A Study of the Mode of Action of Chloramphenicol on the Chick Morphogenesis

The effect of an antibiotic chloramphenicol on the morphogenesis of chick embryos has been reported by BLACKWOOD[1], NEWBURGH et al.[2], BILLET et al.[3] and others; however, its mode of action is little understood. WOLLEY[4] proposed that chloramphenicol could be regarded as a metabolic analogue of phenylalanine. According to ALLFREY et al.[5] cloramphenicol appears to act by inhibiting the transfer of amino acid from amino acyl-RNA to protein, while JARDETSKY[6,7] believes that chloramphenicol interferes with uridine coding (*m*-RNA level) required specifically for the synthesis of phenylalanine. BILLET et al.[3] seem to hold similar views. In view of these explanations, it was felt desirable to study the mode of action of chloramphenicol on chick morphogenesis.

Materials and methods. Freshly fertilized eggs of White Leghorn hens bought from a local poultry farm were incubated at 37.5 °C to obtain definitive primitive streak stages. The glassware used in the culturing of embryos was sterilized. The solutions employed for Pannet Compton saline (PC saline) were separately autoclaved.

The embryos were explanted by the method of NEW[8] and treated with chloramphenicol at a concentration of 0.2 mg/ml which was found suitable. After 6 h of incubation with the chemical treatment, the embryos were plunged into PC saline to remove the chemical completely, subsequently treated with PC saline, mounted, and further incubated for 20–22 h. They served as controls. Embryos were subsequently treated separately with approximately equimolar concentrations of (1) phenylalanine, (2) tyrosine, (3) *ortho*-amino-benzoic acid, (4) alanine, (5) phenyllactic acid, (6) phenyllactic acid and glutamic acid, and (7) *para*-amino-benzoic acid, and kept overnight to serve as experimental objects. Following 20–22 h incubation of the controls as well as the experimental embryos, they were fixed in acetic alcohol for $1^1/_2$ h, processed for whole mounts, stained with hematoxylin, photographed and examined.

Results and discussion. It can be seen from Table I that chloramphenicol affects mainly the differentiation of the nervous system and heart. The brain remains small and undifferentiated into different parts (microcephaly) and the neural tube remains open (Figures 1, 3, 5 and 7). Somites are diffuse in some cases. The heart in many cases remains straight and does not show its characteristic bent (Figures 1 and 3). Similar effects of chloramphenicol have been reported by BLACKWOOD[1] and BILLET et al.[3].

Tables II and III show the results on subsequent treatment in each set with approximately equimolar concen-

Fig. 1.

Fig. 2.

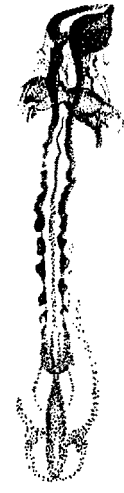

Fig. 3.

Fig. 4.

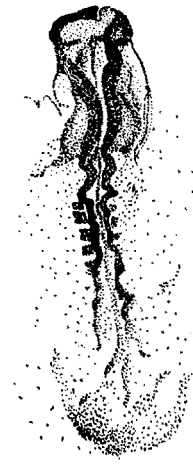

Fig. 5.

Fig. 6.

Fig. 7.

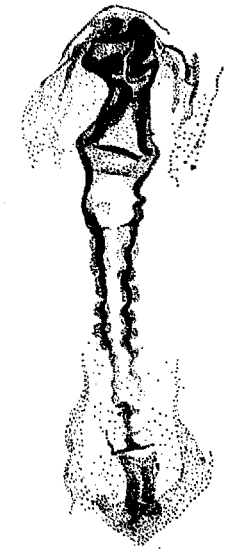

Fig. 8.

[1] U. B. BLACKWOOD, J. Embryol. exp. Morph. *10*, 315 (1962).
[2] R. W. NEWBURGH, A. CLARK, A. WILSON and M. SCHOZZ, J. Embryol. exp. Morph. *12*, 219 (1964).
[3] F. S. BILLET, R. COLLINI and L. HAMILTON, J. Embryol. exp. Morph. *13*, 341 (1965).
[4] D. W. WOLLEY, J. biol. Chem. *185*, 293 (1950).
[5] V. G. ALLFREY, J. W. HOPKINS, H. J. FRENSTER and A. E. MIRSKY, Ann. N.Y. Acad. Sci. *88*, 722 (1960).
[6] O. JARDETSKY, J. biol. Chem. *238*, 2498 (1963).
[7] O. JARDETSKY and G. R. JULIAN, Nature *201*, 397 (1964).
[8] D. A. T. NEW, J. Embryol. exp. Morph. *3*, 326 (1955).

Table I. Effect of chloramphenicol on chick embryos

No. of embryos treated with chloramphenicol (0.2 mg/ml)	Abnormalities			
	Nervous system showing microcephaly and open neural tube	Heart straight, not showing characteristic bent	Somites smaller in size or diffuse	Axis short of bent
23	19	14	12	10

Treatment with chloramphenicol at above concentration for 6 h after which chloramphenicol was replaced by PC saline and embryos incubated for further development for 20–22 h.

Table II. Chloramphenicol-treated embryos subsequently treated with different drugs

No. of chloramphenicol-treated embryos showing abnormalities of nervous system, heart, somites, etc. in sets 1–4	No. of chloramphenicol-treated embryos subsequently treated with:				No. of embryos showing perfect reversal to normal development in:			
	Phenyl alanine	Tyrosine	*Ortho*-amino-benzoic acid	Alanine	Phenyl alanine	Tyrosine	*Ortho*-amino-benzoic acid	Alanine
23, 17, 9, 12	26	20	12	10	26	20	9	–

Table III. Chloramphenicol-treated embryos subsequently treated with different drugs

No. of chloramphenicol-treated embryos showing abnormalities of nervous system, heart, somites etc. in sets 5–7	No. of chloramphenicol-treated embryos subsequently treated with:			No. of embryos showing perfect reversal to normal development in:		
	Phenyllactic acid	Phenyllactic acid and glutamic acid	*Para*-amino-benzoic acid	Phenyllactic acid	Phenyllactic acid and glutamic acid	*Para*-amino-benzoic acid
19, 7, 4	19	8	4	11	2	–

Concentration of Chloramphenicol as in Table I. Subsequent treatment with equimolar concentration of drugs used.

tration of (1) phenyl alanine, (2) tyrosine, (3) *ortho*-amino-benzoic acid, (4) alanine, (5) phenyllactic acid, (6) phenyllactic acid and glutamic acid, and (7) *para*-amino-benzoic acid. It is interesting to find that the effects of chloramphenicol can be reversed by subsequent treatment with equimolar concentration of phenylalanine (Figure 2), tyrosine (Figure 4) and *ortho*-amino-benzoic acid (Figure 6). Alanine (Figure 8) and *para*-amino-benzoic acid are ineffective, while phenyllactic acid alone and phenyllactic acid and glutamic acid are only partially effective in the reversal.

The specific role of the molecule of phenylalanine for the differentiation of neural crest cells in amphibia has been demonstrated by WILDE[9,10]. It has also been shown by COCKBURN et al.[11] that phenylalanine augments the beneficial effects of chloramphenicol in experimental *Klebsiella* infection in mice. In the present investigation it is found that phenyl alanine can be effectively replaced by tyrosine and *ortho*-amino-benzoic acid, and it appears that phenylalanine may merely be a precursor of tyrosine. It does not seem to be a non-specific requirement for aromatic material, since *para*-amino-benzoic acid has failed to substitute phenylalanine. Failure of alanine to substitute phenylalanine and its replaceability by *ortho*-amino-benzoic acid suggests independence of the phenomenon observed on the amino acid moiety of the phenylalanine molecule.

Chloramphenicol is supposed to affect the protein synthesis in chick embryo explants (NEWBURGH et al.[2]), in microorganisms (GALE[12], JARDETSKY et al.[7]) and in isolated cell nucleus (ALLFREY et al.[5]).

As the effects of chloramphenicol are reversed with exogenous supply of either phenylalanine, tyrosine or *ortho*-amino-benzoic acid, it is unlikely that the effects observed are due to metabolite antagonism as suggested by WOLLEY[4]. One would not expect the reversal with *ortho*-amino-benzoic acid if this mechanism was operative. For the same reasons, possible interference with coding of phenylalanine by *m*-RNA (JARDETSKY et al.[7], BILLET et al.[3]) or the transfer of amino acid from 5-RNA-amino acid complex (ALLFREY et al.[5]) is also unlikely to be the site of action of chloramphenicol in the system under investigation.

[9] C. E. WILDE, Ann. N.Y. Acad. Sci. *60*, 1015 (1955).
[10] C. E. WILDE, J. exp. Zool. *133*, 409 (1956).
[11] F. COCKBURN and O. J. KLEIN, Proc. Soc. exp. Biol. Med. *120*, 611 (1965).
[12] GALE, cited by O. JARDETSKY[7].

It is likely that a substance derivable either from phenylalanine, tyrosine or *ortho*-amino-benzoic acid is the agent effective in reversing the effects obtained with chloramphenicol. Chloramphenicol possibly inhibits the endogenous production of such a material. Precise identification of this material will be of interest.

Zusammenfassung. Hühnerembryonen, die in vitro mit Chloramphenicol behandelt wurden, zeigten verschiedene Missbildungen, wie Mikrocephalie, offene Neuralrinne, gerade bleibenden Herzschlauch, verkürzte Körperachse. Nachbehandlung solcher Chloramphenicol-Keime mit äquimolaren Lösungen von Phenylalanin, Tyrosin oder *Ortho*-Aminobenzoesäure ergibt weitgehend oder völlig normale Entwicklung. Durch Behandlung mit Alanin, Phenylmilchsäure oder *Para*-Aminobenzoesäure wird dagegen die Chloramphenicolwirkung nicht aufgehoben. Auf Grund dieser Ergebnisse wird der mögliche Mechanismus der Chloramphenicolwirkung diskutiert.

LEELA MULHERKAR[13], P. N. JOSHI and B. A. DIWAN

Department of Zoology of the University, Poona-7 (India), 20th April 1967.

[13] One of us (L.M.) wishes to thank the Atomic Energy Commission for financial assistance.

Whole-Body γ-Irradiation, Food Intake and Glucuronic Acid Excretion in Rats

In previous work it was found that the sensitivity to naphthalene was increased after whole-body irradiation in rats and that after 1000 R (whole body) of gamma rays the urinary glucuronic acid excretion was diminished[1]. Also it was found by HARTIALA and his group that in vitro local X-ray radiation decreased the ability of tissue from the intestinal tract[2] and liver[3] to conjugate *o*-amino-phenol with glucuronic acid. We found that same effect in the conjugation of anthranilic acid by duodenal tissue[4] in whole-body irradiation. All these results could have the same common denominator. However, a more detailed study of these problems showed that the low excretion of glucuronic acid by these animals is related to the dose of irradiation but mainly to the fact that animals are anorexic after irradiation[4].

The primitive hypothesis that some substances will be excreted as glucuronides after irradiation was found correct when the experiment was conducted with animals in starvation.

The condition of the experiments are reported elsewhere[1,4].

Total glucuronic acid in urine was generally determined by the method described by MEADS et al.[5]. Results are expressed as 'total glucuronic acid' in mg/24 h. The term 'total glucuronides' is used inferring that no free glucuronic acid is excreted by the kidney; free and conjugated glucuronic acid were determined by the method of FISHMAN et al.[6].

In preliminary studies, in which rats were irradiated with 1000 R, the excretion of total glucuronides was found to diminish and to reach the lowest values on the third day after irradiation.

In order to gain more information on this effect, experiments were done in which groups of 8 rats were irradiated with different doses: 1000, 400 and 100 R. Their daily food intake and daily excretion of total glucuronide were measured. The results (Figure 1) show the correlation of irradiation dose with food intake and with the amount of glucuronide excreted.

With 1000 R the food intake decreased severely for 4 days. With 400 R, there was a slight decrease during the first 3 days after irradiation. Therefore, the reduction of glucuronide excretion after irradiation must be correlated with the low food intake of the animals during this period.

An experiment was conducted on 8 rats to test the effect of starvation on the excretion of total glucuronides. Total glucuronide excretion was measured for 3 days before food was withheld. After 7 days food was again supplied. With a standard Purina diet, normal excretion of total glucuronides averages 40.5 mg/day determined

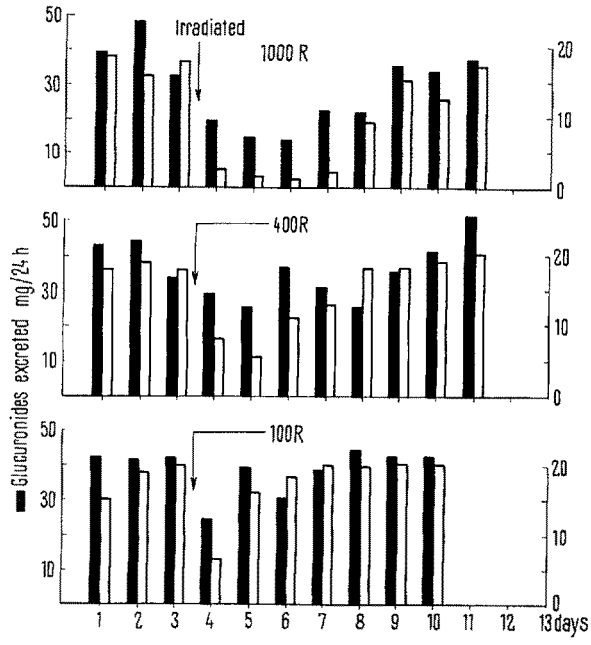

Fig. 1. Daily excretion of glucuronides and food intake □ before and after irradiation.

[1] J. CHIRIBOGA, Nature *198*, 803 (1963).
[2] K. J. HARTIALA, W. V. NANTO and U. K. RINNE, Acta physiol. scand. *43*, 77 (1958).
[3] K. J. HARTIALA, W. V. NANTO and U. K. RINNE, Acta physiol. scand. *42*, 231 (1959).
[4] J. CHIRIBOGA, Puerto Rico Nuclear Center Report No. 80, San Juan, Puerto Rico (1965).
[5] J. A. R. MEADS, J. N. SMITH and R. T. WILLIAMS, Biochem. J. *68*, 61 (1958).
[6] W. H. FISHMAN and S. GREEN, J. biol. Chem. *215*, 527 (1955).