of calibrated red blood cell pipettes. The following preparations were set-up:

(1) Saline + spirochete fluid.
(2) Chalcone 1: 1,333 + spirochete fluid.
(3) Chalcone 1: 1,333 + mapharsen 1: 4,000 + spirochete fluid.
(4) Mapharsen 1: 4000 + spirochete fluid.
(5) Mapharsen 1: 4000 + glutathione 1: 400 + spirochete fluid.
(6) Glutathione 1: 400 + chalcone 1: 1,333 + spirochete fluid.
(7) Glutathione 1: 400 + spirochete fluid.

All dilutions are final dilutions. All reagents were adjusted to pH 7 ±.

The results of six experiments are shown in the

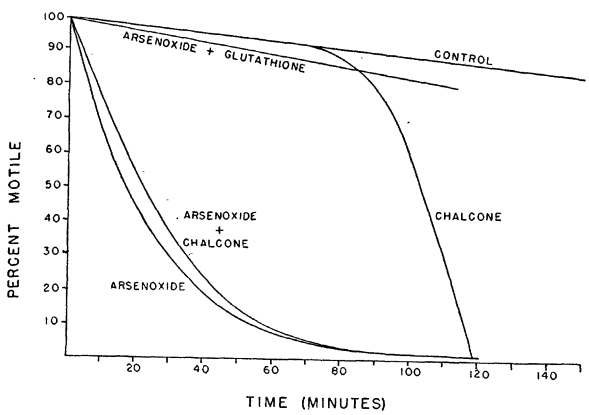

FIG. 1. Effect of various agents and their combinations on the motility of *Treponema pallidum in vitro*.

graph. The curves represent the average percentage of spirochetes retaining motility at the stated intervals. In the concentrations used, methyl chalcone did not inhibit the spirocheticidal action of mapharsen *in vitro*. Methyl chalcone appeared to exert independent spirocheticidal activity between 90 and 120 minutes after mixture with spirochetes. This effect could be abolished by glutathione.

These preliminary observations indicate that the methyl chalcone of hesperidin may be capable of diminishing the toxic effects of mapharsen without inhibiting its spirocheticidal effect, and that it may possess independent spirocheticidal activity to a mild degree. Further studies are in progress to explore these possibilities.

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THE ANTIBACTERIAL EFFECT OF ENZYMATIC XANTHINE OXIDATION[1]

RAISTRICK and his colleagues[2] made the curious observation that an oxidase may display antibacterial activity. They had observed a bactericidal activity with certain protein fractions from *Penicillium notatum* which definitely was not attributable to the ether-soluble, low-molecular penicillin. Eventually this bactericidal effect was traced to glucose oxidase, a flavoprotein which catalyzes the reaction:

$$\text{glucose} + O_2 \longrightarrow \text{gluconic acid} + H_2O_2$$

The generated hydrogen peroxide was thought to be mainly, if not exclusively, responsible for the bactericidal effect of the enzymatic system.[3] The name, notatin, was suggested for this enzymatic bactericide. Simultaneously two similar reports appeared: bactericidal protein-fractions derived from *Penicillium notatum* were described by Kocholaty[4] under the name of penatin and by Doisy *et al.*[5] under the name penicillin B.

Glucose oxidase, although not in its present role, has been known for some time. It is an enzyme found commonly in molds[6] and most abundantly in *Aspergillus niger*. It was recognized as a flavin enzyme.[7] Judging from the latest communications by Doisy *et al.*,[8] Kocholaty,[9] and by Birkinshaw and Raistrick,[3] there seems to be no reasonable doubt that the common mold glucose oxidase is the active principle in penatin and penicillin B as well as in notatin.

The conclusion reached by Raistrick *et al.* that their antibacterial activity was due essentially to the generation of hydrogen peroxide invited the rather obvious deduction that other oxidases may be found which act similarly. To prove this, it was decided to carry out appropriate experiments with xanthine oxidase. This H_2O_2 generating[10] enzyme was chosen because it may be easily obtained relatively free from counteracting factors, such as catalase, peroxidase, etc. Furthermore, the natural occurrence of this enzyme in milk suggested that with its appropriate substrate it may

1 Aided by a grant from the Commonwealth Fund.

2 C. E. Coulthard, W. F. Short, R. Michaelis, G. Sykes, G. E. H. Skrimshire, A. F. B. Standfast, J. H. Birkinshaw and H. Raistrick, *Nature*, 150: 634, 1942.

3 J. H. Birkinshaw and H. Raistrick, *Jour. Biol. Chem.*, 148: 459, 1943.

4 W. Kocholaty, *Jour. Bact.*, 44: 142, 469, 1942.

5 E. C. Roberts, C. K. Cain, R. D. Muir, F. J. Reithel, W. L. Gaby, J. H. Van Bruggen, D. M. Homan, P. A. Katzman, L. R. Jones and E. A. Doisy, *Jour. Biol. Chem.*, 147: 47, 1943.

6 D. Muller, *Biochem. Zeits.*, 199: 136, 1928.

7 W. Franke and M. Deffner, *Ann. Chem.*, 541: 117, 1939.

8 J. T. Van Bruggen, F. J. Reithel, C. K. Cain, P. A. Katzman, E. A. Doisy, R. D. Muir, E. C. Roberts, W. L. Gaby, D. M. Homan and L. R. Jones, *Jour. Biol. Chem.*, 148: 365, 1943.

9 W. Kocholaty, *Arch. Biochem.*, 2: 73, 1943.

10 M. Dixon and S. Thurlow, *Biochem. Jour.*, 18: 971, 1924.

be employed as a milk preservative. It was, however, already known that in whole milk the hydrogen peroxide formed through xanthine or hypoxanthine oxidation would disappear almost as rapidly as it was formed.[11] Nevertheless, addition of hypoxanthine depresses, to some extent, bacterial growth in milk.

Enzyme preparation. It was desirable to use a preparation pure enough to allow hydrogen peroxide to accumulate sufficiently. Too highly purified preparations seemed undesirable because they tend to become unstable. A suitable degree of purity was obtained when we followed the isolation procedure of Corran *et al.*[12] up to their third step. Activity was measured manometrically in the manner described by Ball.[13] The enzyme solution we used for the preparation of our test solution was golden yellow in color; it contained 21 mg protein per ml and its Q_{O_2} was 120 (28° C.). To estimate roughly the amount of pure enzyme therein, the light absorption at 450 mμ, the maximum for riboflavin, was measured with the Beckman Spectrophotometer. About one third of the apparent flavin absorption may be attributed to enzyme-flavin (*cf.* [12]). We found 8.3 γ flavin per ml attributable to xanthine oxidase. Using Ball's figure of 74,000[13] for the molecular weight per mole of flavin, it appears that in our preparation 1.7 out of 21 mg of protein per ml, or 8 per cent. was xanthine oxidase. The enzyme concentrations given in the following experiments refer to content of pure enzyme as estimated in the above manner.

Tests with this preparation showed a satisfactory stability of the enzyme and of enzymatically formed hydrogen peroxide. When three ml of a thirty times diluted solution of this enzyme preparation acted upon 5 micromols of hypoxanthine, the substrate was oxidized to completion in one hour, at which time 70 to 75 per cent. of the theoretically expected hydrogen peroxide was found in the solution.

TABLE 1

GROWTH OF STAPHYLOCOCCUS AUREUS IN BROTH CULTURE

Xanthine oxidase concentration	Additions	
	0	33 mg per cent. hypoxanthine
0	+	+
1 : 12,500	−	−
1 : 115,000	−	−
1 : 1,000,000	−	−
1 : 3,000,000	+	+

Inhibition of bacterial growth by xanthine oxidase. Small inocula of young cultures of a typical Staphylococcus aureus were used for the tests. Readings were taken after 18 hours incubation at 37° C. A sterilization of our enzyme was found unnecessary, the absence of bacteria being due probably to the effects of repeated freezing and thawing. This was fortunate because preliminary experiments showed an almost complete inactivation after filtering through a Seitz filter. The first experiments were done with broth as a culture medium. As shown in Table 1 no growth occurred with the enzyme concentrations down to $1:10^6$. Growth inhibition was, however, independent of addition to the broth of hypoxanthine, because the meat broth already contained, as we found afterwards, appreciable amounts of a substance oxidizable with xanthine oxidase. From the agreement between oxygen consumption and uric acid formation this was considered to be mainly or exclusively hypoxanthine.

In a second series (Table 2) a peptone medium was used, which showed no chemical reaction with the enzyme. With this medium xanthine oxidase alone was without inhibitory effect. Here inhibition was first brought about by incubation with enzyme together with its substrate, hypoxanthine. Tests for hydrogen peroxide with luminol[14] showed its presence in the inhibited samples and its absence in those where growth had occurred.

TABLE 2

GROWTH OF STAPHYLOCOCCUS AUREUS IN PEPTONE CULTURE

Xanthine oxidase concentration	Additions		
	0	hypoxanthine	
	growth	*growth*	H_2O_2
0	+	+	−
1 : 94,000	+	−	++
1 : 850,000	+	−	+
1 : 2,500,000	+	+	−

In the present case the enzyme concentration needed to effect inhibition is about one thousand times higher than that reported for glucose oxidase. We believe this to be due to a greater stability of the mold enzyme.

Experiments with milk. Relatively crude experiments were carried out. The effect of an addition of hypoxanthine to raw milk was evaluated after incubation at room temperature or in the refrigerator. Incubation at 37° resulted in too heavy growth even in the presence of hypoxanthine. Raw milk was infected lightly with *Staphylococcus aureus* and kept for one day at 23–25° C. The count for Staphylococcus was then 500,000 per ml with hypoxanthine against 2,500,000 without. Other organisms were similarly inhibited with the exception mainly of certain streptococci. In another experiment, without addition of extraneous bacteria, after one day in the refrigerator a count of 68,000 per ml was obtained with hypoxanthine against 133,000 without.

In a sense these experiments only confirm the fore-

[11] O. Schales, *Ber.*, 71: 447, 1938.

[12] H. S. Corran, J. G. Dewan, A. H. Gordon and D. E. Green, *Biochem. Jour.*, 33: 1694, 1939.

[13] E. G. Ball, *Jour. Biol. Chem.*, 128: 51, 1939.

[14] O. Schales, *Ber.*, 72: 167, 1939.

gone conclusion that hydrogen peroxide is inhibitory to certain bacteria, mostly anaerobic and facultative anaerobic types devoid of catalase.[15] Many of the pathogenic cocci are of this type. There may, in certain surgical cases, be merits to the slow but steady generation of hydrogen peroxide as brought about by an enzyme system. The difficulties of preparation, however, would outweigh any, probably rather limited usefulness.

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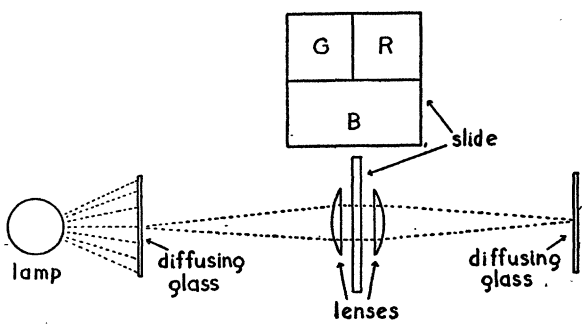
SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE THREE-COLOR MIXER USING FILTERED COLORS

THE method of color mixing most commonly used in psychological laboratories is the rotating disc or color wheel. Though this method is excellent for many purposes, it has certain disadvantages inherent to the use of surface pigments: (1) mixtures are relatively low in saturation (particularly in the case of yellow produced by a mixture of red and green); (2) complementary mixtures are gray rather than white; (3) brightness can not be controlled independently of room illumination without sacrifice of saturation. These disadvantages can be avoided with mixtures produced with filtered colors.

A device which the author recently built uses an extremely simple principle to provide mixtures of three filtered colors. The general design would seem to be of potential value for such uses as (1) measurement of hue (and saturation) discrimination thresholds in both human beings and animals, (2) detection and analysis of color blindness, (3) specification of pigments in terms of three-color components by matching with three color mixtures, (4) classroom demonstrations of color mixing.

The optical system for accomplishing the trichromatic mixture is shown in Fig. 1. Gelatin color filters



Optical System of Color Mixer

FIG. 1

are mounted between glass, forming a square slide. One upper quadrant of the slide is red, the other quadrant is green, and the bottom half is blue. The filters used in the author's apparatus are Wratten gelatin filters of numbers 29 (red), 47 (green) and 61 (blue).

This color mixing slide is supported on a two-way sliding mount between two lenses. Light from one diffusing screen is focused by the lenses upon another diffusing screen. Both screens are of flashed opal glass. Between the two lenses the light rays are parallel, and whatever colors intercept the light beam at this point become uniformly mixed on the second diffusing screen.

Two adjustments of the color-mixing slide are provided by means of levers moving along graduated scales. One lever moves the slide horizontally, thus changing the red-green proportion in the mixture. The other lever moves the slide vertically, thus adjusting the blue component. More specifically, by the two directions of movement, the slide can be adjusted to transmit any one of the three colors singly, or combinations of the three colors in any proportions. On the stimulus screen one can, therefore, obtain any color around or within the color circle. Due to the lack of homogeneity of the filtered colors, however, the resulting mixtures do not quite equal spectral mixtures in saturation. On the other hand, the mixtures far exceed in saturation those obtained on the color wheel.

That the color-mixing principle described here is successful was adequately demonstrated by a single unit built by the author while at the University of Missouri. Color mixtures of good saturation, moderately high brightness and uniform distribution were obtained.

The apparatus is particularly recommended for the demonstration of color-mixing principles. If intended for this purpose, the apparatus should be built so that the stimulus screen can be removed easily and the separate colors being mixed thus exposed to view.

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[15] J. W. MacLeod and J. Gordon, *Jour. Path. Bact.*, 25: 139, 1922.

BOOKS RECEIVED

BLACKWOOD, OSWALD. *General Physics.* Illustrated. Pp. viii + 622. John Wiley and Sons. $3.75.

SOPER, FRED L. and D. BRUCE WILSON. *Anopheles Gambiae in Brazil.* Illustrated. Pp. xviii + 262. The Rockefeller Foundation.