The Inhibitory Action of Antimycin A in the Early Chick Embryo

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WITH ONE PLATE

Antimycin A, an antibiotic obtained from an undetermined species of streptomycetes, was isolated, crystallized, and described by Dunshee, Leben, Keitt, & Strong (1949), and its biological action has been studied by many workers since then. Ahmad, Schneider, & Strong (1950) demonstrated its effects on the growth and metabolism of yeast, on enzyme activities in the succinoxidase system, and on rats given the drug orally. Potter & Reif (1952) confirmed the inhibitory effect of antimycin A on the succinoxidase system in liver, suggested the presence of an 'antimycin A-blocked factor', identical, probably, with the 'Slater factor' and showed that, in certain tissues, there is an antimycin A-insensitive pathway for DPN oxidation. The same workers, Reif & Potter (1954), used the drug to characterize the pathways of DPN oxidation in different tissues. Green, Mii, & Kahout (1955) and Thorn (1956) argue from their experiments that the BAL-sensitive (Slater) factor and the antimycin A-sensitive factor are not identical.

Duffey & Ebert (1957) employed antimycin A as an experimental tool in the investigation of developing tissues in the chick embryo. Using the explantation technique of Fell & Robison (1929) as modified by Spratt (1947) they showed that, like sodium fluoride, antimycin A produced a strong inhibitory effect on the heart-forming mesoderm resulting in complete absence of the heart; somites formed in the presence of the drug but did not persist, otherwise the embryos showed normal development.

In the work described here, the experiments with antimycin A as a metabolic inhibitor were extended to the use of the explantation technique described by New (1955) and the results show that tissues other than mesodermal derivatives may be affected. The location of the abnormalities produced appears to depend on the precise method of application of the drug.

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MATERIALS AND METHODS

All the eggs were from pure White Leghorn stock, and the drug, obtained from the Wisconsin Alumni Research Foundation, was stored as a stock solution (20 mg. in 200 ml. of 95 per cent. ethanol) and diluted for use with Howard–Ringer (Howard, 1953) saline. Confirmatory tests were carried out with the Spratt (1947) technique (Text-fig. 1A) as used by Duffey & Ebert (1957). The New (1955) technique (Text-fig. 1B) was employed for most of the experiments, but the method of introducing the antimycin A varied; in some cases it was mixed with the albumen underlying and applied to the dorsum of the embryo through the vitelline membrane, and in others it was placed on the ventral surface of the disk. A series of experiments was also conducted in which the antimycin A was administered in ovo, either into the albumen or into the yolk.

Eggs were incubated at 37° C. initially for a period between 18 and 22 hours to bring the embryos to stages 4–9 (Hamburger & Hamilton, 1951) and subsequently with antimycin A for 18–24 hours.
RESULTS

Repetition in detail of the work of Duffey & Ebert (1957) confirmed their results. 0.025 γ antimycin A was added to each millilitre of the saline–albumen–agar medium. Of the 21 specimens explanted under these conditions at stages 5–8, none showed heart formation during the next 24 hours' incubation: where somites were present they were irregular and poorly developed. In 14 specimens the central nervous system was normal, in the remainder poorly developed or distorted.

In the first group of experiments using the New technique, the antimycin A, diluted from the stock solution with Howard–Ringer saline, was added to the nutrient medium (albumen). With a dose of 0.03 γ or more for each specimen, there remained no sign of an embryo after 20–24 hours; where damage was less severe and dead, distorted material persisted, lysis of tissue was often marked in the region of the primitive streak. Thirty-four embryos each received 0.025 γ antimycin A; 10 embryos were recovered showing no gross abnormality, 1 had completely disintegrated, and the remainder displayed moderate or gross distortion of the brain often accompanied by lysis of tissue around the central axis and anomalies of the somites. Cardia bifida was recorded once, the only malformation of the heart in this series. Figs. A and B of the Plate illustrate typical findings, and fig. C shows necrosis of neural tissue and of the dorsal part of the somite in a specimen treated in this way.

The pattern of abnormalities obtained when 0.01–0.013 γ antimycin A was applied to the ventral surface of the embryo was quite different. Treatment of specimens which had reached only stages 4 or 5 often caused general inhibition or cessation of growth during the next 18–20 hours, but where development had been less affected it was found that the heart-forming tissue was more seriously damaged than brain, e.g. Plate, figs. D, E: note the relatively healthy appearance of the somites. If the embryos had reached stages 6–8 when explanted, a significant increase in the number of normal specimens was found after 20 hours' incubation with the same dose of antimycin A. Normal, or almost normal, hearts and brains, however, were often accompanied by severe damage to the somites; embryos were recovered showing virtually complete mesodermal degeneration caudal to the heart and brain while, in some, only a transverse segment of somite-forming material was absent, normal tissue existing anterior and posterior to the damaged area (Plate, fig. F). The last-mentioned finding was correlated with and emphasized by the behaviour of one specimen explanted at stage 4 and treated with antimycin A on its ventral surface; after 18 hours it was noted that, although 5 somites were present, no heart had formed and the neural plate appeared healthy. Following a further 4 hours' incubation on the same medium, there was still no heart but 9 somites were now visible and the brain had progressed to the formation of the optic vesicles.

Treatment of embryos in ovo is unsatisfactory compared with in vitro tech-
techniques where the initial developmental stage can be determined accurately and the drug located precisely, but a small series of experiments was carried out varying the dose of antimycin A from 0.1 to 1.0 \( \gamma \) for each egg, the injection being made either into the albumen or into the yolk. It was not possible, however, to decide whether the variation in the site of the injection played a significant part in the type of abnormality produced. Although anomalies of the nervous system were prominent after albumen or yolk injection, they were more frequent in the former; similarly, more of the recorded cardiac abnormalities were in the series of yolk injections. Many specimens, however, showed both types of defect and, especially with the higher dosages, inhibition of growth or even death was common.

**DISCUSSION**

Extensive investigation of antimycin A since its discovery in 1949 has established it as a strong metabolic inhibitor with a specific effect on the succinic oxidase system.

The 2,3-dimercaptopropanol (BAL) sensitive factor (Slater factor) in the oxidation of reduced DPN was identified as its probable site of action by Potter & Reif (1952), while Thorn (1956) postulated a separate antimycin A-sensitive factor. Such specificity might be expected to prove useful in the investigation of tissue metabolism, and indeed Reif & Potter (1954), using heart, kidney, liver, and Flexner-Jobling carcinoma, demonstrated a differential inhibition of respiration in these tissues.

Employing the chick embryo cultured *in vitro*, Spratt (1950) reported that nervous tissue, dependent on enzymes catalysing respiration, is more easily inhibited by oxygen deficiency, cyanide, and iodoacetate, while heart, dependent chiefly on glycolysis, is more sensitive to fluoride. By the same explantation technique, Duffey & Ebert (1957) compared the actions of fluoride and antimycin A and demonstrated an effect which was specific and, except for minor differences, similar for both these substances on the heart-forming mesoderm and somites. Thus antimycin A, a drug known to exert its action on a respiratory mechanism, was found to inhibit, with remarkable specificity, a tissue credited with a considerable dependence on glycolytic mechanisms.

The experiments described here show that the effect of the antimycin A varies with the technique employed; where the drug has been applied to the ventral surface of the embryo, the mesoderm is inhibited with little or no damage to the neural tube; if it is presented to the embryo from the dorsal aspect, brain tissue and the dorsal part of the somite suffer inhibition and often necrosis.

Comparison of the effects of antimycin A at different stages of development in the chick are interesting and important. Applied at stages 4 or 5, it may yield acardiac embryos with normal somites, whereas if applied at later stages, in which the primitive heart may have already appeared, it can destroy or inhibit somite formation without interference with heart or brain. Furthermore, there
may be somite damage localized geographically and, presumably, chronologically as well (Plate, fig. F). Two conclusions can be drawn from these observations: first, that there must be, for heart and for somites, phases of differentiation when each is more susceptible to the action of antimycin A, such phases occurring at or about stage 4 for the heart and over a series of later stages for the somites; and second, that a process of inactivation of antimycin A must occur as a result of its toxic effect on the tissues, allowing normal growth and differentiation to occur subsequently. Neither deduction is original. It has long been recognized not only that organs and tissues experience their initial phases of activity at different stages but also that they are more susceptible to the injurious action of both chemical and physical agents at these times. In regard to the second, Reif & Potter (1953) demonstrated inactivation of antimycin A but stated that it could be reversed by adding serum proteins. Thorn (1956) confirmed the 'pseudo-irreversible' nature of antimycin A by showing that in spite of a strong combination between it and the antimycin A-inhibitable factor, addition of a tissue preparation, in which the succinic oxidase system was specifically and irreversibly inhibited by p-aminophenylarsenoxide, caused reactivation. This renewed activity by the drug was probably due to a redistribution of the antimycin A over the increased quantity of susceptible factor and was inversely proportional to the increased ratio of heart-muscle preparation to inhibitor. On this basis, our experiments showing localized defects in somite formation apparently involved doses of antimycin A so critical that reactivation of the drug subsequent to the production of fresh antimycin A-inhibitable factor in the differentiating tissue was not demonstrable because the concentration of antimycin A had fallen below the threshold value for inhibition.

SUMMARY

Antimycin A has been administered to chick embryos at stages 4–9 in a variety of ways following well-established techniques. The pattern of abnormalities obtained depends essentially on the avenue of approach of the drug to the embryo; if it is applied to the ventral surface, heart and mesoderm are inhibited to a greater degree than nervous tissue; if on the dorsal surface, brain and spinal cord suffer more than mesodermal derivatives.

Heart and somites are susceptible to antimycin A at different stages of development, and Thorn’s demonstration, using heart-muscle preparations, of the reversible nature of the combination between antimycin A and the antimycin A-inhibitable factor in the succinic oxidase system is in agreement with our observations in the chick embryo.

RÉSUMÉ

L’action inhibitrice de l’antimycine A chez le jeune embryon de Poulet

L’antimycine A a été administrée à des embryons de Poulet aux stades 4 à 9 suivant différents procédés dérivant de techniques bien établies. Le type des
anomalies obtenues dépend essentiellement du mode d'administration de la drogue à l'embryon; si elle est appliquée à la surface ventrale, le cœur et le mésodermre sont inhibés plus intensément que le tissu nerveux; si elle est appliquée à la surface dorsale, le cerveau et la moelle souffrent plus que les dérivés mésodermiques.

Le cœur et les somites sont sensibles à l'antimycine A à différents stades du développement et la démonstration apportée par Thorn, à propos des préparations de muscles cardiaques, de la nature réversible de la combinaison entre l'antimycine A et du facteur inhibiteur de l'antimycine A dans le système de la succino-oxydase, est en accord avec nos observations sur l'embryon de Poulet.

REFERENCES


EXPLANATION OF PLATE

Fig. A. Explanted by the New technique at stage 6; 0.025 g antimycin A added to albumen; cultured for 20 hours.

Fig. B. Explanted by the New technique at stage 8; 0.025 g antimycin A added to albumen; cultured for 20 hours.

Fig. C. Transverse section of embryo explanted by New technique at stage 8; 0.02 g antimycin A added to albumen; cultured for 19 hours; showing necrosis of neural tissue and dorsal part of somite.
FIG. D. Explanted by New technique at stage 5; 0·01 y antimycin A applied to ventral surface; cultured for 19 hours.

FIG. E. Explanted by New technique at stage 5; 0·01 y antimycin A applied to ventral surface; cultured for 19 hours.

FIG. F. Explanted by New technique at stage 7; 0·01 y antimycin A applied to ventral surface; cultured for 20 hours.

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