THE EFFECT OF DEOXYPENTOSENUCLEIC ACID ON THE IMPAIRED OVIDUCT RESPONSE TO OESTROGEN IN THE FOLIC ACID-DEFICIENT CHICK

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The involvement of folic acid in the biochemical processes of embryonic development and tissue proliferation in higher organisms has mainly been inferred from experiments in which deficiency states were induced by antagonists of this vitamin. However, the original results of HERTZ[1], demonstrating the specificity of folic acid deficiency in retarding growth in the oestrogen-stimulated oviduct of the immature chick, were established by direct deprivation and not by employing an antagonist. It has been pointed out by JUKES, FRANKLIN AND STOKSTAD[2] that antagonists may produce toxic effects as well as an immediate deficiency of the vitamin when employed in experiments with higher organisms.

The experiments of SNELL AND MITCHELL[3] and STOKES[4] on bacterial growth suggested that folic acid was involved in the synthesis of nucleic acids. Further evidence has been obtained with higher organisms to support this function of folic acid, notably by SNELL AND CRAVENS[5] and NABER, SNELL AND CRAVENS[6] in respect of the chick embryo using aminopterin-induced inhibition of development. These workers showed that it was possible to by-pass the essential anabolic reactions inhibited by aminopterin by the administration of degradation products of the nucleic acids in purified form from natural sources. SKIPPER, BELL AND CHAPMAN[7] also showed that the antileukaemic action of folic acid antagonists could be partially reversed by deoxyribonucleic acid.

In the experimental work described here, an attempt was made to examine whether an essential function of folic acid in oestrogen-stimulated tissue growth, attributable to increased nucleic acid synthesis, could be demonstrated by replacement with intact deoxypentosenucleic acid (D.N.A.) in the immature chick fed a purified diet deficient in this vitamin.

Female chicks were fed a commercial chick mash for fourteen days followed by a purified diet deficient in folic acid for a further period of seventeen days. A combined hormonal treatment with 2.0 mg of oestradiol dipropionate and 1.0 mg testosterone propionate was then given to all birds by intramuscular injection on alternate days during a further eight-day experimental period on the deficient diet. During the first seven days of this period daily intramuscular injections of 100 mg of purified D.N.A. (ex herring sperm) in sterile solution (pH 7.0) or 0.5 mg folic acid were given. The groups of birds on each treatment were given similar amounts of food each day. Several experiments were carried out employing these experimental conditions and the results of two experiments in which positive control groups receiving folic acid were included are given below.

Statistical analysis of the data in relation to oviduct weight in both experiments showed that the D.N.A. significantly increased the growth response to oestrogen in the folic acid deficient chicks. In Expt. 1 where the deficiency was more pronounced than in Expt. 2 as indicated by blood composition, oviduct response of the control group and body gain during the experimental period, the

TABLE I

Experiment	*1*			*2*		
No. of chiks	*10*	*10*	*10*	*10*	*10*	*10*
Body-weight (g) Initial	200	206	206	209	208	207
Body-weight (g) Final	268	260	270	297	293	303
Oestradiol dipropionate (mg)	4 × 2.0	4 × 2.0	4 × 2.0	4 × 2.0	4 × 2.0	4 × 2.0
Testosterone propionate (mg)	4 × 1.0	4 × 1.0	4 × 1.0	4 × 1.0	4 × 1.0	4 × 1.0
D.N.A. (mg)	Nil	7 × 100	Nil	Nil	7 × 100	Nil
Folic acid (mg)	Nil	Nil	7 × 0.5	Nil	Nil	7 × 0.5
Oviduct wt. (g)	0.481 ±0.061	1.775 ±0.421	3.724 ±0.625	0.942 ±0.271	3.200 ±0.309	5.408 ±0.349
Haemoglobin (g/100 ml)	3.54	5.74	10.57	8.28	7.71	10.69

administration of D.N.A. resulted in a significant haematological response and this finding is being further investigated. In this respect this result parallels the sub-maximal haematological response to D.N.A. reported by CARTHWRIGHT, PALMER, TATTING, ASHENBRUCKER AND WINTROBE[8], in the folic acid deficient pig.

The data presented here would indicate that folic acid functions in essential metabolic reactions during synthesis of nucleic acids necessary for tissue proliferation under oestrogen stimulus. It is suggested that the oviduct of the chick rendered deficient by direct deprivation rather than analogue inhibition might prove a useful tissue in which to study the incorporation of isotopically labelled nucleic acid derivatives during growth processes.

Further results relating to the nucleic acid levels in the blood and oviduct tissue obtained in the experiments described here will be reported in greater detail at a later date.

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SUR LA CONSTITUTION CHIMIQUE D'UN ACIDE MYCOLIQUE INSATURÉ ISOLÉ DU BACILLE DIPHTÉRIQUE *(CORYNEBACTERIUM DIPHTHERIAE)**

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Dans des communications précédentes, nous avons décrit l'isolement[1], l'établissement de la structure chimique[2] et la synthèse[3] de l'acide coryno-mycolique, F. 70°, $C_{32}H_{64}O_3$, $[\alpha]_D = + 7.5°$, (I) du Bacille Diphtérique.

En fractionnant les acides insaturés qui accompagnent l'acide coryno-mycolique, nous avons isolé un nouvel acide, que nous proposons d'appeler *acide coryno-mycolénique.*

Nous avons isolé cet acide à partir de son sel de plomb, qui est soluble dans l'éther et dans l'alcool; l'ester méthylique a été chromatographié sur alumine (élution par benzène-éther, 1:1), puis précipité à l'état solide par congélation dans l'acétone à —15°, et enfin distillé sous 0.1 mm. L'*ester méthylique de l'acide coryno-mycolénique* passe à une température du bain de 230–250° sous forme d'une huile incolore, $n_D^{19°} = 1.4680$, $d_4^{20°} = 0.894$, $[\alpha]_{546}^{20°} = + 9.0° \pm 0.3°$. (Trouvé: C 78.18, 77.93%, H 12.58, 12.31%, $-OCH_3$ 6.30, 6.36%; calculé pour $C_{33}H_{64}O_3$: C 77.89%, H 12.68%, $-OCH_3$ 6.09%.)

L'*acide coryno-mycolénique*, obtenu par saponification de son ester méthylique, se présente sous forme d'une huile incolore, $n_D^{19°} = 1.4758$ (trouvé: PM, par titrage: 504; calculé: 494.8). On ne peut pas distiller l'acide sans qu'il se déshydrate partiellement. A l'hydrogénation catalytique il absorbe *une molécule d'hydrogène et donne l'acide coryno-mycolique*, F. 70° (I). Nous avons identifié ce dernier par l'analyse élémentaire (trouvé: C 77.31, 77.13%, H 12.42, 12.53%; calculé pour $C_{32}H_{64}O_3$: C 77.35%, 12.98%) et par son oxydation en *palmitone*, F. 80° (III) (trouvé C 82.50, 82.31%, H 13.78, 13.58%; calculé pour $C_{31}H_{62}O$: C 82.59, H 13.86%). L'identification de la palmitone obtenue à partir de l'acide coryno-mycolénique hydrogéné a été en outre confirmée par des mesures de diffraction

* 4ème Communication sur les constituants du Bacille Diphtérique; 3ème comm. voir[3].