plus précisément aux réactions de synthèses inhibées par l'actinomycine. Or cet agent inhibe les synthèses d'acide ribonucléique gouvernées par l'acide désoxyribonucléique et, notamment, la formation des messagers qui assurent le transfert de l'information génétique aux ribosomes où sont élaborées les protéines spécifiques. L'action inhibitrice de l'actinomycine sur la différenciation montre la relation de dépendance existante entre la différenciation et les synthèses d'acide ribonucléique nucléaire; cette inhibition de la différenciation porte à la fois sur les structures ectodermiques et entomésodermiques et aucune modification de la détermination embryonnaire n'est provoquée par l'actinomycine seule. Cependant, en examinant les effets de faibles concentrations d'actinomycine sur des larves traitées par des agents animalisants ou végétalisants, on observe que cet agent favorise la végétalisation alors qu'il diminue l'expression de l'animalisation. Ces observations suggèrent l'existence d'une différence dans l'état de dépendance vis à vis du noyau des processus de différenciation ectodermique et entomésodermique.

Summary. In the presence of actinomycin D (20–40 μg/ml), the development of the eggs of the sea urchin, *Paracentrotus lividus*, is slowed from the late morula and stopped at the blastula stage. The development is immediately stopped in the blastula treated with actinomycin D (20–40 μg/ml). The inhibitory effects of actinomycin D are prevented by deoxyribonucleic acid. Actinomycin D does not exert animalizing or vegetalizing effects. However, the enhancing of vegetalizing action of lithium and the weakening of animalizing effects of zinc ions and Evans blue have been observed in the presence of actinomycin D. These observations may reflect some difference in the state of dependence of differentiation of entomesodermic and ectodermic structures towards the nucleus[6].

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Effect of α-Methyl-DOPA on Myocardial Catecholamines

Studies in recent years have added considerably to our knowledge about the mechanism of action of noradrenaline (NA) release produced by α-methyl-amino acids. Conflicting results indicate, however, that this mechanism is not yet entirely elucidated.

Smith[1] reported that the administration of α-methyl-DOPA (α-M-DOPA) caused a decrease in brain serotonin but left the NA level unchanged. NA was estimated by means of bioassay. In contrast, it has been subsequently demonstrated by many investigators (i.e. Carlsson and Lindqvist[2], Hess et al.[3], Porter et al.[4]) that α-M-DOPA depresses also the levels of NA in the brain as well as in other tissues. In these experiments a fluorimetric method of NA estimation was used.

It has also been demonstrated that α-M-DOPA and its analogue lacking the *para*-OH group, α-methyl-*meta*-tyrosine (α-MMT), undergo decarboxylation and subsequent β-hydroxylation *in vivo*, and that the amines thus formed (α-methyl-dopamine and α-methyl-noradrenaline, resp. α-methyl-*meta*-tyramine and α-methyl-β-hydroxy-*meta*-tyramine) may be taken up by the tissues; while α-methyl-dopamine was shown to disappear almost completely from the brain 24 h after α-M-DOPA administration, α-methyl-noradrenaline (α-M-NA) seemed to be retained for a longer time[2]. Recent studies of Carlsson[5] showed that α-methyl-β-hydroxy-*meta*-tyramine (metaraminol) was found in the brain of rabbits as late as 7 days after the administration of a single dose of α-MMT (100 mg/kg, i.v.). According to Carlsson, the amines formed from α-methylamino acids would thus displace their physiological analogues

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from the stores, but several investigators questioned this displacement theory (GESSA et al.[6], UDENFRIEND and ZALTZMAN-NIRENBERG[7]).

In view of this, attempts were made (a) to explain the discrepancy between the results obtained from chemical and biological estimation procedures, and (b) to determine whether the displacement theory is applicable or not to hearts of animals receiving α-M-DOPA.

Materials and Methods. L-Noradrenaline (Fluka) and DL-α-methyl-DOPA (Bayer) were purchased commercially. We are indebted to Farbwerke Hoechst Ltd., Frankfurt, for a generous supply of L-α-methyl-noradrenaline hydrochloride.

Male rats, Sprague-Dawley strain, 170–300 g body weight, male and female guinea-pigs (180–330 g) and mice (17–22 g) were used in these studies. α-M-DOPA was dissolved in water. Control animals received the same volume of isotonic NaCl. At various times after drug administration, the animals were killed by a blow on the head. The hearts were immediately removed, rinsed with cold water, blotted on Kleenex tissues and weighed.

The catecholamines were extracted with trichloroacetic acid and adsorbed on alumina (Aluminiumoxyd WOELM, basisch, Akt. Stufe I) according to the procedure of VON EULER and ORWÉN[8] with slight modifications (i.e. all reagents contained EDTA). HCl 0.25 *N* was used for elution.

In chromatographic experiments (paper SS 2043 b, washed) the solvents used were phenol- 0.1 *N* HCl (procedure of CRAWFORD and OUTSCHOORN[9], as modified by VOGT[10]), and *n*-butanol saturated with *N* HCl (as used by many investigators). Elution of the chromatographic strips was carried out with 0.01 *N* HCl or with isotonic NaCl containing 0.01 *N* HCl.

For chemical identification of catecholamines chromatograms were sprayed with 0.1% potassium ferricyanide in 5% aqueous ethylenediamine, dried at 50°C for 5 min and examined for fluorescence (ELLMAN[11]).

The catecholamine content of these extracts was estimated on the blood pressure of the pithed rat (SHIPLEY and TILDEN[12]). The pressor activity of the alumina extracts was expressed in terms of NA and not corrected for an average recovery of 90%. Detailed accounts of the methods were given in a previous study (MAÎTRE[13]).

Results and Discussion. (1) *Effect of α-M-DOPA on total catecholamine content.* α-M-DOPA was administered intraperitoneally in a single dose or repeatedly to rats and guinea-pigs. The pressor activities of the alumina extracts was determined biologically. From the results summarized in Figure 1, it can be seen that the catecholamine contents of rat heart (0.88 ± 0.17 μg/g) and guinea-pig heart (2.68 ± 0.55 μg/g) were not significantly altered (Figure 1).

(2) *Chromatography of alumina extracts.* Paper chromatography was used in order to distinguish between NA and α-M-NA. For these experiments α-M-DOPA was given to guinea-pigs as a single intraperitoneal injection (400 mg/kg).

Five or six hearts were pooled for each extract. All these extracts were chromatographed in the phenol-HCl solvent system together with references. Experiments were repeated for the 16 h and 72 h time and the extracts were then chromatographed in the butanol-HCl solvent system. Results of both series agreed well.

By spraying the chromatograms with ferricyanide, a fluorescent spot with the same colour and Rf value as synthetic α-M-NA was observed. Its fluorescence was particularly strong in chromatograms from hearts of animals which had been given α-M-DOPA 16 h and 72 h previously.

Eluates from the paper strips corresponding to the Rf value of α-M-NA exhibited very strong pressor activity. It was estimated by comparison with synthetic L-α-M-NA. The amounts of α-M-NA found in the heart following α-M-DOPA administration are shown in Figure 2, as compared

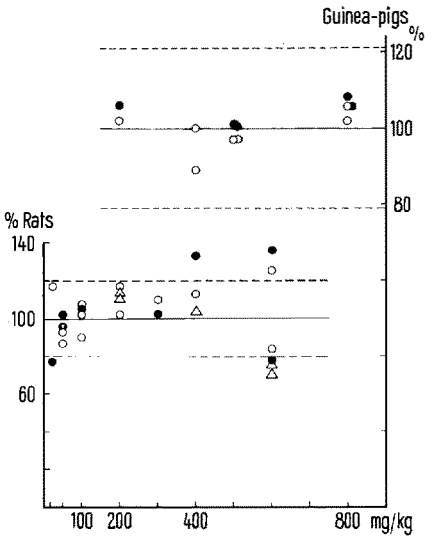

Fig. 1. Effect of α-M-DOPA on catecholamine content to guinea-pig hearts. Catecholamines were estimated in terms of NA and expressed as percentages of control values. The latter are given with the standard deviation of the mean. Each circle, or triangle, represents a single extract. 2 hearts and 2–6 hearts for rats and guinea-pigs respectively were pooled for each extract. The animal had been given: o a single injection 16 h before removing the hearts; • 2 injections, one 40 h and the other 16 h before removing the hearts; Δ 5 injections, one at 64 h, 48, 40, 24, and 16 h before removing the hearts.

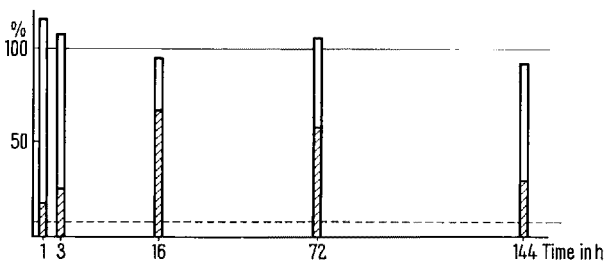

Fig. 2. Vasopressor activity of heart extracts of guinea-pigs treated with α-M-DOPA (400 mg/kg, i.p.). Open columns: Alumina extracts: Activity expressed as % of control values. Hatched columns: After chromatography of the same extracts: Eluates from strips corresponding to the Rf value of α-M-NA. Activity expressed as % of the NA + α-M-NA content. ---- Maximal value for the corresponding strips of control extracts (limit of the sensitivity of the method).

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[7] S. UDENFRIEND and P. ZALTZMAN-NIRENBERG, J. Pharmacol. exp. Therap. *138*, 194 (1962).
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to the corresponding controls and to the corresponding unchromatographed extracts.

The results obtained from the assay of the eluates from chromatographic strips show that there is a big difference between the hearts of control and of treated animals, in contrast to the results obtained from the assay of the alumina extracts. α-M-NA was detected in the hearts of treated guinea-pigs as early as 1 h after α-M-DOPA administration. After 16 h, the pressor activity of the α-M-NA strip accounted for more than 70% of the pressor activity of the whole chromatogram. This activity declined slowly: after 6 days it was as high as 30%. In all instances the corresponding eluates of control hearts had the same activity as isotonic NaCl.

On the other hand, marked activities were found only in the NA strips for control hearts and only in the NA and α-M-NA strips for treated hearts. The assay of the eluates from chromatographic strips shows, therefore, besides the presence of α-M-NA, a profound and long lasting (> 6 days) depletion of NA in the hearts of treated guinea-pigs. The biological assay procedure is thus in agreement with the fluorimetric method. In view of this, it can be expected that a similar process occurs also in rat and mouse hearts. In fact, α-M-NA has been detected in the hearts of mice treated with α-M-DOPA[2].

It is interesting to note that after α-M-DOPA administration the missing NA is replaced in the heart muscle by an equipressor amount of α-M-NA. Moreover, under the experimental conditions used here, the vasoconstrictor effect of α-M-NA was as potent (on a molar basis) as that of NA, and this was true in animals having different sensitivities to NA (Figure 3).

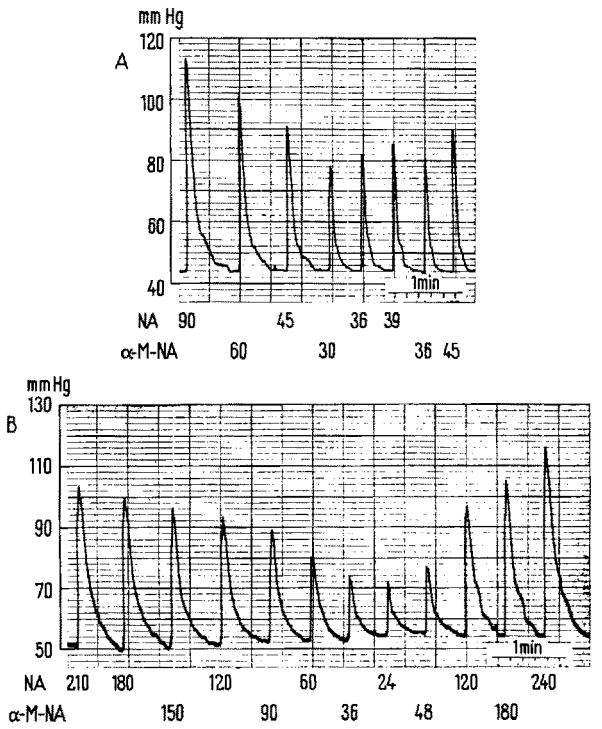

Fig. 3. Pithed rat preparations; arterial blood pressure. Vasopressor activity of NA, as compared with that of α-M-NA; doses are expressed in 10^{-12} *M*. Atropine (2 mg/100 g, s.c.) was given 10–15 min before operation. A: Rat ♂ 190 g. B: Rat ♂ 220 g.

This latter result does not agree with the observation made recently by DAY and RAND[14] who found that α-M-NA had less vasoconstrictor activity than NA on the blood pressure of the rat. In the light of the results reported here it would seem that the hypotensive action of α-M-DOPA does not consist in a depression of the normal pressor potential contained in the NA stores.

It seems interesting that a similar process of NA depletion might occur in the brain since a replacement of NA by α-M-NA has been reported[2] – the amounts of which corresponded roughly to the missing NA – while there is no apparent decrease in the catecholamine content as determined by a biological estimation procedure (SMITH[1]). Our results, obtained from heart extracts, are in agreement with the observation made by SMITH and substantiate, thus, the suggestion that the presence of α-M-NA might interfere in the biological but not in the fluorimetric assay of NA[2].

The time course of the occurrence of α-M-NA in the heart (Figure 2) is correlated with a corresponding depletion of NA in such a manner that the total pressor activity remains unchanged throughout. This supports the view that the NA depletion is a direct consequence of the α-M-NA uptake and might therefore be in keeping with a displacement phenomenon, as observed first by CARLSSON[2] from studies of brain metabolism of α-M-DOPA as well as of α-MMT.

However, CARLSSON's displacement hypothesis is in conflict with the finding that the catecholamine loss produced in the brain and by the heart by α-MMT administration is not balanced stoichiometrically by the levels of α-methyl-*meta*-tyramine or metaraminol taken up and retained by these organs[6,7]. It seems at present not clear why there would be such a discrepancy between the mechanism of NA depletion in the guinea-pig heart produced by α-M-DOPA and α-MMT, since the latter was also found to exert its depleting effect by means of its decarboxylation products.

Résumé. L'α-méthyl-DOPA, même à doses élevées et/ou répétées, ne modifie pas significativement l'activité vasopressive totale – estimée sur la pression artérielle du rat décérébré et démédullé – des amines cathéchiques cardiaques. La quantité de noradrénaline libérée du coeur de cobaye 1 h à 6 jours après administration d'α-méthyl-DOPA y est remplacée par une quantité équipressive d'α-méthyl-noradrénaline.

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RNA and DNA Metabolism in Liver Cells of Normal and Cancer-Bearing Mice

Nucleic acid metabolism has been studied with labelled precursor adenine both *in vivo*[1] and *in vitro*[2] in the hepatic cells of mice and rat.

This paper presents studies *in vivo* on relative nucleic acid synthesis and replacement in the hepatic cells of nor-

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