The removal of copper apparently produces a decrease in protyrosinase which can be restored by the addition of a copper salt (Fig. 1). In agreement

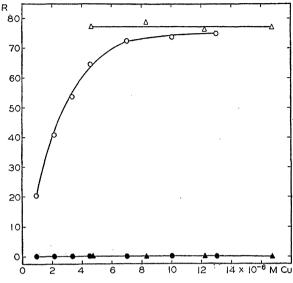


FIG. 1. Resynthesis of protyrosinase. Ordinate, reciprocal  $\times 10^3$  of the time in minutes for the initial uptake of 100 c.mm. of oxygen during the oxidation of  $6.9 \times 10^{-3}$ mM of tyramine catalyzed by tyrosinase from 0.3 c.c. of protyrosinase extract. Abscissa, total concentration of copper. Open symbols, 0.07% Aerosol; closed symbols, no Aerosol. Circles, extract from which copper was removed; deltas, extract from which no copper was removed. pH = 6.8; Temp. = 24.9°C.

with Kubowitz<sup>3</sup> it was found that salts of Fe, Co, Ni, Mn and Zn were unable to replace those of Cu. The protyrosinase yields a tyrosinase which seems to be specifically dependent on copper for its enzymic activity. An excess of activator, Aerosol in this instance, seems to be necessary for conversion of protyrosinase into tyrosinase (Fig. 1). It is worthy of note that twice as much copper, that is 10 rather than  $5 \times 10^{-6}$  M, needs to be present for complete restoration of protyrosinase. The resynthesized protyrosinase and tyrosinase are destroyed by heating at 90° for 5 minutes;  $18 \times 10^{-6}$  M copper sulfate and the same with an excess of Aerosol have no catalytic activity. Thus it seems that copper unites with a copper-free substance to give a thermolabile protyrosinase, which in its turn must be activated before a thermolabile tyrosinase is produced. An anomalous and differential heat effect has also been found for protyrosinase extracts of low copper content. i.e., heat treatment between temperatures of  $60^{\circ}$  and  $70^{\circ}$ not only inhibited but also activated.<sup>6</sup> The heat effects, therefore, appear to be independent of the copper.

Although the potentially active group of protyrosinase reacts with cyanide, it is unable to activate the enzymic oxidation of substrates. With the present knowledge of protyrosinase it seems hazardous to choose between whether the activation of protyrosinase is primary and direct or secondary and perhaps concerned with the removal of some material surrounding a core of tyrosinase. If the latter were so, it may be pointed out that the shell must be permeable to cyanide and its copper compound yet impermeable to substrates. This kind of semi-permeability would seem to be of a very peculiar order. Since Kubowitz<sup>3</sup> has shown that the active group of polyphenol oxidase indulges in electron exchange in order to catalyze the substrate's oxidation, it is suggested that a difference between protyrosinase and tyrosinase may be concerned with the state of its copper. An activator presumably overcomes some hindrance to oxidation and reduction of the active group.

When its copper is partially removed, a protyrosinase extract yields less tyrosinase. The return of copper leads to resynthesis of protyrosinase. The copper of protyrosinase seems to be a potentially active, prosthetic group.

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## THE USE OF FATTY ACIDS IN INSECTI-CIDAL AEROSOLS

In recent investigations<sup>1</sup> it has been shown that some relatively nonvolatile compounds show promise as fumigants against insects when applied in smoke or fog form. This development makes possible the use of safe and inexpensive insecticides that were formerly considered impractical because of difficulties in producing effective concentrations at room temperatures.

In practice a solution of the insecticidal material was sprayed on a heated surface. On coming in contact with the hot surface, the solvent was evaporated with explosive violence, and any dissolved material that did not vaporize readily was reduced mechanically to colloidal dimensions. That is, the insecticide was dispersed as an aerosol consisting of a suspension of the solid or liquid particles in air. By this method of volatilizing it was possible to keep the insecticide dispersed in an enclosed space for a long time. The rate of evaporation was also greatly increased, and the maximum vapor concentration was quickly obtained because of the tremendous surface of these

<sup>1</sup>W. N. Sullivan, L. D. Goodhue and J. H. Fales, Soap, 16 (6): 121, 123, 125, illus. 1940.

<sup>&</sup>lt;sup>6</sup> J. H. Bodine and T. H. Allen, Jour. Cell. and Comp. Physiol., 12: 71, 1938.

small particles. The potency was further increased by the direct contact action of these small particles.

The apparatus used in this work consisted of a small nasal type atomizer mounted four inches above the center of an electric hotplate held at 375° C. A small electric compressor was used to maintain the air pressure that operated the atomizer.

To stabilize and increase the toxicity of these insecticidal aerosols, fatty acids (lauric or oleic) were added to the spray solution. It was shown with biological tests against the housefly that these materials increased the effectiveness of orthodichlorobenzene. The results of these tests are given in Table 1.

Although laurie and oleic acids are substantially inert when used alone, under the conditions of these tests they act as adjuvants when combined with orthodichlorobenzene and greatly increase the effectiveness of the aerosol. Certain fatty acid derivatives, such as salts, esters, and the like, also gave increased insecticidal action. The results were corroborated by room tests against the roach and the bedbug, where a 100 per cent. mortality was obtained by using 1.5 pounds of orthodichlorobenzene containing 5 per cent. of lauric acid per 1,000 cubic feet.

## TABLE 1

RELATIVE EFFECTIVENESS AGAINST HOUSEFLIES OF ORTHODI-CHLOROBENZENE, ALONE AND IN COMBINATION WITH OLEIC AND LAURIC ACID, WHEN DISPERSED IN AEROSOL FORM; EXPOSURE PERIOD 30 MINUTES\*

Material tested	Number of insects tested	Mortality after 2 days, per cent.
Orthodichlorobenzene	609	2
Orthodichlorobenzene plus oleic acid	440	55
Orthodichlorobenzene plus lauric acid	471	60
Lauric acid	216	1
Oleic acid	220	1

\* Orthodichlorobenzene was used at the rate of 0.28 cc per cubic foot and the fatty acid at 0.071 gram per cubic foot.

This method of producing an aerocolloidal dispersion by spraying liquid toxins on a heated surface might be of use to bacteriologists, who have found bacteriocidal aerosols effective in decontaminating rooms.<sup>2</sup>

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

## A BUBBLER PUMP METHOD FOR QUANTI-TATIVE ESTIMATIONS OF BACTERIA IN THE AIR<sup>1</sup>

THE bacterial content of the air of a rheumatic fever hospital has been studied regularly throughout the past winter. For quantitative estimations, an air centrifuge of the type described by Wells<sup>2</sup> was used and occasional runs were made with apparatus similar to that of Hollaender and Dalla Valle.<sup>3</sup> Results were so variable even in successive runs in an apparently stable environment that more refined methods of estimating the number of bacteria in air were sought. The most satisfactory machine in respect to efficiency and ease of operation was a modification of that described by Robertson,<sup>4</sup> Bigg, Miller and Baker in SCIENCE, February 28, 1941. This operated on the principle of the slow bubbling of air through liquid media. Glass beads serve to break up bubbles and release bacteria to the broth which might otherwise escape within the bubbles.

<sup>1</sup> From the Department of Preventive Medicine, Harvard Medical School, and House of the Good Samaritan, Boston, Massachusetts. This work was supported in part by a grant to the House of the Good Samaritan from the Commonwealth Fund.

<sup>2</sup> W. F. Wells, Am. Jour. Pub. Health, 23: 58, 1933.

<sup>3</sup> A. Hollaender and J. M. Dalla Valle, *Pub. Health* Rep., 54: 574, 1939. The apparatus shown in Fig. 1 consists of a sterile 250 cc Erlenmeyer suction flask containing a mea-

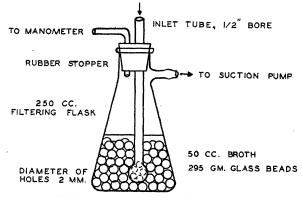


FIG. 1. Diagrammatic drawing of bubbler pump apparatus.

sured quantity of glass beads and broth. Air is drawn through this flask at rates indicated by a U tube manometer at the inlet. At the completion of the tenminute run, one and two ec amounts of the broth are pipetted to sterile petri dishes and blood agar is poured and mixed with the inoculum. A vacuum

<sup>&</sup>lt;sup>2</sup> C. C. Twort, A. H. Baker, S. R. Finn and E. O. Powell, Jour. Hygiene, 40 (3): 253-344, illus. 1940. <sup>4</sup> O. H. Robertson, E. Bigg, B. F. Miller and Z. Baker,

<sup>&</sup>lt;sup>4</sup> O. H. Robertson, E. Bigg, B. F. Miller and Z. Baker, SCIENCE, 93: 213 and 214, 1941.