ANTITUBERCULOUS ACTIVITY OF CYANACETIC ACID HYDRAZIDE

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Cyanacetic acid hydrazide (C.A.H.; "reazide") was investigated by Valdecasas *et al.* (1953), who, considering it to have similar chemical properties to isoniazid, believed that it might have similar anti-tuberculous activity as well. They stated that this was difficult to estimate with accuracy *in vitro* because of instability of the drug in aqueous solution. However, results obtained by Hartl (1954) with tuberculous guinea-pigs suggested that C.A.H. had a high degree of activity *in vivo*, and Scheu (1954), in a preliminary report, claimed that daily doses of 5–10 mg. per kg. given to 11 patients with pulmonary tuberculosis produce responses comparable with those obtained from treatment with isoniazid. Hartl (1954) reported that two isoniazid-resistant strains of *Mycobacterium tuberculosis* were sensitive to C.A.H., suggesting that the actions of the two drugs were different in this respect. Bavin and Seymour (1954) reported some *in vitro* and *in vivo* results, and concluded that the drug appeared less active than isoniazid against *Myco. tuberculosis* H37Rv and was relatively inactive against an isoniazid-resistant strain, though its toxicity was of the same order as that of isoniazid.

We have therefore investigated the toxicity of C.A.H. and its antituberculous activity *in vitro* and *in vivo*, especially against strains of *Myco. tuberculosis* resistant to isoniazid.

Methods

Toxicity.—The acute intravenous and chronic oral toxicities of both C.A.H. and isoniazid were determined in mice.

Strains of *Mycobacteria*.—**Stock Strains:** Human, H37Rv and CN3679; bovine, CN839; avian, CN279. **Other Strains:** Strains made resistant *in vitro*: isoniazid-resistant, H37Rv-I.R.; isoniazid- and streptomycin-resistant, H37Rv-I.S.R. Strains isolated from patients treated with isoniazid: Ht., Et., CN3574, CN3576, CN3578, and CN3617. A variant of H37Rv isolated from a guinea-pig after treatment with isoniazid: CN3616. A strain isolated from a patient before treatment: CN3577.

***In Vitro* Activity.**—The bacteriostatic activity against tubercle bacilli was measured in Dubos medium and in the egg-agar medium described by Peizer and Schecter (1950) and the bactericidal activity by subculture from drug-containing Dubos medium on to jars containing Löwenstein-Jensen medium. The bacteriostatic activity for other organisms was measured in nutrient broth.

Stability Tests.—The rate at which C.A.H. and isoniazid became inactivated by incubation at 37° C. in Dubos medium was measured by microbiological assay of samples taken at weekly intervals.

Development of Resistance.—Resistance to C.A.H. and isoniazid was developed by subculture in drug-containing media.

***In Vivo* Tests.**—**Intravenous Mouse Tests:** Into each mouse (albino, Schofield strain), weighing 17–19 g., 1.2 mg. (wet weight) of organisms from a seven-day culture in Dubos medium, resuspended in 0.5 ml. of this medium, was injected intravenously. The drugs were administered twice daily, except on Saturdays and Sundays, when only one dose was given. The infecting culture was tested for sensitivity to C.A.H. and isoniazid in Dubos medium and on Peizer and Schecter medium. Necropsies were made on the lungs, and tubercle bacilli were recovered and tested for isoniazid sensitivity on Peizer and Schecter medium. **Intra-corneal Mouse Tests:** The inoculation technique was that described by Rees and Robson (1950), and progress of the corneal disease was observed twice weekly.

Results

Toxicity

The intravenous acute LD₅₀ dose in mice was 230 mg. per kg. for C.A.H. and 170 mg. per kg. for isoniazid. This figure for C.A.H. is somewhat lower than the 375 mg. per kg. for rats found by Valdecasas *et al.* (1953). The figure for isoniazid agrees very closely with 153 mg. per kg. for mice reported by Benson *et al.* (1952).

Stability Tests

The minimum concentrations of isoniazid and C.A.H. in Dubos medium inhibiting growth after various periods of incubation at 37° C. before inoculation are shown in Table I. These results suggest that the proportionate loss of activity under these conditions was of the same order for both drugs.

TABLE I.—Effect of Incubation at 37° C. on Antituberculous Activity of Isoniazid and C.A.H. in Dubos Medium. Strain CN3679

Time at 37° C. before Inoculation (days)	0	7	14	21	28
Inhibitory concentra- tion in µg./ml.*	Isoniazid 0.06 C.A.H. 2.0	0.25 4.0	0.5 8.0	0.5 16.0	1.0 16.0

* Read after seven days' incubation.

In Vitro Activity

The minimum concentrations of C.A.H. inhibiting the growth of the isoniazid-sensitive strains of *Myco. tuberculosis* and *Mycobacterium avium* are shown in Table II. Except against *Myco. avium* C.A.H. in Dubos medium was considerably less active than isoniazid in inhibiting growth

TABLE II.—Minimum Concentration (in µg. per ml.) of Isoniazid and C.A.H. Inhibiting Growth of Isoniazid-sensitive Strains of *Myco. tuberculosis* and *Myco. avium*

Organisms	Period of Incuba- tion (Days)	Dubos Medium		Peizer and Schecter Medium	
		Isoniazid	C.A.H.	Isoniazid	C.A.H.
<i>Myco. tuberculosis</i> var. <i>hominis</i> H37Rv	7	0.016	2.0	—	—
	14	0.016	2.0	—	—
	28	0.03	2.0	—	—
	56	0.06	4.0	—	—
<i>Myco. tuberculosis</i> var. <i>hominis</i> CN3679	7	0.06	2.0	—	31
	14	0.12	4.0	0.06	31
	28	0.25	4.0	0.12*	64*
	56	0.5	4.0	0.25*	125*
<i>Myco. tuberculosis</i> var. <i>bovis</i>	7	0.016	1.0	—	—
	14	0.03	1.0	0.12	16
	28	0.03	1.0	0.5*	32*
	56	0.03	1.0	0.5*	64*
<i>Myco. avium</i>	7	16	31	—	500
	14	31	31	31	500
	28	31	62	125*	500
	56	31	125	250*	1,000*

* The growth which appeared after 14 days in tubes previously negative consisted of only a few colonies.

after seven days, though if the incubation was continued the difference between the activities of the drugs became somewhat less marked. On the egg medium the activity of C.A.H. was much less than in Dubos medium, but that of isoniazid was about the same on both media.

Table III, giving the activity of C.A.H. against the isoniazid-resistant strains, shows that, in general, these strains were resistant to C.A.H. also. There were, however, two exceptions; CN3574, which was originally isolated

TABLE III.—Minimum Concentrations (in $\mu\text{g. per ml.}$) of Isoniazid-resistant Strains of *Myco. tuberculosis*. Incubation 14 days

Strains	Dubos Medium		Peizer and Schecter Medium	
	Isoniazid	C.A.H.	Isoniazid	C.A.H.
CN3616 ..	62	>1,000	125	1,000
CN3576 ..	31	31	250	1,000
CN3578 ..	31	125	250	500
CN3574 ..	1	1	4	31
CN3617 ..	16	4	31	31
CN3577 ..	0.2	16	0.2	125
H37Rv-I.R.	40	160	—	—
H37Rv-I.S.R.	40	160	—	—
Ht. ..	0.6	5	—	—
Et. ..	2	160	—	—

from a patient after treatment with isoniazid, was slightly resistant to isoniazid and fully sensitive to C.A.H.; and CN3617, isolated from a patient during treatment with isoniazid, was very resistant to isoniazid and sensitive to C.A.H.

Table IV shows that C.A.H. was bactericidal, and, although its rate of killing was similar to that of isoniazid, the concentration of C.A.H. required to achieve this was some fifty times higher than that needed with isoniazid.

Against *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Salmonella typhosa*, *Bacterium coli*,

TABLE IV.—Viable Counts of *Myco. tuberculosis*, Strain CN3679, on Löwenstein Medium After Exposure to C.A.H. and Isoniazid in Dubos Medium

Time of Exposure to Drug	Isoniazid ($\mu\text{g. per ml.}$)					C.A.H. ($\mu\text{g. per ml.}$)				Control
	1	0.5	0.1	0.05	0.01	10	5	1	0.5	
0 hours	64	160	120	120	160	160	200	200	200	160
6 "	180	150	240	200	220	160	240	200	240	200
30 "	0	0	9	80	>400	0	14	320	240	>400
7 days	0	0	1	>400	>400	0	0	>400	>400	>400

Vibrio comma, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Erysipelothrix rhusiopathiae*, *Pasteurella muriseptica*, and *Clostridium perfringens* C.A.H. showed no bacteriostatic activity at concentrations less than 100 $\mu\text{g. per ml.}$ This examination was made with only one strain of each species.

In Vivo Tests

The results of the experiment with the strain CN3679 are shown in Table V. The mice were protected by 0.2 mg. of isoniazid subcutaneously twice daily, but treatment with

TABLE V.—Chemotherapeutic Experiments on Groups of 10 Mice With *Myco. tuberculosis*, Strain CN3679. Infecting Dose was 1.2 mg. (wet weight) per Mouse

Drug	Route	Daily Dose (mg./mouse)	Average Day Survival (max. 80 Days)
Isoniazid ..	Subcutaneous	2 $\times$ 0.2	80.0
C.A.H. ..	"	2 $\times$ 2.0	25.0
" ..	"	2 $\times$ 1.0	18.6
" ..	Oral	2 $\times$ 2.0	31.8
" ..	"	2 $\times$ 1.0	14.7
Controls ..	Diet	8.0*	17.0
			16.3

* Assuming that each mouse ate 5 g. a day.

C.A.H. subcutaneously or orally gave only incomplete protection, even with a dose ten times greater. (Previous unreported experiments, similarly designed, have shown 100% survival of mice given as little as 0.02 mg. of isoniazid subcutaneously twice daily.)

The results of the experiments with strains showing various degrees of resistance to isoniazid and C.A.H. are given in Table VI. The results confirm *in vivo* the previous findings *in vitro*. With strains resistant to both isoniazid and C.A.H. (CN3576, CN3578, and CN3616) the mice were given little or no protection by either drug. Against strains which were only slightly resistant to isoniazid but fully sensitive to C.A.H. (CN3574) or slightly resistant to both (CN3577) the mice were protected to about the same extent by both drugs as they were against the fully sensitive strain (CN3679) (Table V). The anomaly in strain CN3617, being resistant to isoniazid yet fully sensitive to C.A.H. *in vitro*, was seen also in the animal studies, for the mice treated with isoniazid died almost as quickly as the untreated animals, whereas those treated with C.A.H. did not die so soon, the protection being about the same as in mice given a drug-sensitive infection.

Resistance to C.A.H. and isoniazid after passing through Dubos medium containing C.A.H.

TABLE VI.—*Chemotherapeutic Experiments on Groups of 10 Mice Infected with Strains of Myco. tuberculosis of Various Degrees of Sensitivity to Isoniazid. The Drugs were Given Twice Daily; C.A.H. was Given Orally and Isoniazid Subcutaneously. The Experiments were Terminated at 80 Days*

Strain	In Vitro Sensitivity, Minimum Inhibitory Concentration in $\mu\text{g./ml.}$ (Dubos Medium)		Average Day Survival (max. 80 Days)		
	Isoniazid	C.A.H.	Isoniazid	C.A.H.	Controls
			2 $\times$ 0.2 mg./ mouse/day	2 $\times$ 2 mg./ mouse/day	
CN3576 ..	31	31	35.7	37.6	30.8
CN3578 ..	31	125	13.1	15.2	9.8
CN3616 ..	62	>1,000	9.5	9.0	7.5
CN3574 ..	1	1	65.2	24.6	12.4
CN3577 ..	0.2	16	80.0	21.0	9.6
CN3617 ..	16	4	11.4	18.1	10.8

Intracorneal Tests

Against an infection set up with the isoniazid-sensitive culture of H37Rv, C.A.H. in the diet at the same level of dosage usually adopted for isoniazid in this type of experiment—namely, 0.3 mg. per mouse daily—had no suppressive effect at all. Streptomycin, 4 mg. per mouse daily by subcutaneous injection, combined with C.A.H., had only that effect to be expected from streptomycin alone.

Against an isoniazid-resistant infection with H37Rv-I.R., C.A.H. at the same dosage had no apparent influence on the course of the corneal disease, nor did isoniazid.

With the C.A.H.-resistant infection from CN3576 neither isoniazid at 0.3 mg. nor C.A.H. at 3 mg. per mouse daily in the diet controlled the development of corneal lesions at all.

Development of C.A.H.-Resistant Organisms

Strain CN3679, which normally failed to grow in Dubos medium containing 2 $\mu\text{g.}$ of C.A.H. per ml. during seven days' incubation (Table II), grew in 32 $\mu\text{g.}$ per ml. in the same period after the third serial passage.

This strain also showed an increase in resistance to isoniazid after the passaging through C.A.H.; in one experiment, after the third passage CN3679 grew in 0.5 $\mu\text{g.}$ of isoniazid per ml. in seven days as compared with 0.03 $\mu\text{g.}$ per ml. before passaging, and in another it grew in 2 $\mu\text{g.}$ of isoniazid per ml. in seven days, after only two passages (see Chart).

Discussion

These experiments confirm that C.A.H. has antituberculous activity and suggest that it is some 50 times less active than isoniazid, yet of the same order of toxicity to mice.

The results of cross-resistance studies with C.A.H. and isoniazid and the specific activity of both drugs against the tubercle bacillus strongly suggest that they act in the same way. On the other hand, the relative sensitivities of some strains of tubercle bacilli are not always the same to both drugs. This is seen especially with strain CN3617, which was fully sensitive to C.A.H. but was moderately resistant to isoniazid; similar discrepancies were also reported by Stewart and Wallace (1954).

From these experiments two important points emerge. Firstly, if C.A.H. is used alone in the treatment of tuberculosis there will be a big risk of isoniazid-resistant strains developing; secondly, it will be unusual for a patient with isoniazid-resistant strains to benefit from treatment with C.A.H.

The danger of using C.A.H. in combination with isoniazid is also suggested by some preliminary findings reported by two of us (Bushby and Barnett, 1954), for when the two act together *in vitro* and in mice the effect is not additive, but C.A.H. seems to interfere with the action of isoniazid, reducing the activity of the mixture to the level approximately of that of C.A.H. alone.

Valdecasas *et al.* (1954) have pointed out that, despite the experimental evidence which might suggest otherwise, C.A.H. may yet prove of value in the treatment of tuber-

culous patients. In the light of these laboratory findings, however, reports of clinical trials must be examined with great care.

Summary

In vitro experiments showed that cyanacetic acid hydrazide (C.A.H.) has antituberculous activity, but that this was about one-fiftieth that of isoniazid.

This same relative antituberculous potency was found in studies made with experimental tuberculous infections in mice, to which both drugs were nevertheless equally toxic.

Generally speaking, if a strain of *Myco. tuberculosis* was resistant to isoniazid it was resistant to C.A.H. also. Yet anomalies were found in which such cross-resistance did not exist.

In clinical practice, it is suggested, C.A.H. should not be used alone, nor are there likely to be many instances when it will be more effective than isoniazid. There is, furthermore, a possible danger of using these two drugs in combination.

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LOW BACKACHE IN INDUSTRY

A REVIEW OF 1,163 CASES

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A letter by Dr. L. S. Calvert (1954) advocated the introduction of suitably staffed clinics for the prompt treatment of backache along the lines laid down by Cyriax (1950).

The object of the present paper is to show what can be done by a doctor and a physiotherapist to alleviate suffering and reduce the period of incapacity. That we are employed in industry is irrelevant, for the methods are equally applicable to health centres and group practices. As there was originally no intention of publishing a report we cannot supply carefully tabulated statistical results, yet in spite of this we hope that others will profit from our experience.

There is some confusion concerning the exact aetiology, diagnosis, and treatment of low backache.