*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* | | | | |
| 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) | 16 (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*, $\gamma$/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).

Influence of different treatments and additions on the opsonic power of guinea pig serum

| Additions | Normal serum | Serum inactivated 56°C | Serum + Zymosan | No serum |
|---|---|---|---|---|
| None | 7·16 ± 1·65 | 2·75 ± 0·46 | 2·73 ± 0·40 | – |
| Properdin in barbital buffer . . . | 9·21 ± 2·12 | 7·83 ± 1·75 | 8·63 ± 2·41 | 1·36 ± 0·29 |
| Properdin in barbital buffer + $Mg^{++}$ . . . . . | 13·23 ± 1·36 | 13·74 ± 0·92 | 13·46 ± 0·89 | 2·91 ± 0·66 |
| Barbital buffer . . . . . | 6·09 ± 1·83 | – | – | 2·66 ± 0·50 |
| Barbital buffer + $Mg^{++}$ . . . . . | 12·59 ± 1·54 | – | – | 2·82 ± 0·64 |
| Physiological saline . . . . . | – | – | – | 2·32 ± 0·13 |
| No. of experiments . . . . . | 6 | 6 | 6 | 6 |

The values show the average number of *Staphylococci* phagocytized by one granulocyte ± standard deviation.

HEGEMANN[3], LANDY and PILLEMER[4]) and particularly in animals treated with the somatic antigens (lipopolysaccharides) of Gram-negative bacteria. The correspondence of an increase of properdin blood levels with an enhancement of the natural resistance to the infections has been emphasized (LANDY and PILLEMER).

Properdin does not exert bactericidal action on Gram-positive microorganisms such as *Staphylococcus pyogenes* or *Mycobacterium tuberculosis* (LANDY and PILLEMER), but protection of animals determined by the treatment with lipopolysaccharides of Gram-negative bacteria exists also in the case of *Staphylococcus pyogenes* or of *Mycobacterium tuberculosis* infections (DUBOS *et al.*[5]). It seems then evident that the bactericidal power cannot entirely explain the mechanism of action of properdin.

The properdin system which exerts full bactericidal effect consists of properdin, complement and magnesium ions. It is inactivated by zymosan and by heating at 55–57°C.

The presence in normal sera of a substance (or substances) capable of preparing the bacteria for the phagocytosis by granulocytes was firstly described by SANARELLI (1891), but the first complete description was given by WRIGHT (1904). This author called these substances 'opsonins'.

Opsonin has been described as composed of a thermolabile as well as a thermostable portion[6]. The thermolabile fraction has been thought to be identical with complement (VERNONI)[7].

The possible relationship between the thermolabile fraction of opsonin and properdin is discussed in this paper.

Normal guinea pigs weighing 300–400 g used in this investigation provided the source of serum. The blood was taken directly from the heart of 18-h-fasted animals and the serum was used immediately after separation. Leucocytes were obtained from the peritoneal cavity of other guinea pigs, in which 10 ml of cultural broth were injected; 6 h after this injection, the fluid present in the peritoneal cavity was extracted by means of a Pasteur pipette and centrifuged at 1000 *g* for 20 min. The sedimented leucocytes were washed 3 times and then suspended in 5 ml of physiological saline. Bacterial suspensions were prepared from 24 h broth cultures of *Staphylococcus pyogenes aureus* var. Oxford. They contained about 2 billions living microorganisms in 7 ml of physiological saline. For the measurement of the opsonic capacity of the serum, 0·5 ml of the leucocytes suspension was mixed with 0·5 ml of the serum and with 0·5 ml of the bacterial suspension. The mixture was allowed to stand at 37°C for 20 min, and then centrifuged at 1000 $g \times 15$ min. The sediment was washed once and then stripped on a glass slide. The specimen was then stained by the method of GRAM modified by NICOLLE. The number of cocci phagocytized by an individual leucocyte was counted under a microscope. At least 100 leucocytes were counted each time. The opsonic capacity was expressed as 'opsonic power', i.e. the number of cocci counted within 100 granulocytes divided by 100.

Properdin was prepared from human serum according to the method of PILLEMER[8]. It was stored at −18°C. The amount used in each experiment corresponded to 5 ml of fresh serum; it was dissolved in barbital-acetate buffer, pH 7·4.

Zymosan was prepared from baker's yeast according to the method of PILLEMER[9]. When the serum was treated with zymosan in order to block properdin 4 mg of zymosan were used for 1 ml serum. Incubation lasted for 75 min at a temperature of 17°C. Magnesium ions, when added, had the concentration 0·0012 *M*. The results are summarized in the Table. It seems clear from this table that the treatment of normal serum with zymosan produces a marked decrease of the 'opsonic power'; the degree of this decrease resembles that determined by heating at 56°C for 30 min. Addition of properdin to the serum inactivated by heating or by treatment with zymosan restores completely the normal values. Addition of properdin to fresh serum does not increase the opsonic power. Addition of magnesium always brings about a strong increase. Neither properdin nor magnesium exerted any effect when used alone in the absence of serum. These results confirm that opsonin consists of 2 fractions, the first of which is thermolabile while the second one is not destroyed at 56°C. Since the thermolabile fraction is destroyed also by zymosan, and its full activity can be restored by the addition of properdin, it seems highly probable that the thermolabile fraction of opsonin is identical to properdin.

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*Institute of General Pathology, University of Genoa (Italy), July 9, 1957.*

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*Riassunto*

L'aggiunta di zimosano al siero normale di cavia ne diminuisce notevolmente il potere opsonico; l'aggiunta di properdina al siero inattivato con zimosano o col calore restaura interamente questo potere. La properdina usata da sola senza siero, non ha potere opsonico. Sembra molto probabile che la frazione termolabile delle opsonine sia da identificare con la properdina.

## DDT-Analogs as Synergists for DDT

Diaryl-trifluoromethyl-carbinols $Ar_2C(OH) \cdot CF_3$ have been found to be active as synergists against a strain of moderately resistant houseflies[1]; the most active compound of the series was the di-(*p*-chloro)-derivative. It seemed of interest to investigate analogs containing chlorine and/or fluorine in the methyl group and also to study the importance of the hydroxyl group present in these compounds.

The compounds tested have been described by BERGMANN *et al.*[2], apart from di-(*p*-chlorophenyl)-dichloromethyl-carbinol and 1,1-di-(*p*-chlorophenyl)-2,2-dichloroethane which have been reported by PEPPER and KULKA[3] and by HALLER *et al.*[4], respectively. The housefly species used in these experiments were a susceptible strain ('T') and a 200 times more resistant strain ('R') of *Musca vicina*. Benzene solutions of the chemicals were applied *topically* to groups of 25 flies. Mortality counts were made after 24 and 48 h. Each compound was tested in at least four concentrations with 5 to 8 repetitions. The experimental procedure has been described in detail by TAHORI[1].

The Table summarises the results obtained; it indicates the insecticidal activity of each compound as well as its synergist value (for a ratio of DDT: synergist = 10:1). In order to characterize the slope of the dosage-mortality curves, the $LD_{15}$ and $LD_{85}$ values are given in parenthesis.

Against *susceptible* flies, none of the carbinols approaches DDT in insecticidal activity. It is noteworthy that all those diarylmethyl-carbinols in which the methyl group is fully substituted ($CF_3$, $CF_2Cl$, $CFCl_2$) (VI–VIII) are fairly active on topical application, though on tarsal application no effect has been found for di-(*p*-chlorophenyl)-trifluoromethyl-carbinol[5]. It can be predicted that the same will probably hold true for di-(*p*-chlorophenyl)-trichloromethyl-carbinol. This carbinol and the dichloromethyl analog (IX) have been reported by GUNTHER, BLINN, and METCALF[6] to be of low toxicity to *Musca*. The di-(*p*-chlorophenyl)-trichloromethyl carbinol used by these authors, of Rohm and Haas manufacture, appears to be different from the compound used by REUTER and ASCHER, and to which the same formula had been assigned. On the other hand, the diaryl-*di*halomethyl-carbinols (IX–XIII) exhibit lower activity; this activity rises from the difluoro- over the fluorochloro- to the dichloromethyl compound. One is tempted to assume that, as in the case of DDT, the size of the substituents of the methyl group is the decisive factor, causing a distortion of the tetrahedron of the arylated carbon atom into a trihedral configuration[7]; chlorine is effective, fluorine too small. Another observation may be of interest in this connection. The inactive carbinols are easily dehydrated, the active ones not: the trihalogenomethyl compounds can, of course, not be dehydrated at all, but we have noted that also di-(*p*-chlorophenyl)-*di*chloromethyl-carbinol offers a strong resistance to every attempt at dehydration.

All carbinols tested are more active insecticides than DDT for *resistant* houseflies; again the two groups defined above are clearly distinguished.

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| | Compound | Insecticidal Activity in µg/fly | | Toxicity in µg of DDT/fly + compound at a ratio of 10:1 | |
|---|---|---|---|---|---|
| | | R | T | R | T |
| I | $(p\text{-}ClC_6H_4)_2 \cdot CH \cdot CCl_3$ | 140 (80; 250) | 0·7 (0·4; 1·1) | 140 (80; 250) | 0·7 (0·4; 1·1) |
| II | $(p\text{-}ClC_6H_4)_2 \cdot CH \cdot CF_3$ | 13 ( 8; 21) | 4 ( 2; 7) | 13 ( 8; 22) | 0·2 (0·1; 0·4) |
| III | $(p\text{-}ClC_6H_4)_2 \cdot CH \cdot CF_2Cl$ | 50 (32; 77) | 14 ( 9; 19) | 14 ( 8; 23) | 0·4 (0·2; 0·8) |
| IV | $(p\text{-}ClC_6H_4)_2CH \cdot CFCl_2$ | 115 (68; 190) | 5 ( 3; 8) | 30 (17; 51) | 0·4 (0·2; 0·7) |
| V | $(p\text{-}ClC_6H_4)_2 \cdot CH \cdot CHCl_2$ | > 140 | 4 ( 2; 7) | 140 | 0·6 (0·3; 1·0) |
| VI | $(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CF_3$ | 6 ( 3; 11) | 5 ( 3; 7) | 9 ( 5; 18) | 0·3 (0·2; 0·5) |
| VII | $(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CFCl_2$ | 5 ( 3; 9) | 6 ( 4; 11) | 13 ( 7; 21) | 0·5 (0·3; 1·0) |
| VIII | $(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CFCl_2$ | 5 ( 3; 9) | 6 ( 4; 11) | 13 ( 7; 21) | 0·5 (0·3; 1·0) |
| IX | $(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CHCl_2$ | 4 ( 2; 7) | 1 (0·5; 2) | 15 ( 9; 27) | 0·2 (0·1; 0·4) |
| X | $(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CHFCl$ | 11 ( 7; 18) | 9 ( 5; 14) | 13 ( 7; 21) | 0·4 (0·3; 0·7) |
| XI | $(C_6H_5)_2 \cdot C(OH) \cdot CHF_2$ | 70 (43; 110) | 52 (37; 72) | 66 (38; 115) | 0·4 (0·2; 0·7) |
| XII | $(p\text{-}ClC_6H_4)_2 \cdot C(OH) \cdot CHF_2$ | 27 (16; 46) | 31 (18; 56) | 27 (16; 46) | 0·4 (0·2; 0·7) |
| XIII | $(p\text{-}BrC_6H_4)_2 \cdot C(OH) \cdot CHF_2$ | 31 (19; 49) | 27 (17; 45) | 31 (20; 48) | 0·3 (0·2; 0·7) |
| XIV | $(p\text{-}ClC_6H_4)_2 \cdot C(OOCCH_3) \cdot CF_3$ | 7 ( 5; 13) | 4 ( 3; 7) | 9 ( 4; 18) | 0·2 (0·1; 0·4) |
| XV | $(p\text{-}ClC_6H_4)_2 \cdot C(OOCCH_3) \cdot CF_2Cl$ | 80 (47; 130) | 150 (150) | 36 (18; 70) | 0·4 (0·2; 0·7) |
| XVI | $(p\text{-}ClC_6H_4)_2 \cdot C(OOCCH_3) \cdot CFCl_2$ | > 200 | > 200 | 39 (15; 100) | 0·5 (0·3; 0·8) |

Toxicities of some DDT analogs to 'R' and 'T' strains of *M. vicina* (The values in brackets indicate $LD_{15}$ and $LD_{85}$)