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INHIBITION OF MONOAMINE OXIDASE BY FURAZOLIDONE IN THE CHICKEN AND THE INFLUENCE OF THE ALIMENTARY FLORA THEREON

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1 The addition of furazolidone to the feed at the therapeutic level (0.04% w/w, 10 days) inhibited monoamine oxidase (MAO) activity by 47 to 72% in chicken duodenal mucosa, heart and brain, but in the liver the enzyme activity was unaffected by the treatment.

2 Furazolidone (200 mg/kg) administered by crop tube inhibited MAO activities in duodenal mucosa, liver, heart and brain.

3 Furazolidone (200 mg/kg) injected intramuscularly did not inhibit MAO activity in the chicken.

4 Pretreatment of the chickens with intramuscular neomycin did not antagonize the inhibition of MAO activity produced by furazolidone (200 mg/kg, crop tube).

5 Pretreatment with neomycin by crop tube to suppress the alimentary flora significantly reduced the effect of furazolidone on MAO activity, suggesting that the drug was transformed by the alimentary flora to an active metabolite which subsequently inhibited MAO activity in other organs.

6 Furazolidone in the feed (0.04% w/w, 10 days) or administered by crop tube (200 mg/kg) had no effect on the activity of aminopyrine demethylase in chicken liver.

7 The activity of aspartate transaminase in plasma was unaffected by the addition of furazolidone to the feed (0.04% w/w, 10 days).

Introduction

Furazolidone [(*N*-5-nitro-2-furfurylidene)3-amino-2-oxazolidine] is widely used in the prevention and treatment of certain bacterial and protozoal diseases which affect poultry (Harwood & Stunz, 1954; Rogers, Belloff, Paul, Yurchenco & Gever, 1956). At slightly above the therapeutic level the drug inhibits growth, produces anaemia and arrests spermatogenesis in the chicken (Cooper & Skulski, 1956; MacDonald & Beilharz, 1961; Ehlhardt, Beuving & Haye, 1975) and may also produce cardiac dilatation and ascites (Feron & Van Stratum, 1966). The biochemical lesion associated with these toxic signs has not been defined.

In rat and man, furazolidone inhibits monoamine oxidase (MAO, monoamine:O_2 oxidoreductase (deaminating) EC 1.4.3.4.). However, the drug does not inhibit MAO *in vitro* suggesting that a transformation product, possibly 2-diethylhydrazine, is responsible for the enzyme inhibition *in vivo* (Stern, Hollifield, Wilk & Buzard, 1967; Pettinger, Soyangco & Oates, 1968).

In the present experiments we have investigated the effect of furazolidone on the activity of MAO in the chicken. A preliminary account of some of the work was given to the British Pharmacological Society (Ali & Bartlet, 1979).

Methods

Animals

Thornber chickens (8 weeks old, either sex) were obtained from the Poultry Research Centre, Kings Buildings, Edinburgh. The birds were fed a layer mash (Douglas of Dalkeith, Edinburgh) which was free from growth promoting and anticoccidial drugs. In some experiments furazolidone was administered in the feed at the therapeutic level of 0.04% w/w for 10 days, the controls receiving unmedicated feed. In other experiments the drug (200 mg/kg) was administered by intramuscular injection or crop tube, control birds receiving 0.9% w/v aqueous NaCl or aqueous acacia (20% w/v), respectively.

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Preparation of tissues

Chickens were killed by decapitation and the tissues rapidly removed and chilled on ice. The lumen of the duodenum was washed with chilled saline before cutting it open and scraping away the mucosa with a glass slide. The heart was opened and blotted to remove blood. The tissues were cut into small pieces and homogenized with six strokes in a Teflon homogenizer in an ice bath. Homogenates (20% w/v) of each organ in 0.5 M phosphate buffer (pH 7.4) were prepared for the estimation of MAO activity. For the estimation of aminopyrine demethylase activity, a liver homogenate (25% w/v) in isotonic KCl (1.15% w/v) was centrifuged at 10,000 *g* for 20 min in an MSE High Speed 18 Refrigerated centrifuge at 5°C, and the microsome-rich supernatant used.

Measurement of enzyme activity

Monoamine oxidase activity MAO activity was estimated according to the method of Krajl (1965), in which the production of 4-hydroxyquinoline from the oxidative deamination of kynuramine is measured. The incubation mixture contained kynuramine dihydrobromide (100 μg), 0.5 M phosphate buffer pH 7.4 (0.5 ml), homogenate (equivalent to 0.78 mg duodenal mucosa, 3.13 mg liver or brain or 12.5 mg heart) and de-ionised water to a final volume of 3 ml. An incubate without substrate served as the blank. The reaction mixtures were incubated at 37°C for 30 min under air and with shaking. The incubates were then deproteinised with perchloric acid (0.6 M) as recommended by Century & Rupp (1968) followed by centrifugation at 900 *g* for 10 min at 5°C. Supernatant (1 ml) was mixed with 1 N NaOH (2 ml) and placed in a quartz cuvette. The solution was activated at 315 nm and the peak in the fluorescence scan at 380 nm measured in an Aminco-Bowman spectrofluorimeter. The presence of substances affecting the fluorescence of 4-hydroxyquinoline was sought by mixing equal volumes of tissue blank and 4-hydroxyquinoline solution. In all cases the fluorescence of the mixture was half that of the undiluted solution of 4-hydroxyquinoline, demonstrating an absence of augmentation or quenching of fluorescence.

Aminopyrine demethylase activity was estimated in the 10,000 *g* supernatant fraction of liver. The conditions of the incubation were those described by Mazel (1971), except that the concentrations of aminopyrine and semicarbazide in the incubation mixture were both 10 mM. The formaldehyde produced in the reaction was estimated by the method of Nash (1953).

Aspartate transaminase [AST (EC 2.6.1.1.)] activity was estimated in chicken plasma by the method described by Wilkinson, Baron, Moss & Walker (1972). This measures the rate at which absorbance at 340 nm decreases as NADH is oxidized to NAD. Blood was withdrawn from a wing vein by means of a heparinised syringe fitted with a 21G needle (external diameter 0.8 mm) and spun down in a refrigerated centrifuge (5°C) at 900 *g* for 20 min to separate plasma.

Statistical analysis

The values in the text, tables and figures are means ± the standard error of the mean (± s.e. mean) with the number of observations in parentheses. Significant difference between means was estimated by the *t* test and probability (*P*) values are given. A value of $P < 0.05$ is regarded as significant.

Drugs and chemicals

A premix containing 4% w/w furazolidone (Neftin) was used for the addition of the drug to the feed, and a micronized preparation of furazolidone (particle size 5 μm) for administration by injection and crop tube. Acetylacetone (Koch Light), aminopyrine (4-dimethylamine-antipyrine 97%) (Ralph Emanuel), L-aspartic acid (Sigma), D-glucose-6-phosphate monosodium salt (Sigma), 4-hydroxyquinoline (Sigma), kynuramine dihydrobromide (Sigma), malate dehydrogenase (Sigma), neomycin sulphate (70% soluble powder, veterinary) (Squibb & Sons), nicotinamide (Sigma), nicotinamide adenine dinucleotide phosphate (Sigma), nicotinamide adenine dinucleotide, reduced form (Sigma) and 2-oxoglutaric acid (Sigma) were used. The other chemicals were Analytical Reagent grade.

Results

In the chicken the activity of MAO in heart and liver is reported to increase with age (Ignarro & Shideman, 1968), and our data (Table 1) confirmed this trend.

Table 1 Monoamine oxidase (MAO) activity in homogenates of organs from chickens of different ages

	MAO activity (*4-Hydroxyquinoline, μmol g^{-1} tissue h^{-1}*)		
	1 day	*8 weeks*	P
Duodenum	36.9 ± 5.1 (6)	90.7 ± 12.5 (8)	<0.02
Liver	2.5 ± 0.4 (6)	22.3 ± 1.9 (7)	<0.001
Heart	1.2 ± 0.1 (6)	3.3 ± 0.3 (7)	<0.001
Brain	12.7 ± 1.1 (7)	15.6 ± 2.0 (7)	>0.2

The values in the table are means ± s.e. means (No. of animals).

Thus in our other experiments all the birds were killed at the same age, 8 weeks, at which age the activities of MAO in control organs was consistent.

Effect of furazolidone in the feed on monoamine oxidase activity

When furazolidone was added to the feed at a therapeutic concentration of 0.04% w/w for 10 days, the activity of MAO was reduced significantly in duodenal mucosa, heart and brain, and not in liver (Table 2). Another experiment investigated the recovery in enzyme activity after discontinuing the medicated feed. This experiment confirmed the effect of feeding furazolidone on MAO activity, which was inhibited in the duodenal mucosa ($P < 0.001$), heart ($P < 0.02$) and brain ($P < 0.02$), and not in liver ($P > 0.1$) (Figure 1). Seven days after discontinuing furazolidone in the feed a significant recovery of MAO activity was only evident in the duodenal mucosa ($P < 0.02$). Fourteen days after discontinuing the drug the recovery of MAO activity was complete in each organ, there being no significant difference from the control values ($P > 0.1$ for each organ).

Effect of furazolidone by crop tube or intramuscular injection on MAO activity

The drug was administered at a dose of 200 mg/kg body weight, which is about half the reported LD_{50} in the chicken (Radchuk, 1965). Furazolidone administered by crop tube inhibited MAO activity in the liver as well as that in duodenal mucosa, heart and brain (Table 3). The time course of the inhibition was different in various organs, however, being maximal in the liver 12 h after drug administration and in the duodenal mucosa and brain after 24 h.

In another experiment furazolidone (200 mg/kg) was administered by crop tube or intramuscular injection and MAO activities estimated 24 h later. This experiment confirmed that the drug given by crop tube inhibited MAO activity in the liver as well as in the other organs studied ($P < 0.001$ for each organ, 6 birds). In contrast, furazolidone injected intramuscularly did not affect MAO activity in any of the organs studied ($P > 0.1$ for each organ, 6 birds).

Effect of neomycin on the inhibition of monoamine oxidase by furazolidone

In this experiment the birds were given neomycin, either 20 mg by intramuscular injection or 200 mg by crop tube twice daily for 5 days before MAO estimation. Furazolidone (200 mg/kg) was administered by crop tube to saline- and neomycin-treated birds 24 h before the enzyme estimation. Neomycin did not affect MAO activity and the values obtained from chickens pretreated with saline or neomycin and given acacia mucilage (crop tube) were pooled and used as controls. As shown in Table 4, furazolidone administered by crop tube inhibited MAO activity in each organ as before ($P < 0.01$), and pretreatment

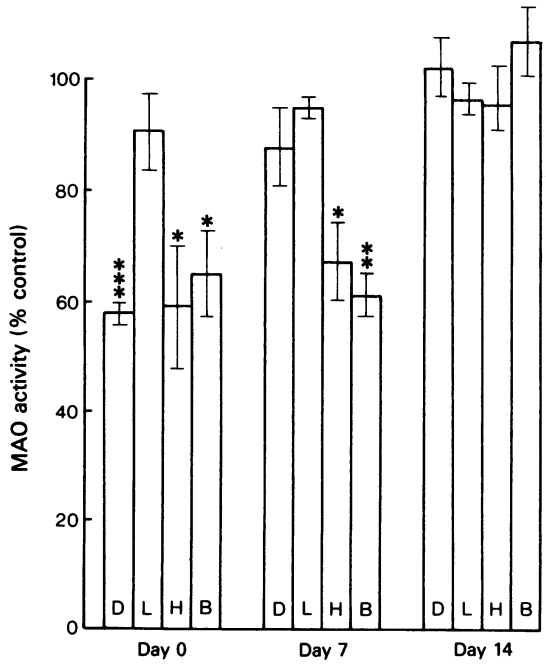

Figure 1 Monoamine oxidase activity (MAO) in homogenates of chicken organs after discontinuing furazolidone in the feed (0.04% w/w, 10 days). Ordinate scale: MAO activity as a percentage of the control value. The columns represent mean values for duodenal mucosa (D), liver (L), heart (H) and brain (B) at 0, 7 and 14 days after discontinuing furazolidone medication. The vertical bar at the head of each column depicts s.e. mean (3 birds). The asterisks mark significant inhibition of MAO, at the 2% (*), 0.2% (**) and 0.1% (***) levels.

Table 2 Effect of feeding furazolidone (0.04% w/w, 10 days) on monoamine oxidase (MAO) activity in homogenates of chicken tissue

	MAO activity (4-Hydroxyquinoline, μmol g^{-1} tissue h^{-1})		
	Control	*Furazolidone*	P
Duodenum	92.6 ± 10.0 (10)	58.9 ± 7.5 (9)	<0.02
Liver	21.9 ± 3.9 (8)	22.3 ± 1.9 (8)	>0.9
Heart	3.4 ± 0.3 (7)	1.7 ± 0.3 (6)	<0.002
Brain	15.5 ± 2.0 (7)	9.4 ± 0.7 (6)	<0.02

The values in the table are means ± s.e. means (No. of animals).

with neomycin by the intramuscular route did not affect the MAO inhibition ($P > 0.1$). On the other hand, pretreatment with neomycin by crop tube significantly reduced the MAO inhibiting action of furazolidone in each organ ($P < 0.01$ to < 0.001). Following oral administration of neomycin and furazolidone the activity of MAO was less than the control value in the liver ($P < 0.002$) and brain ($P < 0.05$) but not in the duodenal mucosa ($P > 0.1$) and heart ($P > 0.05$). Thus the suppression of the MAO inhibiting action of furazolidone by the oral neomycin was complete in the duodenal mucosa and heart and not in the liver and brain.

Effect of furazolidone on aspartate transaminase and aminopyrine demethylase activities

Addition of furazolidone (0.04% w/w, 10 days) to the feed had no significant effect on the activity of aspartate transaminase in plasma (Table 5).

Table 3 Effect of time on monoamine oxidase (MAO) inhibition produced by furazolidone (200 mg/kg) given by crop tube

Time (h) after furazolidone	*MAO activity (4-Hydroxyquinoline, μmol g^{-1} tissue h^{-1})*			
	Duodenal mucosa	*Liver*	*Heart*	*Brain*
0	95.95 ± 6.21 (3)	29.72 ± 4.70 (3)	2.51 ± 0.21 (3)	15.41 ± 1.76 (3)
12	40.43 ± 0.92 (3)	9.93 ± 0.31 (3)	0.90 ± 0.01 (3)	8.80 ± 0.41 (3)
24	34.66 ± 1.67 (3)	11.65 ± 0.53 (3)	0.92 ± 0.10 (3)	7.02 ± 0.85 (3)
48	36.63 ± 2.65 (3)	15.27 ± 1.12 (3)	0.82 ± 0.06 (3)	8.04 ± 0.18 (3)

The values in the table are means ± s.e. means (No. of animals).

Table 4 Monoamine oxidase (MAO) activity in homogenates of chicken organs 24 h after administration of furazolidone (200 mg/kg, crop tube) and the influence of neomycin thereon

	MAO activity (4-Hydroxyquinoline, μmol g^{-1} tissue h^{-1})				P *Intramuscular neomycin [cf (3) and (2)]*	P *Oral neomycin [cf. (4) and (2)]*
	(1) Control	*(2) Furazolidone*	*(3) Furazolidone + *neomycin (i.m.)*	*(4) Furazolidone + **neomycin (oral)*		
Duodenal mucosa	93.71 ± 5.29 (15)	32.83 ± 6.65 (6)	38.10 ± 2.20 (3)	75.19 ± 11.54 (6)	>0.1	<0.01
Heart	2.66 ± 0.13 (15)	0.81 ± 0.09 (6)	1.18 ± 0.30 (3)	2.32 ± 0.14 (6)	>0.1	<0.001
Brain	11.77 ± 0.71 (15)	7.11 ± 0.65 (6)	7.38 ± 0.37 (3)	10.05 ± 0.27 (6)	>0.1	<0.002

The values in the table are means ± s.e. means (No. of animals).
* Neomycin, 20 mg/bird by intramuscular injection, twice daily for 5 days before the MAO estimation.
** Neomycin, 200 mg/bird by crop tube, twice daily for 5 days before the MAO estimation.

Table 5 Effect of furazolidone on the activities of aminopyrine demethylase in liver and aspartate transaminase in plasma from chickens

Expt	*Treatment*	*Aminopyrine demethylase (H.CHO μmol* ml^{-1} h^{-1})*	*Aspartate transaminase (iu/l)*
1	Control feed	0.37 ± 0.04 (6)	56.2 ± 5.2 (5)
	Furazolidone, 0.04% w/w in feed, 10 days	0.37 ± 0.05 (6)	59.1 ± 5.2 (8)
2	Acacia mucilage (crop tube)	0.30 ± 0.03 (5)	
	Furazolidone, 200 mg/kg (crop tube)	0.35 ± 0.08 (6)	

* Per ml of 10,000 *g* supernatant of liver homogenate (25% w/v).
The values in the table are means ± s.e. means (No. of animals).
The drug had no significant effect on the activities of either enzyme.

In two experiments the effect of furazolidone on aminopyrine demethylase activity in a microsome-rich supernatant fraction of chicken liver was investigated (Table 5). In one experiment the drug was administered in the feed at the therapeutic dose, and in the other by crop tube at a dose of 200 mg/kg 24 h before estimation of the enzyme activity. In both experiments the activity of aminopyrine demethylase was unaffected by the drug.

Discussion

Furazolidone administered in the feed at the therapeutic dose inhibited MAO activity in the duodenal mucosa, heart and brain, but in the liver the enzyme activity was unaffected by the treatment. MAO is inhibited by a transformation product of furazolidone (Stern *et al.*, 1967; Pettinger *et al.*, 1968), and MAO inhibition would be expected at the site of transformation. As MAO activity was not inhibited in the liver, it seems improbable that the transformation of furazolidone occurred here. Following withdrawal of medication, the enzyme activity recovered more promptly in the duodenal mucosa than in the other organs. The recovery of activity was probably due to synthesis of new enzyme, as dialysis does not restore activity to MAO inhibited by furazolidone (Stern *et al.*, 1967).

Furazolidone administered by crop tube inhibited the activity of MAO in the duodenal mucosa, liver, heart and brain. Thus furazolidone given as a bolus inhibited the hepatic enzyme although it did not do so when fed continuously. In the hepatocytes, the furazolidone metabolite may inhibit mitochondrial MAO, or, it may be metabolized further and/or excreted with bile. The present observations suggest that clearance of the metabolite was insufficient to protect hepatic MAO from inhibition when the drug was given as a bolus (200 mg/kg), but was sufficient when it was given as a feed additive (0.04% w/w, 10 days). Following furazolidone by crop tube a maximum inhibition of MAO in the liver was found after 12 h and in other organs after 24 h. This observation suggests that hepatic clearance of the furazolidone metabolite resulted in a briefer inhibition of MAO in the liver than elsewhere.

Furazolidone administered by intramuscular injection did not affect the activity of MAO in any of the organs studied. This observation suggests that the conversion of the drug to an MAO inhibitor did not occur in chicken tissues. However, this finding may not extend to other species as furazolidone given by intravenous or intraperitoneal injection is reported to inhibit MAO in the rat (Stern *et al.*, 1967).

The alimentary flora might have produced the metabolite which inhibited MAO activity. To test this hypothesis furazolidone was given by crop tube after suppressing the alimentary flora with neomycin. Pretreatment with neomycin by crop tube antagonized the action of furazolidone on MAO activity, but neomycin injected intramuscularly at 1/10th of the oral dose did not do so. Less than 10% of neomycin is absorbed from the alimentary tract (Waksman, 1953; Freyburger & Johnson, 1956), so in the present experiments absorbed neomycin could not have influenced the action of furazolidone on MAO activity. The inhibition of MAO activity by furazolidone was suppressed by unabsorbed neomycin, which supports the hypothesis that the alimentary flora transforms furazolidone to an active metabolite which is subsequently responsible for the inhibition of MAO activities in other organs.

Inclusion of furazolidone in the feed (0.04% w/w, 10 days) did not affect the activity of aspartate transaminase in plasma. This is a cytoplasmic enzyme, and the present observation suggests that furazolidone did not produce a detectable damage in cell membranes. However, furazolidone at a higher dose (0.05% w/w, 28 days) has been reported to increase significantly the activity of this enzyme in turkey plasma (Staley, Noren, Bandt & Sharp, 1978).

Many drugs inhibit both MAO and mixed function oxidase (Kato, Takanaka & Shoji, 1969). Aminopyrine demethylase activity in chicken liver, however, was unaffected by furazolidone treatment (0.04% w/w, 10 days, or 200 mg/kg by crop tube). Thus furazolidone at the recommended therapeutic dose would not be expected to potentiate the actions of other drugs metabolized by mixed function oxidase.

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References

ALI, B.H. & BARTLET, A.L. (1979). Inhibition of monoamine oxidase by furazolidone in the chicken: influence of the alimentary flora. *Br. J. Pharmac.*, **66**, 491P.

CENTURY, B. & RUPP, K.L. (1968). Comment on microfluorimetric determination of monoamine oxidase. *Biochem. Pharmac.*, **17**, 2012–2013.

COOPER, D.M. & SKULSKI, G. (1956). Effect of feeding furazolidone on fowl—1. Effect on body weight and sexual

development in cockerels. *J. comp. Path.*, **66**, 229–311.

EHLHARDT, D.A., BEUVING, G. & HAYE, U. (1975). Effect of oral administration of furazolidone (Furoxone) on growth, haemoglobin level, packed cell volume and initial egg production of egg- and meat-type replacement pullets. *Arch. Geflügelk.*, **39**, 68–72.

FERON, V.J. & VAN STRATUM, P.G.C. (1966). The effects of furazolidone on broiler chickens fed rations containing amprolium or zoalene—2. Intoxication phenomena at continuous administration during six weeks. *Tijdschr. Diergeneesk.*, **9**, 571–579.

FREYBURGER, W.A. & JOHNSON, L.E. (1956). Blood levels and urinary excretion of orally administered neomycin B and C in dogs. *Antibiot. Chemother.*, **6**, 586–588.

HARWOOD, P.D. & STUNZ, D.I. (1954). Efficacy of furazolidone, a new nitrofuran against blackhead and coccidiosis. *J. Parasit.*, **40**, 24–25.

IGNARRO, L.J. & SHIDEMAN, F.E. (1968). Catechol-*O*-methyl transferase and monoamine oxidase activities in the heart and liver of the embryonic and developing chick. *J. Pharmac. exp. Ther.*, **159**, 29–37.

KATO, R., TAKANAKA, A. & SHOJI, H. (1969). Inhibition of drug-metabolising enzymes of liver microsomes by hydrazine derivatives in relation to their lipid solubility. *Jap. J. Pharmac.*, **19**, 315–322.

KRAJL, M. (1965). A rapid microfluorimetric determination of monoamine oxidase. *Biochem. Pharmac.*, **14**, 1684–1685.

MAZEL, P. (1971). Experiments illustrating drug metabolism *in vitro*. In *Fundamentals of drug metabolism and drug disposition*. ed. La Du, B.N., Mandel, H.G. & Way, E.L. pp. 546–582. Baltimore: Williams & Wilkins Co.

MCDONALD, M.W. & BEILHARZ, R.G. (1961). Effect of furazolidone on growth of chickens. *Aust. Vet. J.*, **37**, 185–187.

NASH, T. (1953). The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.*, **55**, 416–421.

PETTINGER, W.A., SOYANGCO, F.G. & OATES, J.A. (1968). Inhibition of monoamine oxidase in man by furazolidone. *Clin. Pharmac. Ther.*, **9**, 442–447.

RADCHUK, N.A. (1965). Toxic dose of furazolidone for chicks and hens. *Mater.* XIV. *Knof. Leningrad Vet. Inst.*, pp. 133–135.

ROGERS, G.S., BELLOFF, G.B., PAUL, M.F., YURCHENCO, J.A. & GEVER, G. (1956). Furazolidone, a new antimicrobial nitrofuran. A review of laboratory and clinical data. *Antibiot. Chemother.*, **6**, 231–242.

STALEY, N.A., NOREN, G.R., BANDT, C.M. & SHARP, H.L. (1978). Furazolidone-induced cardiomyopathy in turkeys. *Am. J. Path.*, **91**, 531–541.

STERM. I.V., HOLLIFIELD, R.D., WILK, S. & BUZARD, J.A. (1967). The anti-monoamine oxidase effects of furazolidone. *J. Pharmac. exp. Ther.*, **156**, 492–499.

WAKSMAN, S.A. (1953). Activity of neomycin in experimental animals. In *Neomycin*. pp. 132–146. New Brunswick, New Jersey: Rutgers University Press.

WILKINSON, J.H., BARON, D.N., MOSS, D.W. & WALKER, P.G. (1972). Standardization of clinical enzyme assays: a reference method for aspartate and alanine transaminases. *J. clin. Path.*, **25**, 940–944.

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