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Combined Action of Sulfadiazine and Trimethoprim

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It has been claimed that trimethoprim, 2,4-diamino - 5 - (3',4',5' - trimethoxybenzyl)-pyrimidine, exerts a "synergistic" effect when combined with sulfonamides (Hitchings, *Drugs, Parasites and Hosts*, p. 202, J. and A. Churchill, Ltd., London, 1962) and polymyxin (Garrod and Waterworth, J. Clin. Pathol. **15**:328, 1962) in experimental infection of mice and natural infection of man (Noall, Sewards, and Waterworth, Brit. Med. J. **2**:1101, 1962), in both instances with *Proteus* sp. Because of the promising theoretical background and practical results of the use of trimethoprim, we used it in combination with sulfadiazine to treat an experimental staphylococcal infection of mice, to extend the above observations and illustrate a method of analysis of the results of the combined action of drugs (Plackett and Hewlett, Ann. Appl. Biol. **35**:347, 1948).

The drugs in 0.5 ml of distilled water were injected through a tube attached to a syringe into the stomachs of 5.5- to 6.5-week-old randomly distributed mice (originally CFW; bred here for 7 years). After 3 hr, the mice were inoculated intraperitoneally with 0.25 ml of a 10^{-7} dilution of an overnight broth culture of the Smith strain of *Staphylococcus aureus* (obtained from A. K. Miller, Merck Institute for Therapeutic Research, Rahway, N.J.; originally described by Smith and Dubos, J. Exptl. Med. **103**:87, 1956), mixed with 0.5 ml of a 5% suspension of granular hog mucin (1701-W; Wilson Laboratories, Chicago, Ill.). Appropriate controls were included (Table 1). Mice found dead the next morning were assumed to have died from gastric intubation and were excluded from the experiment. Deaths from the above dose of the Smith strain occur characteristically at 48 hr and within a day or so thereafter. The mice were examined daily for 10 days.

TABLE 1. *Survival of mice inoculated intraperitoneally with Staphylococcus aureus Smith and mucin after sulfadiazine and trimethoprim treatment*

Sulfadiazine	Trimethoprim	Expt 1	Expt 2	Expt 3	Expt 4	Total	Per cent survivors
mg	*mg*						
3	0	13/20*	9/19	8/20	16/24	46/83	55
3	5	18/20	19/21	6/17	47/50	90/108	83
3	10	15/19	19/20	5/19	46/50	85/108	79
3	20	—†	19/20	16/18	—	35/38	92
0	5	—	5/17	—	6/24	11/41	27
0	10	8/18	—	—	9/23	17/41	41
0	20	—	18/19	7/16	7/19	32/54	59
0	0	3/39	3/20	1/15	7/50	14/124	11

* Numerator = survivors; denominator = number of mice inoculated.
† Not performed.

A number of experiments were carried out with variable results; the last four experiments seemed to produce relatively reproducible results and are shown in Table 1.

The types of combined action of two independently acting drugs producing the same quantal response may be as follows. In type I, the probabilities of response of individual subjects to the two drugs are completely positively correlated. Thus, used separately, the weaker of the two drugs would produce a response only in subjects susceptible to the stronger drug, or, $p = p_1$, $(p_1 > p_2)$, where p is the proportion of subjects responding to the drugs in combination and p_1 and p_2 represent the proportion responding to the drugs used separately. In type II, the probability of an individual subject's responding to the first drug is inversely proportional to the probability of its responding to the second (i.e., complete negative correlation); thus, $p = p_1 + p_2$,

$[(p_1 + p_2) \lesseqgtr 1]$. In type III, there is no correlation between the probability of response to one drug and the probability of response to the other; hence, $p = p_1 + p_2 - p_1p_2$.

Type III is intermediate between the other two types. Types I and II are extreme values, and all degrees of relationship between them can be envisaged. We may say that, when the combined action of drugs gives a result less than that predicted by our type I action, an unexplained "antagonistic" action exists, $p < p_1$, $(p_1 > p_2)$; conversely, when the joint action exceeds that predicted by type II action, there is an unexplained "synergism," $p > (p_1 + p_2)$, $[(p_1 + p_2) \lesseqgtr 1]$.

In our own results, the infected mouse is the subject; survival is the response. The results show that the observed combined effect of the drugs is either approximately equal to or less than type II combined action (even when 11% survival is allowed for); thus, there does not appear to be unexplained or "synergistic" action. The observed combined action is greater than type III combined action, indicating that there may be some degree of negative correlation of the probabilities of response of subjects to the two drugs.

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