to 9.6 days. These findings would seem to suggest that certain physiological changes, associated with age, tend to inhibit the full expression of parthenogenetic development. The exact reason for the decline in level of embryo production and in embryo viability in eggs produced the second season is not clear. It is suggested, however, that the same factors responsible for lowered reproductive performance in older birds generally may also be operative in the case of parthenogenetic embryos.

Summary. Unfertilized Beltsville Small White turkey eggs produced by the same hens during their first and second laying year were incubated for 9-10 days and subsequently examined for parthenogenesis. Involved were 163 hens and more than 13,000 unfertilized eggs. On the average, parthenogenetic development encountered in eggs of younger birds yielded a higher percentage of embryos. It was likewise found that, on the average, embryos in eggs of younger birds survived longer within the shell than did those of older birds.

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The Lightening Effect of Actinomycin D on the Skin of *Amphiuma tridactylum.** (31807)

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While investigating the effects of actinomycin D on ribonucleic acid (RNA) and protein synthesis of the hepatic melanocytes of amphiuma, it was observed that the antibiotic causes a lightening of the skin within 4 to 6 hours following injection. The present paper describes the effects of actinomycin D, melanocyte-stimulating hormone (MSH), cycloheximide and melatonin on the skin of amphiuma. The data suggest that the actinomycin D probably inhibited the secretion of MSH by the pituitary.

Materials and methods. Since it was difficult to identify the sexes of *Amphiuma tridactylum* grossly, both males and females were used in the present study. The weight of the animals varied from 200 to 400 g. The animals were maintained in a polyethylene tank filled with demineralized water which was changed twice a week. They were fed beef liver once a week. The room in which the animals were kept had 12 hours of fluoresent light and 12 hours of darkness. The experiment was carried out in a room in which the light condition was kept constant for at least 16 hours.

Actinomycin D was dissolved in absolute ethyl alcohol in a concentration of 1 mg/ml and was injected intraperitoneally to a group of 14 animals at a dose of 5 and 10 μg/g of body weight. The animals were observed for 24 hours. The initial skin color of these animals was similar. An equivalent amount of absolute ethyl alcohol was given intraperitoneally to a group of 6 animals.

The skin biopsies from the experimental and control animals were taken under ice anethesia, fixed in 10% buffered formalin and stained with hematoxylin-eosin.

To investigate whether the lightening of the skin was due to an inhibition of melanin dispersing action of melanocyte-stimulating hormone (MSH) by actinomycin D, a group of 3 animals was injected intraperitoneally with 10 mg/animal of synthetic β-MSH in

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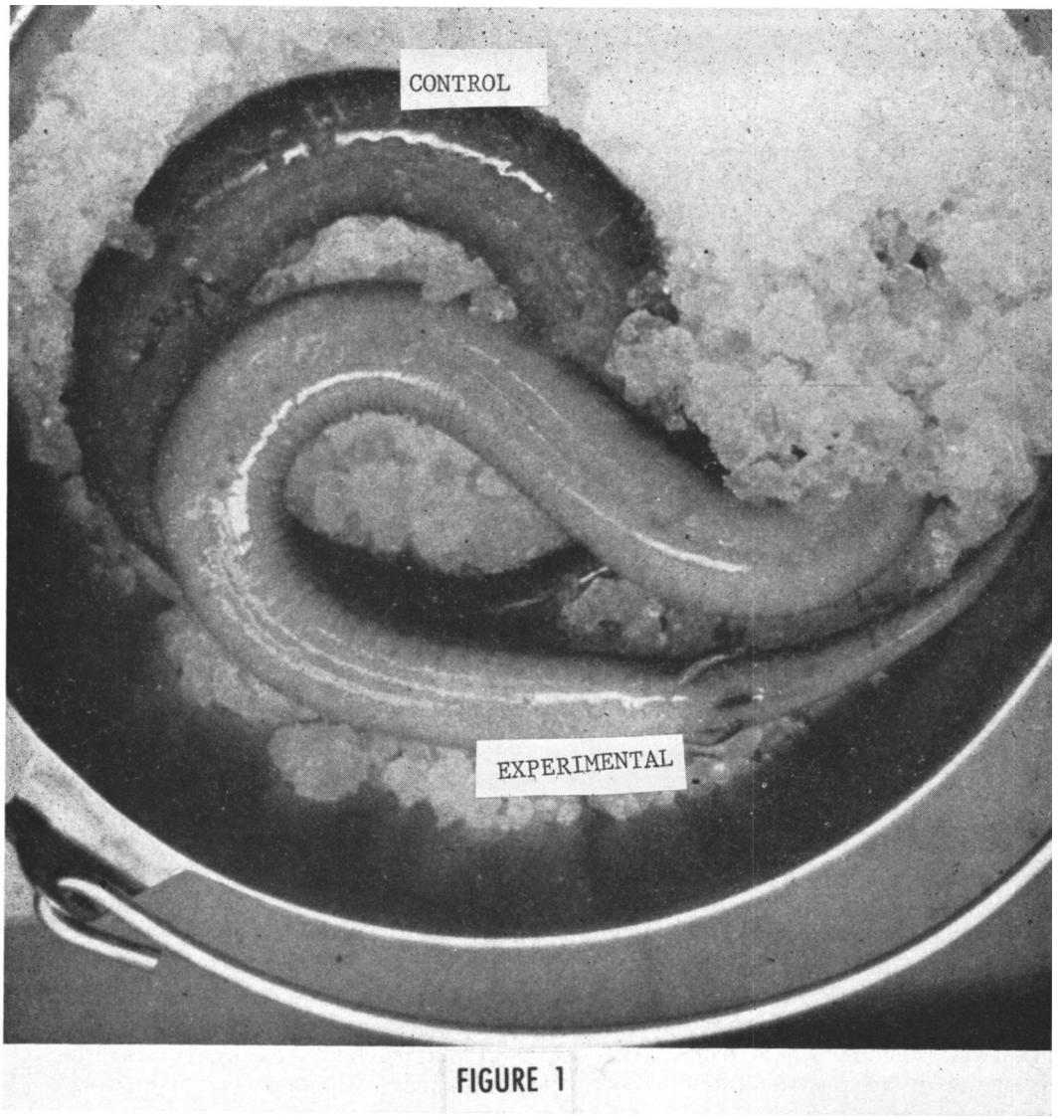

FIG. 1. Ventral surface of *Amphiuma,* which received actinomycin D intraperitoneally in a dose of 5 μg/g of body weight; the control animal was given ethyl alcohol. Both were selected on the basis of their similar coloration. Lightening of the skin is seen in antibiotic-treated animal 6 hours after injection, but no change is seen in control.

saline at a time when the skin appeared pale. To study whether the paling of the skin was due to an inhibition of MSH synthesis, cycloheximide, an inhibitor of protein synthesis(1,2), was used. The drug was dissolved in 95% ethyl alcohol and was injected intraperitoneally to a group of 6 animals at a dose of 5 and 25 mg/100 g of body weight.

To show whether melatonin, a potent lightening agent for the frog skin(3), also produces such an effect in amphiuma, the drug was dissolved in 95% ethyl alcohol and injected intraperitoneally in a group of 2 animals at a dose of 25 mg/100 g of body weight.

Results and discussion. Actinomycin D in doses of 5 and 10 μg/g produced a paling of the skin within 4 to 6 hours after injection. The difference between the antibiotic-treated animals and controls was more

marked ventrally and laterally than dorsally. Fig. 1 shows a marked paling effect on the ventral surface of the skin of experimental animals 6 hours after administration of the antibiotic, whereas no such effect was seen in the controls. Histology of the skin showed that the melanin granules which were dispersed in the melanophores of the control (Fig. 2a) animals were aggregated in both the epidermal and dermal melanophores of actinomycin D-treated animals (Fig. 2b). The paling of the skin was spontaneously reversible within 8 to 12 hours after it was first observed.

If β-MSH was given to the actinomycin-treated animals at a time when the skin appeared pale the color of the skin returned to an initial level within 2 hours instead of 8 to 12 hours. Animals that did not receive the antibiotic did not show any change in skin color with the same dose of synthetic β-MSH.

Cycloheximide did not produce a paling of the skin in amphiuma. The animals which received a dose of 25 mg/100 g of body weight died in 2 days, whereas those receiving a lower dose died in 3 to 4 days.

Melatonin also did not produce a paling of the skin in amphiuma. Since actinomycin D has been known to inhibit DNA-dependent RNA synthesis(4) it has been used in recent years to understand the mechanism of hormone action. The results to date from such an experimentation(5-15) have led to the postulation of two broader views: (a) the effects that result in the synthesis of new RNA, and (b) the effects that do not involve new RNA synthesis. In the present study, the melanin-dispersing action of exogenous MSH is not interfered with in the presence of the antibiotic and, therefore, it may not involve the synthesis of new RNA. The mechanisms of lightening of the skin by actinomycin D remain obscure, but the experiment with synthetic β-MSH suggests that the paling effect was not due to overcoming the melanin-dispersing effect of MSH. It has been reported(16) that hypophysectomy produced a paling of the skin in amphiuma within 4-5 hours after surgery and this effect was irreversible. This suggests that after hypophysectomy the biological half life of endogenous MSH in 4-5 hours decreases to a level at which the hormone can no longer maintain its effectiveness for melanin-dispersing action. In the present study the timing of paling of the skin following administration of actinomycin D coincides with those of hypophysectomy. This indicates that the paling effect in both experimental conditions has been mediated *via* MSH and the antibiotic probably inhibited the secretion and/or synthesis of MSH to an extent similar to that of hypophysectomized animals. However, the possibility yet remains that actinomycin D-induced lightening of the skin may have been mediated *via* pathways other than MSH.

Since MSH is a polypeptide, it was thought that cycloheximide, an inhibitor of protein synthesis, may be useful in showing whether the lightening effect was related to the lack of MSH secretion or synthesis. This drug did not produce paling of the skin which indicates that this effect was not related to an inhibition of MSH synthesis. Furthermore, the fact that the paling effect was a transient one suggests that actinomycin D causes an inhibition of MSH secretion rather than its synthesis.

The failure of cycloheximide in producing the lightening of the skin deserves one more comment. It has been presumed that MSH being a polypeptide has the same mechanism of synthesis as other polypeptides which have been studied extensively in both *in vivo* and *in vitro* systems. However, in the present study unlike other polypeptides, the synthesis of MSH seems insensitive to cycloheximide treatment. Although the present evidence for such an observation is an indirect one, it does indicate a possibility that the mechanism of synthesis of MSH in the pituitary may be different from those polypeptides which are sensitive to the treatment of cycloheximide.

Summary. A lightening of the skin of amphiuma was observed within 4 to 6 hours after injection of actinomycin D in doses of 5 and 10 μg/g body weight. This effect was associated with the aggregation of melanin-granules in both the epidermal and der-

mal melanophores and was spontaneously reversible within 8-12 hours after it was first observed. However, the administration of synthetic β-MSH reversed this effect within

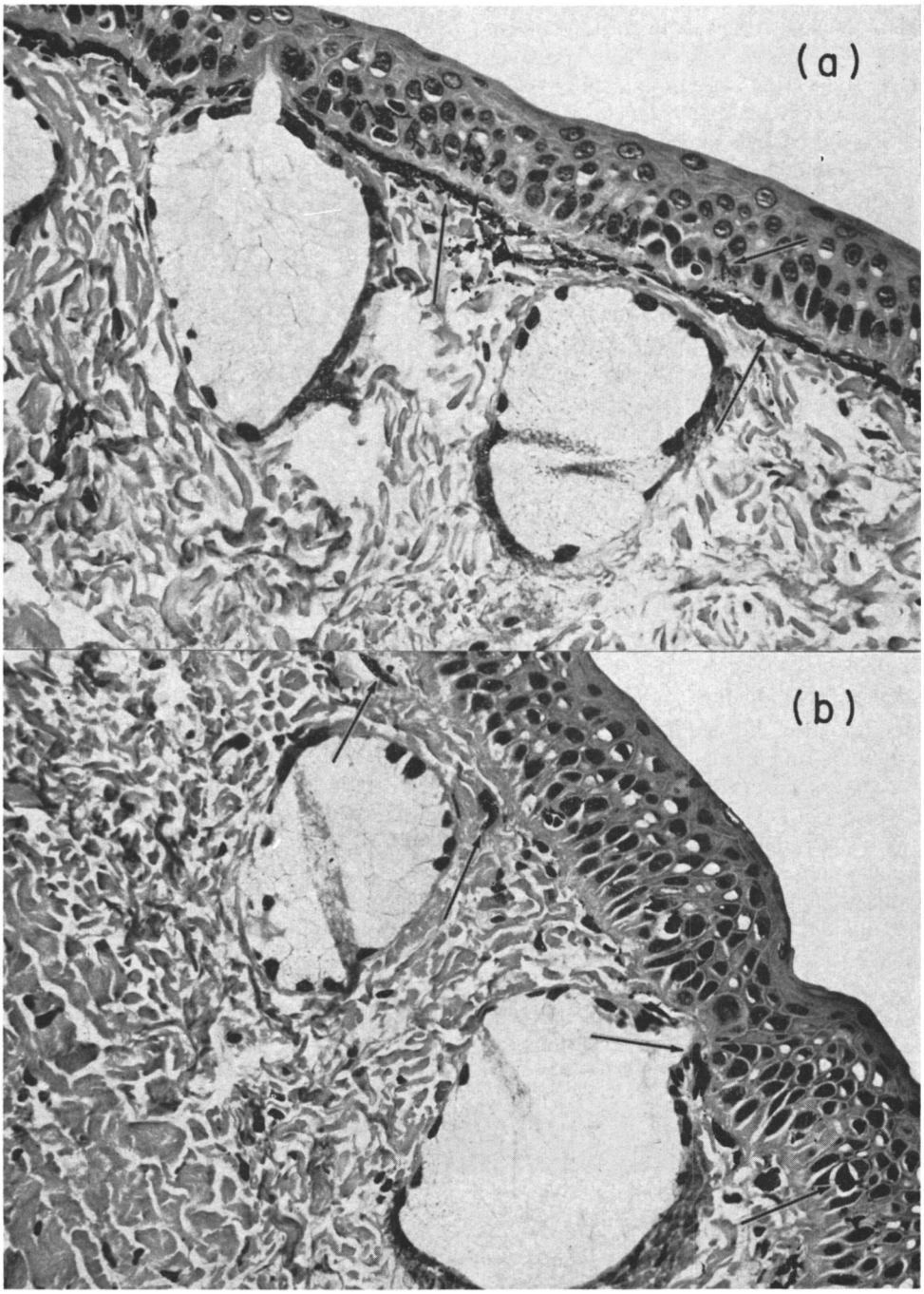

FIG. 2a. Histology of dorsal skin of *Amphiuma* which was given ethyl alcohol. Melanin granules are dispersed in melanophase (indicated by arrows) (× 160).

F G. 2b. Histology of dorsal skin 5 hr after injection of actinomycin D. Melanin granules are aggregated toward the nucleus of both epidermal and dermal melanophase (indicated by arrows) (X 160).

2 hours. Cycloheximide, an inhibitor of protein synthesis did not produce paling of skin in amphiuma. Melatonin which has been known as a potent lightening agent in frog did not produce such an effect in amphiuma. The possibility that actinomycin D produced paling of the skin by blocking the secretion of MSH by the pituitary and that *in vivo* the mechanism of synthesis of this hormone may be different from other polypeptides has been discussed.

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Limitations of Antiadrenergic Therapy for Refractory Traumatic Shock.* (31808)

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The generally accepted principle that prolonged and excessive vasoconstriction is a self-defeating defense mechanism led to the use of antiadrenergic therapy in experimental traumatic shock. There was no appropriate pharmacologic agent of sufficient potency until phenoxybenzamine became available. The effectiveness of these and other related substances as prophylactic agents has been repeatedly affirmed(1-3), but their effectiveness when applied during shock remains in doubt(4-6) because none of them can consistently achieve a significant salvage rate in the experimental animal or in man. On this account we made a study of coeliac blockade as a method for maximum exploitation of the therapeutic principle involved, for it achieves immediate and total release of sympathetic activity in the splanchnic viscera, and so facilitates preferential diversion of flow to the region most in need of it(7).

In this communication we report a comparison of the effects of this therapy with that of phenoxybenzamine in endotoxic and hemorrhagic shock.

Procedure and results. 1. *Endotoxic shock in rabbits (Table I).* Adult white rabbits received an MLD/100 *E. coli* 0111:B4 endotoxin intravenously. Three types of antiadrenergic therapy were studied, in each instance in a group of 12 rabbits and at different times, as follows:

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