derate green fluorescence, the individual fibers having varicose-like enlargements. The fibers could be seen to traverse the reticular ventral nucleus in the medulla oblongata, the parvocellular reticular nucleus of the pons, the caudal and oral reticular nucleus of the pons, medial to nuc. mot. n. trigemini, and nuc. subcuneiformis of the mesencephalon. The course of these fibers is very similar to that of the ascending noradrenaline (NA) tracts in the rat[17,18]. SLADEK[19] has recently described this tract in kittens but interpreted it to represent CA nerve terminals due to the strong fluorescence in the varicose-like enlargements. It is known, however, that in young animals the fiber tracts have a relatively strong fluorescence intensity[20,21].

The present data indicate that in the monkey the principal architecture of the central monoamine neurons is similar to that in the rat and other mammals such as the rabbit and the cat. Thus, the CA and 5-HT neurons in the monkey are reticular lower brain stem neurons with inter alia long ascending monosynaptic connections with the tel-diencephalon[22].

Zusammenfassung. Verteilung und Morphologie von Katecholamin-(KA) und 5-Hydroxytryptamin-Neuronen im Affengehirn stimmt mit früheren Befunden über das Rattengehirn gut überein. Die Zahl der KA-Zellkörper in Area Subceorulea ist jedoch bedeutend grösser bei Affen, und eine neue Art terminaler KA-Nervenfasern von starker Fluoreszenzintensität und im Durchmesser variierender Varikosität wurde aufgefunden.

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Alleviation of the Toxicity of Actinomycin D by Uridine and Thymidine on the Morphogenesis of Chick Embryos Cultivated in vitro

Teratogenic effects of Actinomycin D in various ambryonic systems have been reported by BRACHET et al.[1], BRACHET and DENIS[2], WALLACE and ELSDALE[3], FLICKINGER[4,5], and SAMESHIMA et al.[6] in amphibia and PIERRO[7,8], KLEIN and PIERRO[9], POHL[10], and GALLERA[11] in chick embryos and GROSS[12] and COUSINEAU[12] in sea urchin embryos.

The malformations in amphibians and chick embryos involved the nervous system, the eyes and cardiovascular system etc. In the present work actinomycin D has been shown to cause similar abnormalities in the chick embryos and can be reversed to a greater extent with subsequent treatment with thymidine or uridine.

Materials and methods. Fresh and fertilized eggs of white leghorn hens were obtained from a local farm and incubated at 37.5 ± 1 °C for required number of hours so as to obtain the proper stage for experiments. The experiments were performed at 2 different stages of development namely 1. primitive streak stage and 2. head process stage (stages No. 4 and No. 5, respectively, HAMBURGER and HAMILTON[13]). The glassware employed in the experiments was sterilized and the culturing solutions were autoclaved.

Stock solution of Actinomycin D (Merck, Sharp and Dohme) (500 µg/ml) was suitably diluted to give an effective concentration (0.05 µg/ml).

The embryos were explanted by the method of NEW[14] and treated with the above concentration of actinomycin D. Care was taken to add the antibiotic gently by the side of the blastoderm so that it is evenly exposed to the antibiotic. The preparation was kept at room temperature for 1 h for proper diffusion of the antibiotic before incubation. After 6 h of incubation with the antibiotic, the embryos were plunged 3 times separately into fresh PC saline to remove the antibiotic completely. The embryos were then divided into 2 experimental groups A and B in series 1. In group A the embryos were mounted in PC saline only. These embryos served as controls. In group B, the embryos were subsequently exposed to uridine (0.05 µg/ml). In series 2, the controls were run (A_1) as in the above series and the embryos in experimental group were subsequently treated with thymidine (0.05 µg/ml) (B_1) instead of uridine. 30 embryos were used in each set of controls and experimentals.

Identical sets of experiments were done using head process stage of embryos (stage V), first treated with actinomycin D and then subsequently treated either with uridine or thymidine at the same concentration (Series 3 and 4).

It is seen in the present work that actinomycin D causes microcephaly in 60% of the embryos treated at the primitive streak stage, inhibits the formation of somites and heart in 63% and 80% cases, respectively (Figure 1). Shortening of axis is also observed to the extent of 56%.

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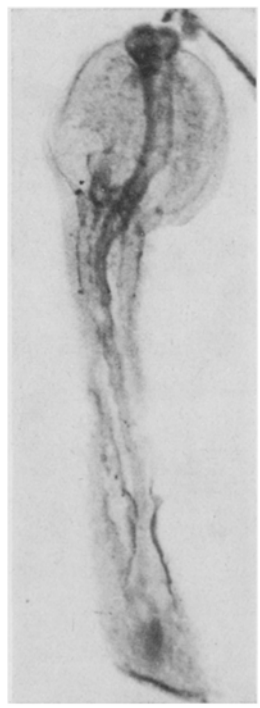

Fig. 1. Entire chick embryo (primitive streak stage) treated with actinomycin D. × 24.

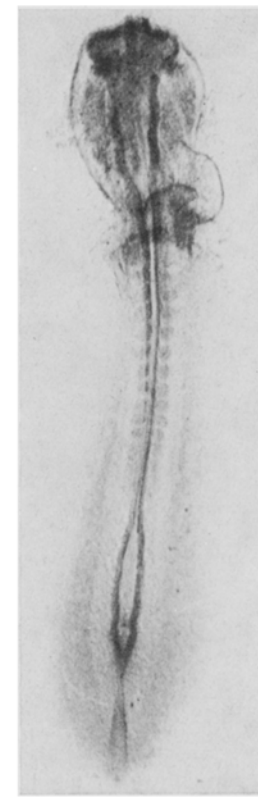

Fig. 2. Entire chick embryo of the above stage treated with actinomycin D and subsequently with uridine. × 24.

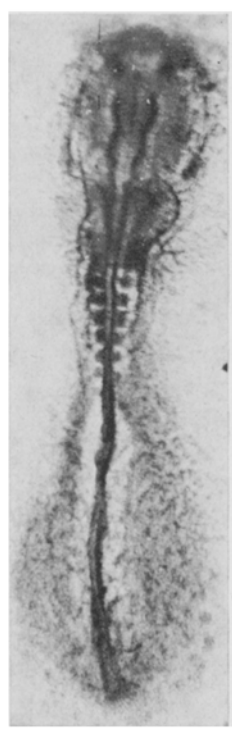

Fig. 3. Entire chick embryo of the above stage treated with actinomycin D and subsequently with thymidine. × 24.

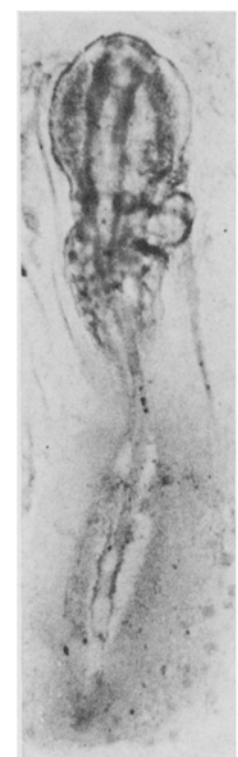

Fig. 4. Entire chick embryo (head process stage) treated with actinomycin D. × 24.

Subsequent treatment with uridine reverses the abnormalities of brain, somites and heart by 38%, 28% and 48%, respectively.

Similarly subsequent treatment with thymidine reverses the abnormalities caused by actinomycin D to the extent of 27%, 43% and 33% in brain, somites and heart, respectively. Shortening of axis is also reversed in both the cases.

As the experiments were done uniformly on the same breed, namely white leghorn, inherent variations in the response of individual embryos to actinomycin D are likely to be insignificant. Alleviation with uridine as well as with thymidine appears to be statistically significant. In general overall performance with uridine is slightly better than with thymidine which has been corroborated by appropriate statistical tests, namely test for the significance of proportions, on the assumption of asymptotic normality.

Thus teratogenic effect of actinomycin D in the present system (Figure 1) is seen to be alleviated not only by uridine (Figure 2), a precursor of RNA but also by thymidine (Figure 3) a precursor of DNA indicating that actinomycin D affects DNA biosynthesis as well. Embryos treated with actinomycin D at the head process stage (stage V HAMBURGER and HAMILTON[13]) in which anterior structures are already determined show abnormalities of the posterior axis in that somite formation is very much affected or completely absent while brain, foregut and heart formation though abnormal is not drastically affected (Figure 4). The differential response in the stages No. 4 and 5 towards toxicity of actinomycin D suggests that actinomycin D primarily affects the biosynthesis of the new nucleic acids rather than their functioning. Subsequent treatment with both uridine and thymidine of actinomycin D-treated embryos at the head process stage also show reversal to normal development.

Actinomycin D is known to exert a profound influence on cellular nucleic acids and it interferes with DNA dependent RNA polymerase enzyme (REICH et al.[15], GOLDBERG and RABINOWITZ[16], DENIS[17], BRACHET et al.[1]). Recently it has been suggested by BOVARNICK et al.[18] that actinomycin D affects cell division and possibly DNA replication in *Euglena*. COWELL and WESTON[19] have also shown in avian cell cultures that the uptake of thymidine like that of uridine is suppressed as judged by the pick-up of H^3-TdR in presence of actinomycin D, suggesting inhibition of DNA as well. Teratogenic action of actinomycin D in syrian hamster was found to be reversed to a greater extent with DNA rather than RNA, by ELIS et al.[20]. Our observations are in general accord with the above interpretations as the toxicity of actinomycin D is alleviated by uridine as well as by thymidine. Preliminary studies with guanosine were found to be ineffective in reversing the effect of actinomycin D. It will be of interest to study the effect of actinomycin D by subsequent treatment with deoxy guanosine and cytosine. Further work along this line is in progress.

Zusammenfassung. Untersuchung über die Wirkung von Actinomycin D auf Primitivstreifen- und Entwicklungsstadien der Kopfregion bei Hühnerembryonen in vitro: Anormale Entwicklung des Zentralnervensystems, der Somiten und des Herzens; diese Effekte konnten durch Thymidin oder Uridin zum Teil aufgehoben werden.

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Specific Anti-Antibodies

The concept that antibody globulins show no immunologically recognizable differences from normal globulins has been refuted. Various investigators have demonstrated that anti-antibodies can be produced[1, 2, 6, 7]. The mechanism of their action in blocking specific antibody production is not known; it is believed that anitbodies are directed against the binding site of the antibodies used as antigens[3–5].

Anti-antibodies would be of great significance in a new approach to the treatment of certain allergies and autoimmune diseases and in the prevention of transplant rejection. Induced immunotolerance to white blood cells may pave the way to the treatment of malignant diseases by transfusions or transplantation of immunocompetent cells.

In the past, we have proposed that it might be possible to train individuals' lymphopoietic systems to produce anti-antibodies against anti-bodies in certain immune diseases or in homograft rejection[8]. For this purpose we used an 'immunological triangle' in which 3 animals are involved: a donor and a recipient of the same species and an intermediate animal of a different species. The donor's tissue, red blood cells, was used as antigen to elicit anti-donor antibody in the intermediate species. The anti-donor antibody was then isolated and used as an antigen to elicit anti-antibody in the eventual recipient. This antibody was intended to block antibody formation in the recipient against the donor erythrocytes. This experiment was successful in several animals in which repeated trans-

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