due to insecticides plus herbicides were statistically analyzed by the *t*-test.

In the first experimental series, synergistic effects of herbicides on insecticides were studied by exposing fruit flies, houseflies, or mosquito larvae to parathion, *p,p'*-DDT, one of the four herbicides, or insecticide-herbicide combinations as indicated in Table 1. The insecticide dosages chosen were such that insect mortalities obtained after 24 hours of exposure to insecticides alone were relatively low. The results indicate (Table 1) that in most cases all four herbicides increased the toxicity of the insecticides. This increase was greater with parathion than with DDT. Atrazine was most effective, increasing significantly (at the 0.1 percent level) the toxicity of parathion to fruit flies and mosquito larvae by a factor of 5.3, and to houseflies by a factor of 4.1. Least effective was 2,4-D, which increased insecticide toxicity in only two out of six cases (DDT toxicity with fruit flies and parathion toxicity with mosquito larvae). No synergistic effects on the toxicity of DDT toward mosquito larvae were observed with any of the herbicides. Although enough DDT was added to the water to give a concentration of 0.18 part per million (ppm), this concentration was probably never obtained because of its low water solubility (0.001 ppm). It is, therefore, questionable as to what an extent the larvae had a chance to come into contact with the insecticide.

In the second experimental series, the synergistic effects of atrazine on the toxicity of 12 insecticides were studied with fruit flies. The insecticides included nine organophosphorus compounds, one carbamate, and two chlorinated hydrocarbons (Table 2). Insects were exposed to dry deposits of atrazine, one of the 12 insecticides, or atrazine-insecticide combinations. Atrazine was always applied at 40 μg per bioassay jar and the insecticides in amounts as indicated in Table 2. The toxicity of all the insecticides was significantly increased by atrazine. This increase ranged from a factor of 2.2 to 8.6, depending on the insecticide present. From the data reported above (Tables 1 and 2) it appears that the phenomenon of synergism of insecticides by selected herbicides is rather general, therefore suggesting that the mode of action of the herbicides was not related to the blocking of specific detoxifying enzyme systems within the insect body. Other factors, such as an increase in insecticide penetration through the insect cuticle, could possibly have played a role in these phenomena.

In a third experimental series fruit flies were exposed to fixed amounts of insecticides (carbofuran, 0.5 μg; DDT, 4 μg; parathion, 0.35 μg; or diazinon, 0.2 μg) and increasing amounts of atrazine (from 2.5 to 40 μg per bioassay jar) in order to ascertain a potential dose-response relation of the synergistic effects of the herbicide. Results presented in Fig. 1 indicate that with increasing amounts of atrazine, increasing insect mortalities occurred during the 18-hour exposure period. Exposure of the fruit flies to carbofuran, DDT, parathion, or diazinon alone resulted in mortalities of 7.5, 9.5, 8, and 10.5 percent, respectively. The dosage-mortality curves indicate that 50 percent mortality of the insect populations would have been achieved with the addition of 23, 40, 6, and 10 μg of atrazine, respectively.

Results with fruit flies as shown in Tables 1 and 2 and Fig. 1 were obtained in different tests conducted over a period of several months. Since the susceptibility of the insects to pesticides fluctuates to some extent from week to week, quantitative differences in various data are due to these fluctuations.

This report further illustrates the necessity for continued investigations of the interactions of pesticides with other chemicals in biological systems. Although it is imperative to study the fate and behavior of single environmental chemicals, it is also apparent that these problems must be approached within the concept of a whole system in which chemicals can interact with each other.

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## Peroxidase Mediated Antimicrobial Activities of Alveolar Macrophage Granules

Abstract. *The 20,000g pellet obtained by centrifugation of a homogenate of rabbit alveolar macrophages has antibacterial activity in the presence of a hydrogen peroxide–generating system and iodide. Peroxidase activity has been demonstrated in this fraction. Addition of 3-amino-1,2,4-triazole diminished the antibacterial activity of the pellet–hydrogen peroxide–iodide system.*

The precise mechanism (or mechanisms) by which phagocytes inactivate intracellular microorganisms has been a subject of study for almost a century. Renewed interest in this subject is mainly due to the finding of a myeloperoxidase (MPO)–$H_2O_2$–halide antimicrobial system in the polymorphonuclear neutrophilic leukocytes (PMN). Individually the components of the reaction are devoid of antimicrobial activity at the concentration employed. They appear to function only together (*1*).

To our knowledge, the antimicrobial activities of alveolar macrophages (AM) fractions have not yet been reported. Since these macrophages are generally reported to have either no peroxidase or insignificantly low activity (*2*), possible functioning of a peroxidase–$H_2O_2$–halide antimicrobial system in these cells has not yet attracted much attention. Species differences in peroxidase activity of mononuclear cells have been demonstrated (*2, 3*). In this communication we demonstrate that the fraction prepared from the homogenate of AM centrifuged at 20,000*g*, which contains most of the peroxidase activity, in the presence of $H_2O_2$ and $I^-$, is able to kill *Escherichia coli*. We also show that 3-amino-1,2,4-triazole (AT), an inhibitor of AM peroxidase activity

(*4*), inhibits the bactericidal activity of the 20,000*g* pellet-$H_2O_2$-$I^-$ system. Finally, we present evidence suggesting that peroxidase from AM is similar to that of horseradish in its antimicrobial activities.

Alveolar macrophages were isolated by tracheobronchial lavage (*5*). The cells were suspended in 0.25*M* sucrose and homogenized. The homogenate was centrifuged (refrigerated Sorvall $RC_2$) at 20,000*g* for 30 minutes. The pellet was resuspended in 0.25*M* sucrose. Peroxidase activity was assayed by the guaiacol oxidation method (*2, 4*). Bactericidal activity of the pellet fraction was measured in lactate buffer, *p*H 4.5 (*6*). Glucose and glucose oxidase were used for $H_2O_2$ generation (*6, 7*). In the case of intact cells, Krebs Ringer phosphate buffer (KRPB), *p*H 7.4, was the reaction medium. Bacterial survival was estimated with the use of a semimicro pour plate procedure (*8*).

Iodide, when necessary, was added in halide-free phosphate buffer. When $Cl^-$ was desired as the halide, KRPB was used. Decarboxylation was assayed by the formation of $^{14}CO_2$ from L-[1-$^{14}$C]-alanine (New England Nuclear). The procedure has been described (*8, 9*). All reagents were of reagent grade or better.

Peroxidase activity, as judged by guaiacol oxidation, indicated that the 20,000*g* pellet fraction contained 60 to 80 percent of the total in the cell. If the assay was carried out in the absence of sucrose, the peroxidase of the pellet fraction was increased twofold, when compared to the corresponding activity in the presence of 0.25*M* sucrose. This finding confirms the work of Romeo *et al.* (*4*). Peroxidase activity of the 20,000*g* pellet from rabbit AM was approximately 0.08 guaiacol unit per $10^8$ cells, compared to a value of 0.5 for PMN.

The bactericidal activity of intact alveolar macrophages and 20,000*g* pellet fraction in the presence of two different halides, chloride or iodide, is presented in Table 1. Intact cells have significant bactericidal activity. However, pellet alone or pellet in combination with an $H_2O_2$-generating system and chloride have very little or no bactericidal activity. In contrast, if $I^-$ is added to the reaction mixture containing pellet and an $H_2O_2$-generating system, only 3.7 percent of the bacteria survive after 1 hour of incubation.

Addition of AT to the pellet-$H_2O_2$-$I^-$ system increased bacterial survival tenfold, apparently because of the inhibition of peroxidase activity by AT (*4*).

The decarboxylation activity of the 20,000*g* pellet from rabbit AM in the presence of $Cl^-$ or $I^-$ was also estimated. There was no decarboxylation in the presence of either $Cl^-$ or $I^-$ by the 20,000*g* pellet of rabbit AM. The decarboxylation in each of these systems was similar to that of the reagent control in the absence of any pellet ($< 1.0$). The work of Romeo *et al.* demonstrates that when peroxidase is measured at 37°C with higher $H_2O_2$ concentration and with cetyltriethylammonium bromide, its activity in AM is significant (*4*). Our studies have been carried out at 37°C and in the presence of an efficient $H_2O_2$-generating system. These data would suggest that the peroxidase–$H_2O_2$–halide antimicrobial system previously described to be operative in the PMN may also be functioning in the AM. Interestingly, the AM peroxidase may not be identical to MPO in its mode of action. The MPO-mediated antimicrobial system utilizes either $Cl^-$ or $I^-$ as the halide (*1*). Antimicrobial activity of the AM peroxidase in the 20,000*g* pellet occurs only with $I^-$. Since the MPO-$H_2O_2$-$Cl^-$ system is thought to exert its antimicrobial activity by its ability to oxidize appropriate substrates to aldehydes (*9, 10*), it is interesting that AM pellet peroxidase does not participate in this reaction. In this respect, the AM pellet peroxidase appears to resemble horseradish peroxidase (*9, 10*). This latter enzyme does not function in the peroxidase-mediated antimicrobial system when the halide employed is $Cl^-$. On the other hand, it does when $I^-$ is the halide (*8, 9*).

Our data on bactericidal activity coupled with the biochemical data from Rossi's (*4*) and Gee's (*11*) laboratories indicate that an MPO–$H_2O_2$–halide antimicrobial system described for PMN may also be operative in the AM. Further, the antimicrobial activity mediated by peroxidase noted in these cells is more similar to that of horseradish peroxidase than it is to MPO (*8–10*). Finally, the antimicrobial system for the AM pellet fraction should not be considered as the sole mechanism by which this mononuclear phagocyte can inactivate microorganisms. Certainly other systems do operate, especially with other organisms and in the intact cells. For example, killing by the intact cells has been described in the absence of added iodide (*12*) (Table 1).

Table. 1. Antimicrobial and L-[alanine-1-14C]-decarboxylation activities of rabbit alveolar macrophages and peroxidase in the pellet after centrifugation at 20,000*g*. All incubations were carried out at 37°C for 60 minutes. Lactate buffer (30 μmole), *p*H 4.5, in a total volume of 2.0 ml was used for studies with the 20,000*g* pellet. With intact cells KRPB, *p*H 7.4, was used. Additions were as follows: 20 μmole of glucose and 0.005 unit of glucose oxidase; 0.4 μmole of KI; 300 μmole of NaCl; 20 μmole of AT, 0.1 to 0.5 ml of pellet from a homogenate from $2 \times 10^7$ AM per milliliter (0.002 to 0.008 guaiacol unit), and $2 \pm 10^6$ *E. coli* cells. For killing by intact cells, the ratio of AM to bacteria was 1 : 5. Autologous serum (10 percent) and $2.5 \times 10^6$ to $4.0 \times 10^7$ *E. coli* cells were used. No significant decarboxylation of [1-$^{14}$C]alanine was observed from 5.4 μmole of alanine. Under similar conditions MPO from PMN would decarboxylate approximately 200 μmole of alanine.

| System | Survival (%) |
|---|---|
| Bacterial control | 100.00 |
| Intact cell | 2.35 |
| 20,000*g* pellet | 62.30 |
| Pellet + $H_2O_2$-generating system | 52.40 |
| Pellet + $H_2O_2$-generating system + $Cl^-$ | 100.00 |
| Pellet + $I^-$ | 71.40 |
| Pellet + $H_2O_2$-generating system + $I^-$ | 3.70 |
| Pellet + $H_2O_2$-generating system + $I^-$ + AT | 37.20 |

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