adrenal gland to produce the hormones necessary to prevent arthritic symptoms in the scorbutic guinea pigs. This is in agreement with the theory that vitamin C may be essential in the production of the oxy-type of adrenal-cortical hormones.

The results of the chemical and histopathological studies of the tissues of these experimental animals will be published later.

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Antitubercular Diazine Carboxamides

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The effectiveness of nicotinamide in suppression of experimental tuberculosis was discovered by Chorine (*1*) in 1945. Several years later McKenzie *et al.* (*2*) independently reported the same result. In that report and in an associated paper by Kushner (*3*) tests were described on 27 related compounds. Of these only five monosubstituted amides of nicotinic acid possessed a fraction of the activity of the parent amide. We have been engaged in examination of other nicotinamide relatives and nicotinamide isosteres. As a recent account (*4*) of the antituberculosis activity of pyrazinoic acid amide ("Aldinamide") anticipates a similar report from this laboratory (*5, 6*), we are prompted to publish a summary of our results.

In a standardized mouse assay under suitable test conditions, pyrazinoic acid amide, administered in diet, was found to be approximately three times as potent as *p*-aminosalicylic acid (*6*); it is also about three times as effective as nicotinamide. The following pyrazine derivatives, administered either orally in diet or by parenteral injection at maximum tolerated levels, had no significant activity: pyrazinoic acid, sodium pyrazinoyl hydroxamate, sodium N,N′-dipyrazinoyl hydrazine, pyrazine-2,3-dicarboxylic acid and the corresponding diamide, pyrazine-2,5-dicarboxylic acid and diamide, 2-hydroxypyrazine-3-carboxamide, 2-aminopyrazine-3-carboxamide, 2,5-dimethylpyrazine, and sodium pyrazine sulfonate.

Carboxamides of other heterocyclic ring systems were tested. The pyrimidine, imidazole, thiazole, thiophene, and quinoxaline compounds listed in Table 1 were found ineffective. Pyridazine-3-carboxamide is definitely antitubercular, at least as effective as nicotinamide on parenteral administration, whereas the isomeric 4-carboxamide is inactive. Benzamide and *m*-nitrobenzamide are also inert.

A number of nicotinamide relatives have been examined: nicotinamide methochloride, N-methyl-2-pyridone-5-carboxylic acid and amide, N-methyl-2-pyridone-3-carboxylic acid and amide, thio-nicotinamide, N,N′-dinicotinoyl methylenediamine, 3-amidinopyridine hydrochloride, 3-cyanopyridine, 3-hy-

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TABLE 1

	Antitubercular activity	pKa†	Reduction potential (polarigraphic half-wave) (in 0.1 *N* NaOH, 25°)		Nucleotidase inhibition‡	
			$E_{1/2}$ (volts)	I_d/C (μ amp/mg/ml)	Conc	Inhibition (%)
Nicotinamide	+	3.1 ± 0.2	−1.768	56.4	10^{-2}	100
					10^{-3} *M*	50, 47
Pyrazinoic acid amide	+++	−0.5 ± 0.3	−1.195	56.4	10^{-2}	1
Pyrimidine-5-carboxamide	0		−1.597	106		
Pyridazine-3-carboxamide*	+	1.0 ± 0.2	−1.301	50.6	10^{-2}	16
Pyridazine-4-carboxamide*	0	1.0 ± 0.2	−1.077	32.4	10^{-2}	0
			−1.359	32.7		
			(Two-step reduction)			
Imidazole-4-carboxamide	0	3.7 ± 0.2 and 11.8 ± 0.2	Not reducible		10^{-2}	13
Thiazole-5-carboxamide	0	0.6 ± 0.3	−1.765	31.3		
Quinoxaline-2-carboxamide	0	−0.4 ± 0.3	−0.970	48.1		

* The preparation of pyridazine-3-carboxamide (mp, 186°) and the 4-carboxamide (mp, 192°) will be described elsewhere.
† Values determined by ultraviolet absorption.
‡ A preparation of nucleotidase from guinea pig lung was employed. Coenzyme-I was determined spectrophotometrically.

droxymethylpyridine, 3-aminomethylpyridine dihydrochloride, 3-acetylpyridine and N-propyl-*o*-dihydronicotinamide. Included in the above list are several metabolites of nicotinamide and the interesting nicotinic acid antagonist, 3-acetylpyridine (*7*), but neither these compounds nor the others listed had any demonstrable effect in tuberculosis. Tuberculosis tests on several of the above materials have recently been reported by Fox (*8*).

The possibility that the antitubercular amides function by inhibition of nucleotidase was considered and rejected. Nicotinamide is a known inhibitor of this enzyme (*9–11*), but, although the inhibition by nicotinamide was duplicated (Table 1), it was found that pyrazinoic acid amide has no inhibitory action at similar levels. Furthermore, pyridazine-3-carboxamide is no more effective as an inhibitor than imidazole-4-carboxamide, which is not antitubercular.

The possibility that the active amides function by draining labile methyl was likewise disproved. Administration in diet of choline (0.2%), methionine (1%), and mixtures of these compounds (methionine 0.2%, choline 1%) had no effect on the antitubercular action of pyrazinoic acid amide. Also, it was found that high dosages of glycocyamine (1% in diet) did not influence tuberculosis infection in mice.

The pKa values of the various amides have been determined and are tabulated. There is, however, no obvious relation between these results and the tuberculosis data. More interesting, perhaps, are considerations of structure and reduction potential. In formation of pyridine nucleotides, nicotinamide is converted by reduction and alkylation to an N-phosphoribityl-3-carboxamido-1,2 (or 1,6)-dihydropyridinium moiety. Of the other heterocyclic amides only pyrazinoic acid amide and the 3- and 4-carboxamides of pyridazine are reducible to dihydro compounds (*12*). As the pyridazines undoubtedly reduce in the same way, judging by the similar reduction potentials, only one can have the same *o*-dihydro form as the reduced nicotinic and pyrazinoic acid amides. This suggests the possibility that the activity of the amides is due to excessive formation of pyridine or pseudopyridine nucleotide. The metabolism of the tuberculosis organism may be adversely affected either by this excess of nucleotide or by a resultant depletion of ribose or adenine required for other purposes. Related to this hypothesis, which is now being investigated, is the observation of McKenzie that riboflavin can replace approximately one third of the nicotinamide required in treatment of infected mice. We have not attempted to verify this but do not observe a similar supplementation with pyrazinoic acid amide.

The heterocyclic amides have been assayed microbiologically for nicotinic acid and antinicotinic acid activity. Although pyrazinoic acid has been reported effective in human pellagra (*13, 14*), neither pyrazinoic acid amide nor any other amide showed vitamin activity with the test organism, *Lactobacillus arabinosus*. Pyridazine-4-carboxamide was observed to inhibit slightly utilization of both nicotinic acid and nicotinamide, but the apparent antagonism may have been due to traces of pyridazine-4-carboxylic acid. This acid is a much more potent inhibitor. The inhibition index for both nicotinic acid and nicotinamide is approximately 7000 at half maximal inhibition.

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Examination of Human Fat for the Presence of DDT

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A program for analysis of DDT deposits in human fats was undertaken in this laboratory in 1949. The first samples examined were those from persons having known high exposure to DDT and other halogenated hydrocarbons. The material isolated from these fat samples showed the typical blue or blue-purple color given by DDT upon being analyzed by the Schechter-Haller method. However, upon extending this study to fat samples from persons having had no known excessive exposure to DDT or samples from persons who had not been exposed to large amounts of DDT for some time previous to biopsy, off-colors were encountered. The majority of these samples gave reds or purples of various intensities, indicating that the materials isolated contained a large proportion of degraded DDT.

It is noteworthy that in animals exposed experimentally to DDT it seems clearly established in the literature that DDT is stored primarily as such in the body fat. DDA [bis-(*p*-chlorophenyl) acetic acid] has been shown to be excreted in marked amounts in the urine of both man and animals under experimental conditions (*1, 2*) of DDT ingestion. In general, no evidence appears to have been presented indicating pronounced storage of degradation products of DDT in the body fat in the case of experimental animals.