CHEMOTHERAPEUTIC EXPERIMENTS WITH COAL-TAR DYES ON SPONTANEOUS MOUSE TUMORS

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We are aware that these attempts to influence the growth of tumors may seem to partake of the haphazard and casual. Many different substances and of widely different chemical structure have been injected into tumor-bearing animals, with a therapeutic purpose.1 Most of these attempts have been failures or have had but temporary or equivocal success. The basis or justification of such experiments is clear and follows the obvious and ancient tradition of therapeutics,—to give medicine. That many such attempts must be wholly or partly empiric goes without question. The great importance which would attach to any clue or leading suggestion obtained from any substance whatever capable of repeated administration to the animal organism without serious injury, justifies persistent essays to test the effect on cancer of the most various groups of chemical agents. Obviously in many such cases no cogent a priori reason could be given for expecting other than adverse or negative results. In view of the usefulness of accidental observations and unexpected results throughout the field of experimental science and the barren field of medication in cancer, it seems justifiable and expedient to investigate even unpromising fields.

We have aimed to test representatives of all the major chemical groups into which the innumerable coloring matters derived from coal-tar are classified and to this end have selected upwards of seventy actual or potential dyes. No attempt has been made to select toxic compounds, to combine them with metals, or to confine to agents having medicinal, vital staining,

1 See Weil, J. Cancer Res. 1918, i, 95.
or tumoraffine properties, but rather to seek any organic bodies in this group which might in any way influence growth of spontaneous tumors and hence furnish a possible clue to specific or general action in chemotherapy. A few pharmaceuticals not used as dyes have been included. We wish here to record the results with about thirty of these tests reserving the unfinished remainder for a future report, to include a classificatory tabulation of the dyes and some references to the bibliography of the subject.

Except where otherwise indicated, all the dyes used are stock preparations of the National Aniline and Chemical Company of New York. The Schultze number refers to the fifth edition of the "Farbstofftabellen."

The results have been entirely negative. Nevertheless, it is obvious that no particular negative has been established. Perhaps handled in another concentration and varied endlessly in the details of administration some of these bodies might yield a different and better result.

METHODS

The dye has been dissolved in distilled water in suitable concentration and injected intravenously. Intravenous injections by way of the tail vein in mice can not be repeated indefinitely, on the same animal. The tail often becomes highly colored hiding the vessels, and severe necrosis may set in, even to the sloughing of the entire member. In such cases the continued injections were subcutaneous until a result appeared. In a few cases subcutaneous doses alone were given. An approximate determination of a minimal lethal dose was first made and the tolerance was approached in the therapeutic dose. Injections were made once daily with an occasional intermission of a day or two. These were continued for days, usually for weeks, sometimes for months, until it appeared that the tumor was unmistakably growing. It was aimed to use tumors plainly visible or palpable, but in the earlier stages, as the size of a pea or hazel nut. Except where otherwise stated in the protocols, only such early tumors have been used.
Male mice of about thirty grams weight will endure daily intravenous injections of one cubic centimeter of distilled water for at least ten days, and probably much longer, without apparent harm. A well grown adult usually recovers from the shock of a single dose of 2 or 3 cc. Four cc. at one time are usually fatal. The dose of dissolved dye usually was contained in a fraction (1/10 to 5/10) of a cubic centimeter. In some cases the dye was non-toxic or was but slightly soluble and a lethal dose could not be given. A saturated solution was then used, or a suspension of excess dye was shaken, drawn up into the needle and injected. The dose is stated in milligrams of dye.

The growth against which the injections are made is the spontaneous adenocarcinoma of the mammary gland in the female albino mouse. The mice and tumors have the uniformity which results from long inbreeding. In a few cases transplants of the same growth have been used in a supplementary way, and are detailed in the proper place.

**Mercurochrome**

Intravenous only: Two tumor mice, each one mg. six times at intervals during one month. Tumors grew rapidly. Another received one mg., then 2 mg. twice during the next three weeks. At death in the fourth week the tumor was slightly smaller.

A fourth, with a large tumor, received 2 mg. doses seven times in seven days, followed by two daily subcutaneous doses, when the tumor was found slightly smaller, but death occurred two days later. A fifth was given five daily doses of 2 mg. each, 3 intravenous, 2 subcutaneous. Death next day, tumor unchanged.

Subcutaneous only: Tumor of hazel nut size. 38 doses, 2 mg. each, during 45 days. Death 8 days after cessation, with mercurochrome ulcers. Tumor much larger.

**Naphthol Yellow**

(Schoelkopf Aniline & Chemical Company)

Tumor just palpable. Dose 3 mg: 12 intravenous, 50 subcutaneous, during 72 days. Tumor grew rapidly to large size.
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during injections and was enormous at death 10 weeks after cessation.

"FUCHSIN"
(Source unknown)

One intravenous milligram is promptly fatal. Three mice with tumors pea size or smaller. During 13 days one was given 2 mg. divided in 6 intravenous, followed by 1.7 mg. in 4 subcutaneous doses; another 1.2 mg. in 4 intravenous, 2 mg. in 5 subcutaneous doses; the third 1.5 mg. in 5 intravenous, 1.5 mg. in 4 subcutaneous doses. All the tumors grew under the treatment. All mice lived for weeks after cessation. The smallest tumor at death had reached only pea size, the other two walnut size or larger.

ACID FUCHSINE (RUBINE S). SCHULTZ NO. 524
(Total Dye Content 65 Per Cent)

An initial intravenous dose of 17 mg., followed by 14 subcutaneous doses each of the same amount and 11 of 34 mg. each, during 30 days. The tumor grew much larger during this period. Death three weeks after the injections ceased. Tumor not stained.

FUCHSINE, NEUTRAL. VITAL STAIN

Toxicity about as the preceding. Mice will not endure for long a daily subcutaneous dose of one milligram.

Subcutaneous only: One half mg. 19 times in 21 days, when death occurred, the tumor having grown and ulcerated.

MAGENTA ROTH (GRÜBLER)

During 30 days, doses of a half milligram; 10 times intravenous, 17 times subcutaneous. The tumor of pea size grew slightly larger. Death two days after injections ceased.

GENTIAN VIOLET

During 15 days three tumor mice were treated. One received two intravenous doses of a half milligram each, and seven subcutaneous of 16 mg. total. At death on the sixteenth day
tumor was slightly smaller. A second was given 3 intravenous doses of a half milligram each and 4 subcutaneous of 17 mg. total. Death nine days later, the tumor unchanged. The third received six intravenous doses of a half milligram each and 16 mg. in 7 subcutaneous doses. A month later mouse was in bad condition and was killed, the tumor having grown considerably.

**METHYL GREEN (GRÜBLER)**

Three tumor mice, each with a tumor the size of a large pea, were treated alike. During 11 days, 8 intravenous doses of 1/10 mg. each; six subcutaneous doses, one of 1/10 and 5 of 1/5 mg. each. All three tumors grew much larger under the treatment.

**METHYL GREEN. VITAL STAIN**

A tumor of pea size. 1/10 mg. was given intravenously daily for 9 days, followed by 43 subcutaneous doses during the next 52 days. Death two days after last injection. The tumor grew slowly throughout the two months of almost continuous daily treatment.

**WASSER BLUE (GRÜBLER)**

The tail is soon colored by the dye, making tail vein injections difficult. Two intravenous injections of 10 mg. each, then 22 subcutaneous each of 10 mg., during the next 25 days. Death the day after last injection, with the tumor of pea size unchanged, though colored throughout by the dye. Organs and viscera irregularly and unequally colored.

**BISMARCK BROWN (GRÜBLER)**

Three tumor mice. One received 15 mg. once, 10 mg. 6 times, intravenously; and 15 mg. twice subcutaneously. Total time 21 days. The tumor had become somewhat smaller when the mouse died four days after the last dose.

A second received six intravenous doses of 10 mg. each, and three subcutaneous of 15 mg. each, in 19 days. The tumor had grown much larger.

The third received three intravenous doses of 10 mg. each;
subcutaneously, one of 10 mg. and five of 15 mg. each. Total
time 19 days. The original tumor became slightly larger, and
a new tumor arose during the course of injections.

**BRILLIANT YELLOW (GRÜBLER)**

Nine intravenous daily doses of $7\frac{1}{2}$ mg. each. Tumor un-
changed in size.

**ACID YELLOW XX. SCHULTZ NO. 139**

(Orange IV, Tropaolin 00)

Two adjacent small tumors on one host. During two months
54 intravenous injections of $\frac{1}{2}$ mg. each. The tumors grew
constantly during this treatment. During the next ten days
nine subcutaneous doses of one milligram each. Two weeks
later the mouse died with the tumors coalesced and much larger.

**DIAZINE GREEN. VITAL STAIN**

(Janus Green)

Highly toxic, whether intravenous or subcutaneous. It is
unsafe to give more than 1/50 milligram. This intravenous
doctrine was given daily for 12 days without checking the growth.
A subcutaneous dose of the same amount was given 29 times in
the next 36 days, while the tumor grew from pea to walnut size.
The mouse lived two months after cessation of treatment.

**TRYPAN BLUE. VITAL STAIN**

The first intravenous injection, of 3 mg., colored the tail a
deep blue, preventing repetitions. Fourteen subcutaneous in-
jections of 3 mg. each followed during 16 days. Death the
following day. Tumor deeply colored by the dye and not
recognizably changed in size.

**TRYPAN RED. VITAL STAIN**

The tumor and tissues in general save central nervous system
are highly colored by the dye.

Five intravenous doses, followed by 12 subcutaneous, of 2
mg. each, during 19 days. Death one day later. Tumor
moderately larger.
BORDEAUX RED (GRÜBLER)

During 40 days, 38 mg. in 5 intravenous doses, 300 mg. in 30 equal subcutaneous doses. Two days later the mouse died with the tumor slightly larger.

ISAMINE BLUE. VITAL STAIN

("Original Formula")

Dose 2 mg. Intravenous once, subcutaneous 40 times, during 47 days. Soon after the first injection a second tumor arose, and at cessation of treatment both had grown considerably. Mouse lived 48 days longer, the earlier tumor having reached enormous size. The other was the size of a hazel nut. Both were colored throughout by the dye save for a large necrotic cyst.

A second animal was treated during 22 days. Dose 2 mg., intravenous 6 times, subcutaneous 13 times. The tumor grew slowly during and after the injections. Death 33 days after cessation. Only the capsule of the tumor was then found stained. A second tumor was present, probably developed after the treatment.

The dye colors highly the skin and subcutaneous tissues, making repetitions of tail vein injections difficult. A normal male of 30 grams, which died after receiving three initial and 41 subcutaneous doses of 4 mg. each, showed at autopsy: Skin bright blue; subcutaneous tissues and abdominal wall well colored, but skeletal muscles on section only moderately stained. Bladder and genitalia, except seminal vesicles, well colored. Periosteal stain makes brain case appear blue, but neither bone nor brain is stained.

AZO RUBIN

Tumor of hazel nut size. Injections covered 51 days. Intravenous doses of 15 mg. each 10 times, 10 mg. each 21 times, and 12 subcutaneous of 10 mg. each. Death next day. Tumor much larger, ulcerated, not stained.
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CARMINATE-SODIUM. VITAL STAIN

Dose 5 mg., subcutaneous only, 23 times during 28 days. Death next day. Tumor considerably larger, colored. Color general in tissues, except the brain.

RHODAMINE. VITAL STAIN

During 62 days, five intravenous and 47 subcutaneous doses were given. During these two months the original tumor of pea size grew to walnut size and three additional tumors developed at intervals.

METHYLENE BLUE (BAUSCH & LOMB)

During 25 days, 7 intravenous and 16 subcutaneous doses of one mg. each. The animal died next day with the tumor unchanged.

A second mouse received 9 intravenous and 25 subcutaneous doses of one mg. each during 39 days. At the end of the intravenous series the tumor was visibly larger. Death 8 days after injections ceased, with the tumor much larger and not colored.

METHYLENE BLUE (EHRLICH). VITAL STAIN

Two intravenous and 24 subcutaneous doses of one mg. each during 29 days. The small tumor was slightly larger when the mouse died the day after last injection. Neither tissues nor tumor were stained.

TOLUIDINE BLUE. VITAL STAIN

Dose one milligram: Intravenous 12 times, subcutaneous 32 times, during 53 days. The animal became cachectic and developed ulcers at injection sites. The two small original tumors regressed slightly, but a third developed. At death 11 weeks after cessation there were four tumors.

A second mouse with a barely palpable tumor was given four intravenous and 25 subcutaneous doses of one mg. during 35 days, in which the tumor grew to hazel nut size. The animal lost weight, developed skin ulcers from the injections and died three weeks after they ceased, the tumor remaining unchanged.
BRILLIANT CRESYL BLUE. VITAL STAIN

Subcutaneous only. Eight doses of 3 mg. each, one of 2 mg., 15 of one mg. each, during 27 days. Death the day after last injection, the tumor scarcely changed in size. The dye produced ulcers, the mouse lost weight and fell off in condition. No color.

NEUTRAL RED (EHRlich). VITAL STAIN

Subcutaneous only. One dose of four mg. and 6 of 2 mg. each, during 7 days. Death untimely one day later, doubtless from the dye. Tumor not visibly affected.

A second mouse was given 5 intravenous and 11 subcutaneous injections, of one mg. each, during 18 days. Animal became sick and died two days after last injection. Tumor unchanged in size and without stain.

NIGROSIN (EIMER & AMEND)

One intravenous dose of 3.75 mg. The colored tissue then obscured the veins. One subcutaneous dose of 2.5 mg., one of 10 mg. and 9 of 12.5 mg. each. Mouse died three days later. Tumor same size, on section distinctly stained. A small tumor in the lungs distinctly blue. Muscles and most of the organs except lungs were deeply colored.

A second animal was given, during 11 days, 2 intravenous doses of 2.5 mg. and 3.75 mg., two subcutaneous doses of 10 mg. each, and 6 of 12.5 mg. each. Death next day. Tumor larger.

ACRIFLAVINE

(Diamino-methyl-acridinium-chloride) Abbott Laboratories, Chicago

A tumor of 14 mm. Six intravenous doses of one mg. each, 11 subcutaneous doses of 1½ mg. each, during 20 days. Death four days after last injection. Tumor much larger.

ALIZARIN RED S.

(Alizarin Mono Sodium Sulfonate)

Two intravenous doses of one mg. each in two days. Half the tail sloughed in a day or two. During the next 54 days 48
subcutaneous doses of one mg. each. A second tumor became palpable 16 days after beginning of treatment. At death the day after last injection both tumors were much larger and not colored.

**ALIZARIN BLUE S (EHRlich). SCHULTZ NO. 804**

This dye is a sodium bisulphite compound with the insoluble alizarin blue. It dissolves readily but splits by oxidation and soon throws out the insoluble dye, which is far more toxic. An intravenous minimal lethal dose of freshly dissolved S dye for a large mouse is somewhat under 10 mg. About 1/10 mg. of a suspension of alizarin blue may be given intravenously, and much larger amounts under the skin. All the trials with tumor mice were made from the precipitate of an alizarin blue S solution, the injected agent being a suspension of minute crystals of

**ALIZARIN BLUE**

Two supposed tumor mice received many intravenous and subcutaneous doses of 1/5 mg. The shrinkage of one tumor and the disappearance of the other led to more extended tests. One tumor was certainly, the other probably, an abscess.

**Spontaneous Tumors**

A mouse with two tumors of size 29 ½ 18 mm., and 19 ½ 12 mm. Intravenous dose 1/5 mg., and the same amount subcutaneously, daily for 6 days. Then one subcutaneous mg. 21 times during 24 days. During this period the larger tumor increased greatly and ulcerated, while the smaller diminished slightly. Mouse died a week later, the same condition holding.

Another mouse with a tumor as large as a hazel nut received one subcutaneous milligram daily for six days. The tumor was then slightly smaller. Unfortunately the mouse died next day.

Another with a tumor 8 mm. in diameter was given 14 intravenous doses of 1/10 mg. each, and two subcutaneous of 1/5 mg. each, during nine days. The tumor grew distinctly larger.

A breeder with a tumor 12 × 19 mm. received both a subcutaneous and intravenous dose of 1/10 mg. 8 times in 10 days,
then twice a day for 12 days. The tumor then measured $14 \times 22$ mm.

Transplanted Tumors

Subcutaneous only: Six mice with tumors of various sizes, only one larger than a pea, were given injections beginning 27 days after the implantation. Four received daily doses of one mg. each 20 times, the others 16 and 8 times. All the tumors grew during the treatment save one, which became slightly smaller.

Intravenous only: Three mice with growths of two weeks, scarcely beyond palpable size, were treated twice daily for five days. The usual dose was $1/10$ mg., a few being $1/20$ mg. The tumors became recognizably larger during this period.

Despite the few instances that equivocally suggest some therapeutic effect, it is improbable that alizarin blue is an effective agent against tumor growth. It should be remembered that even spontaneous mammary tumors in the mouse regress in somewhere near one per cent of cases; the spontaneous tumor perhaps ten times as often.

Amaranth. Schultz No. 168

Seven intravenous followed by 3 subcutaneous doses of 10 mg. each were given during 12 days. This dosage is too high for continuous treatment. The mouse died next day, the tumor of pea size unchanged.

Doses of 8 mg. each were given a second mouse, 23 intravenous and 32 subcutaneous, alternating at intervals during 50 days, as the tail recovered condition. The tumor grew rapidly and a second tumor developed during the first three weeks. Both were growing at cessation of treatment and at death a month later.

Azo Fuchsin

Dose 15 mg. Three daily intravenous injections caused the tail to slough. During the next month 23 subcutaneous doses were given. There were marked subcutaneous reactions, the mouse became sick and died five days later, but the small tumor had grown slightly.