PHARMACOLOGY

Anticoccidial Activity of Nicotinamide Antagonists

THE life-cycle of poultry coccidia can be inhibited by certain *p*-aminobenzoic acid[1–3], folic acid[3–6] and thiamine[7] antagonists. This knowledge led to our testing three known antagonists of another vitamin, nicotinamide, for anticoccidial activity. These substances were 3-acetylpyridine[8], pyridine-3-sulphonamide[9] and 6-aminonicotinamide[10].

To evaluate the activity of the compounds, they were mixed in chick starter mash of known composition and fed to one-week-old cockerels, starting one day before they were inoculated orally with approximately 200,000 sporulated oocysts of *Eimeria tenella* or *E. necatrix*, or 50,000 sporulated oocysts of *E. acervulina*. In each test one infected and one uninfected control group of birds were fed unmedicated ration. The criteria to assess activity were a comparison of mortality and oocyst production[11]. The toxicity of the compounds was assessed in both infected and uninfected groups by a comparison of death rates and weight gains of the birds individually weighed at intervals.

3-Acetylpyridine at 0·1 per cent w/w in the food was inactive against both *E. tenella* and *E. acervulina*.

Pyridine-3-sulphonamide at 0·025 per cent w/w was inactive against *E. tenella* and *E. necatrix*, and showed no signs of toxicity when fed for eight days. It was active against *E. acervulina*, but less effective than sulphaquinoxaline, the reference compound (Table 1). The results of exp. 3 indicate the possibility of different strain responses. The activity was neutralized by simultaneous feeding of an equal concentration of nicotinamide (exps. 4 and 5, Table 1). In another single experiment the activity of pyridine-3-sulphonamide was not antagonized by equal concentrations of *p*-, *o*- or *m*-aminobenzoic acid.

6-Aminonicotinamide showed a different spectrum of activity to pyridine-3-sulphonamide. It was active against *E. tenella* at low concentrations comparable with those of the control drug amprolium (1-(4-amino-2-*n*-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride) (Table 2), but there was little margin between the active and toxic concentrations. This nicotinamide analogue was active against *E. necatrix* and inactive against *E. acervulina* at the maximum tolerated concentration. Lower concentrations were not examined against *E. necatrix*.

Table 1. ACTIVITY OF PYRIDINE-3-SULPHONAMIDE AGAINST *E. acervulina* AND THE EFFECT OF ADDING NICOTINAMIDE

Exp. No.	Per cent drug in food P3S	NC	SQ	Strain of *E. acervulina*	Millions of oocysts passed per chick*
1	—	—	—	Ongar†	222·65
	0·0125	—	—		0·15
	0·006	—	—		10·47
	—	—	0·0125		0
2	—	—	—	Ongar†	88·26
	0·0125	—	—		0
	0·006	—	—		1·18
	—	—	0·006		0
3	—	—	—	Ongar†	113·02
	0·0125	—	—		2·14
	—	—	0·006		0
	—	—	—	Houghton‡	189·18
	0·0125	—	—		101·66
	0·006	—	—		210·75
	—	—	0·006		0·02
	—	—	—	Andover§	111·26
	0·025	—	—		0·02
	0·0125	—	—		37·60
	0·006	—	—		79·60
	0·003	—	—		85·79
	—	—	0·025		0·72
	—	—	0·0125		116·18
4	—	—	—	Ongar†	261·49
	—	—	0·006		0
	0·0125	—	—		0·76
	0·006	—	—		14·30
	0·003	—	—		78·90
	—	0·0125	—		179·68
	0·0125	0·0125	—		113·55
5	—	—	—	Ongar†	32·23
	0·05	—	—		0
	0·025	—	—		1·15
	—	0·05	—		25·03
	0·05	0·05	—		14·07
	0·025	0·025	—		22·28

P3S, pyridine-3-sulphonamide; NC, nicotinamide; SQ, sulphaquinoxaline.
* From fourth to thirteenth days post-infection.
† Isolated 1958.
‡ Isolated 1956.
§ Isolated 1960.

Table 2. 6-AMINONICOTINAMIDE ACTIVITY AGAINST *E. tenella* AND TOXICITY IN CHICKS

Exp. No.	Drug	Per cent in food	Deaths* by seventh day after infection: Acute coccidiosis	Toxicity
1	Nil (control)	—	13/15	—
	6-aminonicotinamide	0·001	0/15	13/15
		0·0005	0/15	0/15
		0·00025	11/15	0/15
	Amprolium	0·002	0/15	0/15
		0·001	3/15	0/15
		0·0005	7/15	0/15
2	Nil (control)	—	8/10	—
	6-aminonicotinamide	0·0008	0/10	0/10
		0·0004	3/10	0/10
		0·0002	5/10	0/10

* Numerator, No. of chicks dead. Denominator, No. of chicks per group.

Table 3. ANTAGONISM OF THE ACTIVITY AGAINST *E. tenella* AND THE TOXICITY IN THE CHICK OF 6-AMINONICOTINAMIDE (6-ANC) BY NICOTINAMIDE (NC)

Per cent drug in food 6-ANC	NC	Deaths* due to acute coccidiosis in infected groups after one week	Uninfected groups: Deaths* due to toxicity after 1 week	2 weeks	Av. wt. (g) gain after 1 week	2 weeks
—	—	15/15	0/28	0/28	39·3	103·3
0·002	—	0/15	15/28	21/28	5·8	20·2
0·001	—	0/10	1/20	6/20	14·0	37·1
0·0005	—	3/5	0/10	0/10	31·3	86·4
—	0·004	5/5	0/10	0/10	40·8	99·8
—	0·008	5/5	0/8	0/8	46·0	99·8
—	0·02	5/5	0/10	0/10	32·0	95·4
—	0·1	3/5	0/10	0/10	35·0	102·4
0·002	0·0005	0/5	8/10	10/10	0·6	—
0·002	0·001	0/9	4/18	15/18	3·6	17·4
0·002	0·002	0/10	7/18	13/18	14·2	46·5
0·002	0·004	0/10	2/18	9/18	15·8	67·6
0·002	0·008	4/5	0/8	0/8	33·3	81·6
0·002	0·02	5/5	0/10	0/10	32·4	93·9
0·002	0·1	5/5	0/10	0/10	32·8	92·1

Results of three experiments added together. Groups balanced for weight at the start of each test.
* As Tables 1 and 2.

The results in Table 3 show that one-half to twice the concentration of nicotinamide added to 6-aminonicotinamide has some effect in offsetting its toxicity as judged by the deaths and depression of gain in weight; but four times or more the amount of the vitamin was required to neutralize the anticoccidial activity and toxicity of this analogue.

These observations suggest that nicotinamide is a growth factor for *E. tenella*, *E. acervulina* and presumably for *E. necatrix*.

It is interesting to note that pyridine-3-sulphonamide showed activity only against *E. acervulina*, which is also generally more sensitive than *E. tenella* and *E. necatrix* to sulphanilamide derivatives. This may indicate that for *E. acervulina* transport of drugs to the site of action is enhanced by a weakly acidic group such as $—SO_2NHR$.

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