Table I: Degree of immunity against diphtheria

	One stimulans method		Two stimulans method		Toxic challenge method		
	Di-Te	Di-Te-Per	Di-Te	Di-Te-Per	Dilution	Di-Te Total death	Di-Te-Per Total death
Arithmetic mean of A.U.	3·44	3·064	4·47	6·38	1/100	1/20	1/20
Mean of log. A.U.	0·4856 ± 0·242	0·3714 ± 0·372	0·620 ± 0·101	0·8086 ± 0·104	1/200	19/20	19/20
Geometric mean of A.U.	3·05	2·35	4·17	6·41			
Difference between samples	$0·5 > P > 0·4$		$P < 0·001$				

Table II: Degree of immunity against tetanus

	One stimulans method		Two stimulans method		Toxic challenge method		
	Di-Te	Di-Te-Per	Di-Te	Di-Te-Per	Dilution	Total death	Total death
Arithmetic mean of A.U.	2·57	2·66	18·82	17·34	1/50	0/20	0/20
Mean of log. A.U.	0·10 ± 0·661	0·1616 ± 0·869	1·2066 ± 0·169	1·157 ± 0·199	1/100	8/20	8/20
Geometric mean of A.U.	1·251	0·689	16·10	14·30			
Difference between samples	$0·5 > P > 0·4$		$0·5 < P > 0·4$				

The pertussis vaccine with addition of aluminium phosphate and diphtheria and tetanus toxoids proved significantly better than the pertussis fluid vaccine ($0·01 > P > 0·001$). In additional experiments, the same vaccine batch to which only aluminium phosphate was added proved also better than the fluid vaccine.

Conclusion.—Laboratory tests of a combined diphtheria-tetanus-pertussis preparation consisting of 30 Lf of purified diphtheria toxoid mixed with 10 Lf of purified tetanus toxoid and 20,000 millions of *H. pertussis* germs, adsorbed on 10 mg of aluminium phosphate, showed that the composition of the preparation was well balanced and that efficient immunity was conferred at the same time against diphtheria, tetanus and whooping-cough.

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Zusammenfassung

In Laboratoriumsuntersuchungen wurde der gegenseitige Einfluss der einzelnen Komponenten des auf Grund purifizierter Anatoxine zubereiteten und auf Aluminiumphosphat adsorbierten Mischimpfstoffes gegen Diphtherie, Tetanus und Pertussis festgestellt.

Pyridoxin and the Acute Toxicity of Isoniazid and Other Acid Hydrazides in Guinea Pigs[1]

In an earlier communication from this laboratory[2], it was demonstrated that the acute toxicity produced in guinea pigs with acid hydrazides is different from that of free hydrazine compounds. It was further shown that the toxicity of the acid hydrazides alone responded to the administration of pyridoxin. Isoniazid and cyanacetic acid hydrazide were the two substances investigated in that study, and this article represents the results of further experimentation with the hydrazides of pyridine carboxylic, benzoic and acetic acids.

The methods used have been described earlier[2]. A total of 273 guinea pigs were used in this experiment. All compounds, dissolved in water, were administered by intraperitoneal injection. Pyridoxin, when given, was injected immediately before by the same route. The animals were then observed for a period of 20 h for signs of toxicity and the survival time noted.

The results of these experiments show that the central stimulatory action of isoniazid could also be produced by other acid hydrazides whether they are derived from a pyridine carboxylic acid, benzoic acid, or an aliphatic carboxylic acid, such as acetic acid, although the degree of toxicity of these compounds was variable.

From the results presented in Table I, it is seen that pyridoxin exerted a protective effect against the toxicity of all the hydrazides studied. It seems, therefore, that there exists no specific metabolite-antimetabolite relationship between pyridoxin and isoniazid alone. The possibility was considered that pyridoxin simply exerts its effect by forming a hydrazone which may be excreted, as postulated by BIEHL *et al.*[3]. In order to investigate this problem, ethylidene-isoniazid representing the hydrazone of isoniazid and acetaldehyde, was studied for its toxicity. This substance, it was presumed, cannot chemically interact with pyridoxal as the free end of the hydrazine group is bound. Results indicate that ethylidene-isoniazid was less toxic than isoniazid, and only at higher doses produced symptoms of toxicity, which were modified by pyridoxin. It is possible that ethylidene-isoniazid was hydrolysed within the body to yield free isoniazid, which then produced the toxic effect.

[1] The authors are indebted to J. R. Geigy S.A., Basle, for the preparation of the acid hydrazides studied.

[2] M. O. TIRUNARAYANAN and W. A. VISCHER, Exper. *12*, 291 (1956).

[3] J. P. BIEHL and R. W. VILTER, Proc. Soc. exp. Biol. Med. *85*, 389 (1954).

Table I

Effect of Pyridoxin on the Acute Toxicity of Acid Hydrazides in Guinea Pigs

mg/kg	Minimal mean survival time, in hours																	
	Iso-nicotinic acid hydrazide		Picolinic acid hydrazide		Nicotinic acid hydrazide		Benzoic acid hydrazide		Acetic acid hydrazide		Ethylidene-INH		Iso-nicotinic acid		Iso-nicotinamide	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
100	> 20 (5)	–	> 20 (4)	–	–	–	> 20 (3)	–	–	–	–	–	–	–	–	–
150	–	–	15 (4)	> 20 (3)	–	–	7 (6)	> 20 (3)	> 20 (3)	–	–	–	–	–	–	–
175	–	–	4·5 (3)	> 20 (3)	–	–	1·5 (3)	> 20 (3)	1·5 (2)	> 20 (2)	–	–	–	–	–	–
200	> 20 (5)	> 20 (5)	7·5 (3)	16·6 (3)	–	–	1·3 (3)	> 20 (6)	1·5 (2)	> 20 (2)	> 20 (2)	> > 20 (1)	> 20 (2)	–	> 20 (2)	–
250	>	–	1 (5)	17 (3)	> 20 (3)	–	1·5 (3)	1·2 (3)	1 (3)	15 (3)	> > 20 (1)	> > 20 (1)				
300	2·5 (5)	> 20 (5)	1·25 (1)	2·6 (3)	2·3 (3)	> 20 (4)	1 (3)	1 (3)	1 (3)	14 (3)	> > 20 (2)	> > 20 (2)	–	–	–	–
400	2 (5)	> 20 (5)	1·25 (2)	0·75 (3)	1·5 (3)	2·3 (3)	1 (3)	1 (2)	1 (2)	11 (2)	7 (9)	19 (11)				
500	1·75 (5)	3 (5)	1·1 (2)	1·6 (3)	1·3 (3)	1·5 (3)	1 (2)	1 (2)	–	2·3 (1)	4 (9)	13 (10)	> 20 (2)	–	> 20 (2)	–
600	–	–	–	–	0·75 (3)	1·25 (3)	–	–	–	–	1 (9)	13 (10)	–	–	–	–
750	–	–	–	–	–	–	–	–	–	–	–	–	> 20 (2)	–	15 (2)	–

A = no vitamin; *B* = with pyridoxin, 300 mg/kg. Figures in ( ) indicate the number of animals used.

Table II

In vitro Tuberculostatic Activity of Acid Hydrazides

	Minimal inhibitory concentration*, γ/ml	
	H37Rv	H37Rv isoniazide-resistant
Isoniazid	0·1	> 100
Nicotinic acid hydrazide	> 100	> 100
Picolinic acid hydrazide	10	> 100
Benzoic acid hydrazide	> 100	> 100
Acetic acid hydrazide	> 100	> 100
Ethylidene-isoniazid	0·1	> 100
iso-Nicotinic acid	> 100	> 100
iso-Nicotinamide	> 100	> 100

* Minimal inhibitory concentration determined in Youmans medium after three weeks incubation at 37°C

Results presented in Table II show that, among the substances tested, only picolinic acid hydrazide and ethylidene-isoniazid had antituberculous activity comparable to that of isoniazid, they were also ineffective against isoniazid-resistant tubercle bacilli.

From the results of these experiments, it can be concluded that the acute toxicity of isoniazid is not specific as it is produced by other acid hydrazides; the acute toxicity of all acid hydrazides studied is modified by pyridoxin; ethylidene-isoniazid, as an isoniazid-hydrazone, has diminished toxicity comparable to a combination of pyridoxin and isoniazid; and the antituberculous activity of the acid hydrazides and their toxic effects are produced by two different mechanisms.

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Zusammenfassung

Die Autoren konnten nachweisen, dass Isoniazid und Säurehydrazide die gleichen toxischen Symptome verursachen. Pyridoxin zeigt eine schützende Wirkung gegen die Toxizität aller Säurehydrazide. Ferner konnte nachgewiesen werden, dass die antituberkulöse Aktivität der Säurehydrazide und ihre Toxizität verschiedenen Wirkungsmechanismen angehören.

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Opsonic Activity of Properdin

It has been recently suggested that properdin, a protein present in the serum of normal mammals, is involved in the mechanism of the natural immunity against infections (Landy and Pillemer)[1]. In fact, this substance possesses a marked bactericidal action on Gram-negative bacteria and is able to produce also complete inactivation of several types of pathogenic viruses. Modifications of the properdin content of the sera have been reported to occur in several pathological conditions (Frank *et al.*[2],

[1] M. Landy and L. Pillemer, J. exp. Med. *104*, 383 (1956).

[2] E. Frank, J. Fine, and L. Pillemer, Proc. Soc. exp. Biol., N.Y. *89*, 223 (1955).