TABLE 1

Treatment	No. of mice	No. of tumors	Per cent. with tumors	Average tumor age, mo.	Average age of non-cancerous mice at death, mo.
4° 1 hour	18	9	50	16	18
24° 2 hours	17	6	35	15	18
37° 1 hour	19	4	21	16	19
60° 1 hour	18	0	0	..	20
90° 1 hour	18	0	0	..	20

$(NH_4)_2SO_4$ precipitation resulted in various fractions which gave rise to only 1 tumor out of 50 animals. This must be interpreted as meaning that the salt *per se* or the manipulations involved in the fractionation brought about an inactivation of the agent, or possibly that the petroleum ether removed some inhibitor.

Experiments in which phosphate buffer extracts of tumor material were incubated at room temperature at various pH values throw some light on the pH stability of the agent. Tumors have been noted from extracts kept at pH 5.0, 5.5, 6.3, 6.9, 8.7 and 10.2 for 1 or 2 hours. No tumors were noted after treatment at pH 4.5 for 2 hours, but such a negative finding will require confirmation. In general, the material soluble after incubation gave rise to more tumors than did the precipitates, even at pH 5.0, but these differences probably are not significant.

Another experiment which is not yet complete deals with acetone or petroleum ether extraction of lyophilized tumor material. So far 2 of 13 animals given ether-extracted material and 4 of 15 animals given acetone-extracted material have developed tumors. No tumors have developed from the acetone extracts, but in one group given an ether extract 2 of 10 animals have had tumors. This last material, however, differed from the other acetone and ether extracts in that it showed a granular precipitate as well as an oil when the solvent was removed.

Two recent experiments, both incomplete at the present time, deal with an attempt to precipitate the agent with the basic protein, salmine. The animals used in these experiments were all between 4 and 6 weeks of age. The starting material in both experiments was spontaneous tumor tissue, which in one case was used fresh and in the other was kept in the frozen state for several weeks. The tissue was extracted with saline or distilled water and the supernatants collected after centrifuging at about 2,000 g for 20 to 30 minutes. In one case the supernatant was adjusted to pH 5.5 and the soluble fraction treated with various concentrations of salmine. In the other case the original supernatant, which had a pH of 6.8, was treated with salmine. Tumors have already appeared from all fractions tested in both experiments with the exception of one of the sediments occurring after salmine treatment. The combined results of the two experiments are summarized in Table 2.

TABLE 2

Material	No. of mice	No. of tumors	Per cent. with tumors	No. still living	Average tumor age, mo.
Orig. S.N.	17	8	47	4	12
S.N. pH 5.5	10	4	40	2	13
Sed. pH 5.5	10	3	30	4	13
Salmine S.N.	38	17	45	11	12
Salmine Sed.	38	4	10	19	13

S.N. denotes supernatant; Sed. denotes sediment.

Perhaps the most important conclusion to be drawn from this experiment is that the one gram equivalent of tumor tissue, which all mice received, is much more than the minimal effective amount since tumors are developing from all fractions tested despite careful washing of all sediments. Experiments are now in progress to determine the minimal effective amount.

Summary: These experiments appear to show that the milk agent is destroyed at temperatures of 60° C and above, that it is stable at pH values between 5.0 and 10.2 but not at pH 4.5, that it is not inactivated by petroleum ether or acetone and not appreciably soluble in these solvents, and that it is partially, though perhaps only slightly, precipitated by salmine at pH values of 5.5 and 6.8.

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INHIBITION OF THE BACTERIOSTATIC ACTION OF MALACHITE GREEN BY ITS LEUCOBASES

THE bacteriostatic action of triphenylmethane dyes for Gram positive microorganisms is a well-known fact (see Churchman[1]). On the other hand, it is also generally acknowledged that the leucobases of these dyes are not bacteriostatic.

We carried out bacteriological assays with different leuco-derivatives of malachite green, such as leucobases, carbinol bases, bisulphite and hydrosulphite derivatives (leucosulphonic and leucosulphinic acids) and we found that:

(a) Carbinol bases, bisulphite and hydrosulphite derivatives possessed a bacteriostatic activity, no less, than the original malachite green dye (chloride or oxalate), *i.e.*, they were active until dilutions of 10^{-6} inclusively.

(b) The leucobases of malachite green had not only no bacteriostatic action in a dilution of 10^{-4}, but it

[1] Churchman, in Jordan-Falk's "The Newer Knowledge of Bacteriology and Immunology." Chicago. 1928.

was also able to diminish the action of malachite green and that of its active derivatives (Table 1).

TABLE 1

Dilution of the bacteriostatic substances:	10^{-5}	10^{-6}	10^{-7}
Malachite green alone	–	–	++
Malachite green + leucobase diluted 10^{-4}	–	++	++
Carbinol bases alone	–	–	++
Carbinol bases + leucobase diluted 10^{-4}	+	++	++
Malachite green bisulphite alone	–	–	++
Malachite green bisulphite + leucobase diluted 10^{-4}	–	++	++

– no growth; + weak growth; ++ regular growth.

These assays were carried out with a strain of scarlatinous streptococcus (Dochez) in peptone-glucose broth and in Goodman medium.

Such antagonism may be caused (a) by neutralizing the active compound directly through some chemical or physicochemical process (formation of new compounds or complexes, adsorption, etc.); (b) by opposite influences upon the milieu; (c) by some biochemical mechanism, as by inhibition of entering the toxic compound into the cell through competition or by opposite influences upon metabolistic processes, etc.

We are now investigating these possibilities and can already state that malachite green and its leucobase do not change oppositely the redox potential of the milieu (*cf.* Ingraham[2]).

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

RELATIVE POTENCY AS APPLIED TO THE ASSAY OF PENICILLIN

A SIMPLE estimation of the relative potency of penicillin, insulin[1] and other drugs can be made under conditions where the biological indicator gives a graded linear response when plotted against the logarithm of the dose. An unknown, U, is compared with a standard, S, at two concentration levels such that the dilution ratio $U_2 : U_1 = S_2 : S_1$. The two doses of the unknown are selected so that they will have the same potency as those of the standard, in so far as this can be determined in advance. The four doses are applied at random to sets of four quite similar biological units. The sets in turn are repeated until the potency of the unknown has been determined with the desired precision.

The data are given in Table 1 for a cylinder-plate assay of penicillin which meets these requirements. Four glass cylinders were placed on the inoculated agar of each petri dish. Two were filled with different doses of an unknown preparation (U_1, U_2) and two with corresponding doses of a standard (S_1, S_2). The diameter in millimeters of the cleared area about each cylinder on the following day is shown for each plate or set in the left side of the table.

In this experiment the calculations are shortened through the adoption of a ratio between concentrations such that $U_2 = 3.16\ U_1$ and $S_2 = 3.16\ S_1$, giving a log-ratio of $I = 0.500$. This modification was first proposed by Dr. Lloyd C. Miller, to whom I am indebted for the data in Table 1, and it has been used successfully for penicillin assays at the Winthrop Chemical Company, Inc.[2] Both U_2 and S_2 are prepared initially to contain 2.0 U/cc of penicillin, the unknown in the present case having an assumed potency of 400 U/mg. The weaker dilutions are obtained by adding 2.31 cc of S_2 and U_2 to vessels containing 5.00 cc of buffer solution.

The first step in the analysis is to compute four differences for each plate, as shown in the second part of Table 1. The successive differences are those of the unknown minus the standard at the high (D_1) and at the low (D_2) dosage levels and of the high minus the low dose for the unknown (D_3) and for standard (D_4). These initial differences are then used to obtain the basic computing units (y). The values for $y_1 = D_1 + D_2$ total the effect of the differences between the two preparations, while those for $y_2 = D_3 + D_4$ total the effect of the differences between the two dosage levels. The differences $y_3 = D_1 - D_2 = D_3 - D_4$

TABLE 1

Plate No.	Diameter in mm for				$D_1 = U_2 - S_2$	$D_2 = U_1 - S_1$	$D_3 = U_2 - U_1$	$D_4 = S_2 - S_1$	$y_1 = D_1 + D_2$	$y_2 = D_3 + D_4$	$y_3 = D_1 - D_2 = D_3 - D_4$
	U_2	U_1	S_2	S_1							
1	25.8	20.8	25.6	20.4	.2	.4	5.0	5.2	.6	10.2	–.2
2	25.8	21.0	25.2	20.4	.6	.6	4.8	4.8	1.2	9.6	0
3	25.4	20.4	24.8	20.0	.6	.4	5.0	4.8	1.0	9.8	.2
4	25.8	20.8	25.2	20.4	.6	.4	5.0	4.8	1.0	9.8	.2
									3.8 = T_1	39.4 = T_2	.2 = T_3

[1] C. I. Bliss and H. P. Marks, *Quart. Jour. Pharmacy and Pharmacol.*, 12: 182, 1939.

[2] Ingraham, *Jour. of Bact.*, 26, 573, 1933.

[2] L. C. Miller, J. H. Bailey and W. F. Warner (in preparation).