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Effect of Methylene Blue on Tumors.*

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This paper deals with the results of experiments obtained with rat carcinoma-sarcoma No. 256/94 A from the Crocker Institute inoculated subcutaneously into a selected strain of rats, and with mice as described below. In the rats, the per cent of non-takes was 6.5. The spontaneous regressions, *i. e.*, cases in which tumors were never larger than about 1 mm. in size and disappeared after a few days, were about 10%. These were not used in making computations in either set of controls or dye-treated animals. Only those tumors which reached a size larger than this and then disappeared were considered "regressions".

Previous experiments which will be described in the full report were unsatisfactory because of the high percentage of non-takes and spontaneous regressions. This paper will deal only with a group of animals in which this difficulty has been largely eliminated.

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There were 51 control animals and 87 in the treated group. Treatment, which was started when the tumor had reached a size of about 0.5 cm., consisted of daily injections into the tumor of 0.01% aqueous solution of methylene blue (Merck's medicinal). This dye was found to be the most effective of a group of oxidation-reduction indicators varying in redox-potential according to Clark's scale. The amount injected varied with the size of the tumor, which was difficult to gauge accurately, but was usually about 1/10 cc. for each mm. length of tumor; however, not more than 2 cc. was used for the large tumors to avoid possible toxic effects of the dye on the animal.

The average length of life of those animals having tumors which did not regress was 49.4 days in the controls and 45.2 in the dye treated. Those animals whose tumors regressed lived indefinitely and are therefore not included in the calculation of the above averages. The average size of the tumors of those animals which died was 6.25 cm. in the controls and 5.4 cm. in the dye-treated. The tumors of the dye-treated animals usually did not become so large as those of the controls.

The per cent regressions in the controls was 5.5; that in the dye-treated was 44. The regressing tumors usually became necrotic, sloughing off and gradually shrinking with scab formation, and finally disappearing.

Experiments with mice were not so satisfactory as those on rats just described, because the dye had to be given in smaller concentrations to avoid toxic effects on the animals. The most satisfactory group was a strain containing a mammary tumor from Memorial Hospital. These tumors never regressed in the controls but eventually killed the animal. However, in a group of 14 dye-treated animals, there were 4 regressions, while 10 animals bred at the same time and used as controls for this set gave no regressions.

The injection of methylene blue into tumors of rats and mice appears to favor regression of the tumors. This may result from one or both of the following actions; first, poising of the tissue at a redox-potential unfavorable to tumor survival, and second, the catalytic effect of methylene blue acting as a donor of activated oxygen to the tissues. Since this second effect depends upon cyclic oxidation and reduction of the dye, it can only occur when the redox-potential of the tissues lies at an appropriate level, namely, that at which the dye itself tends to poise the tissue. The two effects are therefore interdependent. It seems important in this connection that other dyes, above or below methylene blue in the redox scale, are relatively ineffective. The importance of the amount and type of oxidation

as a factor in tumor physiology is apparent from the work of Warburg on glycolytic metabolism of tumors. The present work does not, however, afford sufficient basis for excluding the possibility of other factors being operative, nor for further elaboration of hypotheses as to the interrelationship between the effects of methylene blue, redox potentials, type and amount of oxidation, and tumor physiology.

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Effect of Cholesterol Feeding under Varying Conditions upon Lipids of Rat Livers.

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Our interest has been drawn during the past few years to the question of the effect of various dietary deficiencies or excesses upon the deposition of cholesterol and cholesterol ester in tissues; manifested pathologically as fatty livers, arteriosclerotic arteries and gall stones. The following is a preliminary report of the first of a series of studies of tissues of rats fed cholesterol with diets of known but varying fat content, vitamin intake, etc.

The litters of rats used were cut to 8, 4 males and 4 females, shortly after birth. At 21 days of age, 2 males and 2 females from each litter were placed upon the diets containing cholesterol, while the littermate controls were placed upon the same diets without the cholesterol. After a feeding period of approximately 60 days the rats from both groups were killed by cutting the spinal cord at the base of the brain. The tissues were separated as quickly as possible and samples weighed for determination of moisture and of lipid content and in some cases for histological examination. The samples for lipid determinations were ground with sand and extracted to exhaustion with alcohol-ether.

The present report is limited to the composition of the livers of 2 groups of 16 animals each fed 1% cholesterol with basal diets consisting of 20% baked and extracted casein, 4% Osborne-Mendel salt mixture, 4% agar. In the first series this was supplemented with 15% Crisco and 47% starch, while in the second series 10% Crisco and 62% starch were used. For the experimental