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THE POSSIBILITIES AND DANGERS IN USING MALACHITE GREEN IN PISCICULTURE.

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Introduction.

For many years a dye has been used in the practise of pisciculture and pond management, which is known by the name of malachite green, and is used in all of the following three ranges:

1. To combat fungus on fish eggs (the case with Saprolegnia).
2. To combat fungus on fish (the case with Saprolegnia).
3. To combat external animal parasites on fish.

Hopes also to be able to stem other diseases of fish or to destroy unwanted water plants with malachite green have not been fulfilled.

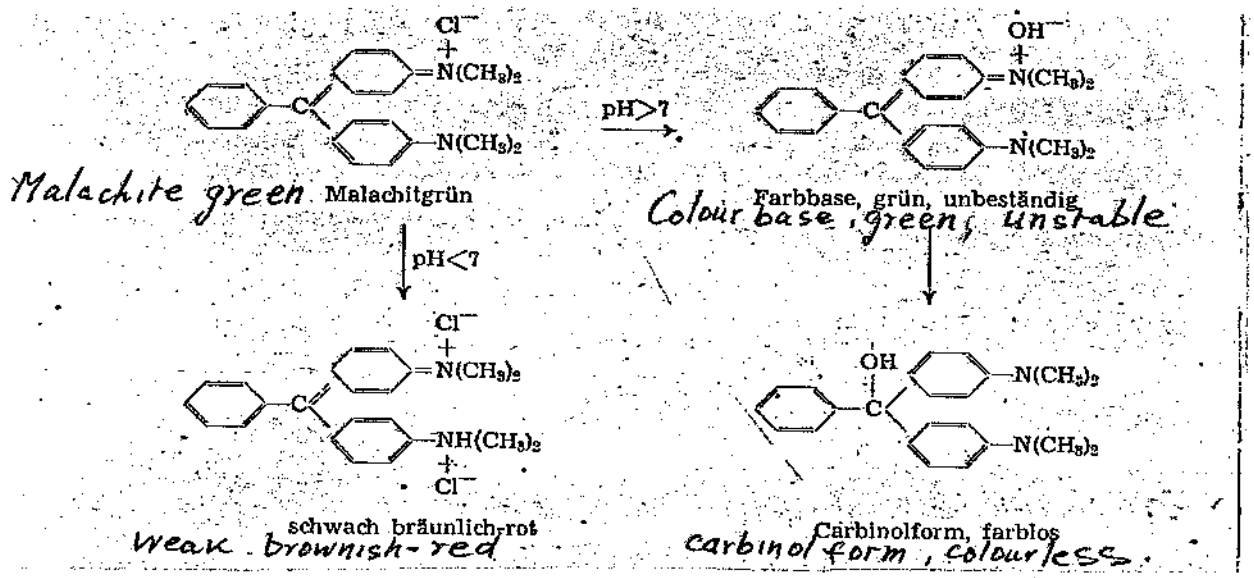
Recently several works (19, 35, 36) have appeared, showing that with malachite green some danger is attendant in a physiological aspect. For example it can cause extensive, retarded damage in rats, and in Chironomidae larvae can give rise to structural alterations in the chromosomes of the salivary glands. On these grounds it seemed advisable to us to describe in its entirety the problem of the application of malachite green in pisciculture and to undertake special, complementary research.

The stability and chemical constitution of malachite green.

Malachite green is a basic triphenylmethane dye, which is on the market as a chloride, oxalate, sulphate or double salt of zinc chloride. This dye should not be confused with malachite - the green copper spar $[(CuCO3.Cu(OH)2)]$ - which unfortunately occurs frequently in reference works.

Malachite green belongs to the less acid and alkaline-stable dye. In an acid medium there follows from salt formation the accumulation of a proton on the triple-bound amino group, through which the colour changes from

green to brownish-red with substantial reduction of their intensity. In an alkaline medium the malachite green en the unstable green colour base is transformed into the colourless carbinol form. These transformations are illustrated by the following formulae:



In both cases there thus occurs decolorisation respecting the reduction in intensity of colour.

In AMLACHER'S (2) aquarium experiments and also in our own experiments it was noticeable that the solutions of malachite green became colourless in a more or less short time. We have therefore determined the pH-value of these solutions, and thereby established that, following the intensive aeration of the basins, the dissolved CO2 was expelled, through which the pH-value rose from 7.3 to 8.1. By this means the malachite green was transformed into the colourless carbinol form. The dye could be restored to its intensity of colour through the careful acidification with 0.1_N sulphuric acid. As this transformation can be relevant in circumstances for the effectiveness of the malachite green, experiments were undertaken to evaluate the speed of transformation depending on the pH-value, (Tables 1 & 2). For this the oxalate and the sulphate of malachite green were available to us. Solutions with 1 mg/1 of malachite green in each case were prepared. Mains water served as the medium of solution; in two cases distilled water was used. The transformation of the malachite green was determined by measuring

the extinction in the linear colorimeter. The highest extinction value reached under our experimental conditions was set at just 100%, and the other values were related to this. Thus in the tables it is a question of constantly related data.

While the malachite green oxalate remains unaltered in a weakly acid pH-range (Tab. 1), the corresponding sulphate appears to possess the greatest durability in the almost neutral pH-range, (Tab. 2). In other pH-ranges the transformation of the malachite green follows in more or less high quantity as far as a definite equilibrium, whereby this equilibrium does not set in until after several hours.

If the malachite green solutions with the weakest colour intensity were brought to a pH-value which allowed the strongest colour intensity to be expected, the expected extinction would constantly be more nearly approached.

Table 1

Transformation of malachite green oxalate (by %) depending on the time and the pH-value of the solutions.

Hours pH-values (distilled water).

Tabelle 1

Umwandlung des Malachitgrünoxalats (in %)
in Abhängigkeit von der Zeit und vom pH-Wert der Lösungen

nach Stunden	pH-Werte 7,3	6,45	6,0 (Aqua dest.)	4,65	2,7
0	83	87	97	100	82
3	65	83	97	100	70
7	32	80	97	100	67
24	28	79	97	97	62
72	27	79	95	97	59

Table 2

Transformation of malachite green sulphate (by %) depending on the time and the pH-value of the solutions.

Hours — pH-values (distilled water).

Tabelle 2

Umwandlung des Malachitgrünsulfats (in %)
in Abhängigkeit von der Zeit und vom pH-Wert der Lösungen

nach Stunden	pH-Werte 8,1	7,3	6,5	6,4 (Aqua dest.)	4,6	2,9
0	19	20	26	23	17	12
1	20	42	80	72	33	7
4	23	64	99	85	54	7
21	—	81	100	85	65	6
28	—	80	100	87	70	7
45	26	80	100	83	64	6

The durability of malachite green, therefore, extends in each case only over a small pH-range. Supervision of the pH-value is recommended in all cases with the use of this dye in pisciculture, as the discolouration of the water definitely represents no criterion for the breaking down of the dye molecule, and the products resulting from transformation could also have toxic effects.

The quantitative composition of both malachite green salts was moreover determined. On this occasion it was shown that the formula $H_4\left[(C_{23}H_{25}N_2)_2(C_2O_4)_3\right]$ is appropriate for malachite green oxalate, while the formula $(C_{23}H_{25}N_2)HSO_4$ exists for the corresponding sulphate as an acid salt. Consequently malachite green sulphate possesses a higher content (77.3%) of colour components as the oxalate (71.1%), which - as later experiments will show - leads to a greater effectiveness of this combination.

Combating fungus on fish eggs with malachite green.

With the incubation of fish eggs in fish hatcheries, losses are constantly arising through the death of individual eggs, losses which in their number depend on the respective species, the individual parent animals and the prevailing environmental conditions. This apparently normal phenomenon is of considerable significance for the practice, since the deceased eggs are often attacked by fibrous fungi (Saprolegniaceae). These also spread very easily to healthy eggs which happen to be nearby, so that the pisciculturist is constantly compelled to select white eggs which have gone bad. This involves a considerable waste of time, since most of the work is done with pipettes or tweezers. This operation has been simplified through the Scharfling egg-sucker, which works on the principle of the siphon, and with which eggs of the most different sizes, which have died proportionately quickly, can be separated (10). Faulty mechanical selection can before everything lead to injuries to the healthy eggs in the first period of the incubation to the appearance of the eye points, whereby the losses are increased.

It is not surprising, therefore, that for a long time already means have been sought to prevent the damaging development of Saprolegniaceae. BURROWS (3) found that Malachite green in a solution of 1:200,000 (5 mg/1) applied hourly wards off the appearance of fungus on the eggs. According to his statements, the fish eggs have a wide range of tolerance against this dye, and moreover it was shown that the costs of the treatment are low. BURROWS (3) conducted his experiment on the eggs of Salmonidae, allowing a specified amount of the original solution to flow into the incubator from a Mariotte flask by siphoning, in a given time. During the operation the flow through the incubator troughs was not altered. The method became widespread fairly quickly and is used today in almost all countries in which fish eggs are incubated. In the literature the use of malachite green as a natural measure is mentioned in numerous instances. It is naturally only possible to go briefly into the most important fact within the scope of this work. Malachite is used to protect the eggs of trout, char, salmon and other species, which are incubated on filters in plants along the current,

under the current, and similar apparatus. HUBLOU (17) constructed a permanent inflow receptacle out of plexiglass, which possesses some advantages compared with the apparatus used by BURROWS (3). It can be set up on the upper board of the incubatory trough with the help of a clamp, is of unbreakable material, and can be filled with 1800 c.c. of the original solution, which is sufficient for treatment lasting one hour. Obviously only the possibility is also given here, of providing an incubatory apparatus with the fungoid substance or several standing one beneath the other. JOHNSON and others (18) go one step further and mix the entire necessary amount of water in the incubatory building centrally with malachite green. They describe a pump installation, with the help of which a measurable amount of the original solution of malachite green can be fed from the mains supply tank to the incubator plant. Also in this case a concentration of 5 mg/1 is placed in the water supply for one hour. Subsequently the pump and mains are flushed out with clean water. GOTTWALD (14, 15) attains a similar effect through letting the contents of a wicker bottle of 30 l. capacity flow through a siphon in the supply to the incubator building.

Should small eggs, i.e. eggs of pike (Esox lucius) and whitefish (?) (Coregonus albula), whose incubation takes place in automatic selectors, be treated with malachite green, then it appears expedient to provide the mains supply with the dye, and not the plant by itself, as for example B.CUMMINS (5) did with the incubation of the American sander (Stizostedion vitreum). The original solution of malachite green can, however, also be led separately to each individual incubation glass. We reached this in our own experiments, in which we brought the supply vessel over the automatic selector, and from above inserted the drain pipe, to which a glass tube had been secured, so far into the apparatus, that its end projected into the glass neck of the incubator. Here the inflowing water supply and original solution were then mixed without interruption.

VIBERT (34) recommends the use of this means with the operation of large vertical apparatus, in which the individual vessels for the eggs are arranged vertically over one another. It is easy to see that with systems

of this kind the chemical treatment of the eggs has special advantages, since mechanical selection is very difficult.

The concentration used and the permanence of the effectiveness are quite separate. In general it becomes so confused, that the inflow is not cut off, which leads to losses being incurred through forgetfulness. The original solution which is added is then so selected, that the required concentration develops in the incubatory apparatus. In this case the amount of inflowing water in a unit of time must be considered, and determined in advance. As BURROWS (3) did, JOHNSON and others (18) and VIBERT (34) also worked with a concentration of 1:200,000 (5 mg/1). The operation was carried out twice weekly for one hour. ROBERTSON (25) treated the eggs of Salvelinus namaycush twice daily for one minute. The concentrations used by him were obviously high, but unfortunately cannot be sufficiently reconstructed. Hybrids of Salmo and Salvelinus, which SOWARDS (31) bred, received five minutes' daily treatment with malachite green while in the egg stage. The concentration is given with 1:15,000 (66 mg/1). DEUFEL (6) lets 500 mg/1 for one minute, but all five days, work on the eggs when the inflow is cut off. The trough with a current passing through was similarly treated, and with an equal concentration the malachite green takes effect at the most for some minutes. Whitefish eggs in Zuger (?) glasses can, according to the same author, be also treated for one minute all five days with 500 mg/1. The process in this way becomes so advanced that the inflow is cut off, half the water is removed from the apparatus, and it is filled up with 0.1% (1000 mg/1) malachite green solution. There then prevails a concentration of 500 mg/1. After an action of one minute the inflow is again started. GOTTWALD (14, 15) recommends the following: for all two days pike eggs are exposed for 15 minutes to a concentration of 1:100,000 (10 mg/1), whitefish and trout eggs all three days for thirty minutes to a concentration of 1:100,000 or 1:200,000 (10 respectively 5 mg/1), and sander (Lucioperca lucioperca) eggs all two days for fifteen minutes to a concentration of 1:40,000 (25 mg/1). SHARP and others (30) had already treated pike eggs similarly in 1952. They allowed 5 mg/1 to act for one hour, and indeed about five times during the incubation period. GENINA and

MARTINSON (11) used 10 mg/1 on pike eggs every two days and daily for 30-45 minutes at increased temperature up to the appearance of the eye points. By this means the losses could in many cases be reduced to about half (e.g. from 50-60% to 25-30% or from 95% to 50%). WOYNAROVICH (37) could prevent attacks by Saprolegnia on nests where roach (Rutilus rutilus) eggs had been laid, if he rinsed them with malachite green in concentrations of 10-100 mg/1. The growth of fungus can be prevented, according to CLEMENS and SNEED (4), on the eggs of the American Welsart (?) Ictalurus punctatus if 1 mg/1 of malachite green is applied. The treatment should be undertaken in the incubator with the current flowing through, in such a way so that the dye is completely expelled after one hour (repeat as necessary).

The temperature at which the eggs were treated are only rarely sufficiently specified. The hybridisation experiments of SOWARDS (31) had the water at an average of 5.6°C. The incubation of pike outlined by SHARP and others (30) took place at temperatures of 4.5°C to 11°C. Naturally the clearance space in all these cases can not be very large.

Far more important is the question, of whether only the eggs or also the freshly hatched breed can be exposed to the malachite green. In general the dye should be added principally during the actual incubation. GOTTWALD (14) treated the eggs of pike and whitefish regularly up to the appearance of the eye points. DEUFEL (6) recommends that no more malachite green should be used in the last 4-5 days before hatching, since the eggs otherwise burst prematurely and the newly hatched breed very quickly dies through the concentrations (500 mg/1) which have been applied to them. MAZURANICH and NIELSON (21) report that malachite green is no longer given in the salmon hatcheries in the state of Washington, if the fish hatch or stay shortly before. On the other hand SOWARDS (31), who uses a concentration of 66 mg/1 daily for five minutes, has no scruples about treating young Salmonidae up to the start of their ability to feed. JOHNSON and others (18) proceeded similarly, in allowing 5 mg/1 to act twice weekly for one hour, not only on the eggs of Oncorhynchus, but also on the fry up to the start of their ability to feed.

As malachite green can exist in different forms, it does not after all seem necessary to pay attention to what kind of preparation is used. In American papers it is in some cases stressed that the oxalate is used. ROBERTSON (25) writes that he used zinc-free oxalate. DEUFEL (6) refers to the difference in effectiveness which may be encountered in substances from different firms.

Some experiments could have been undertaken in recent years in three different trout hatcheries. Mostly the malachite green was received for us as an oxalate, at times, however, work was also done with the sulphate. As a rule the dye took effect thereby in a concentration of 1 mg/l daily for one hour on the eggs. In some cases the eggs were treated with 2 mg/l. The flow through the incubator was uninterrupted, the necessary malachite green was added to the inflowing water after careful calculation in the form of a concentrated solution. In order to have possibilities of comparison with untreated controls, malachite green was added in an incubator only to a few apparatus along the current, in two successive years, while the eggs in all other plants did not come into contact with the medium. We allowed the original solution to mix with the inflowing water supply out of a clear bottle. This simple method was sufficient for the thorough mixing, but there were small quantitative inaccuracies, since at the start the outflow was greater, while at the finish it was less, owing to the reduced pressure. This was, however, of little consequence, and led to no impairment of the effectiveness. In both the other hatcheries the original solution arrived at a central position in the distributory channel, so that all eggs in the incubator were supplied with the adequate solution of malachite green. In this case attention must be paid to a thorough mixing taking place in the distributory channel. This is achieved only if the water is rushing violently through a considerable natural fall, otherwise an agitator must eventually be used.

The calculation was made by treating a single apparatus along the current in the following way: the cabinet has water running through it at a rate of 18/1 minutes an hour of around 1000 l. Should the concentration amount to 1 mg/l, 1 g. of malachite green must be added every hour. An

original solution should be used, which contains 1 g. of substance/100ml. We filled up 100ml. of this original solution to 5l. with clean mains water Care must now only be taken that this 5l. solution, which contains 1 g. of the medium, should flow through the incubator in exactly one hour. A concentration of 1 mg/l is then prevalent in it during this time.

The medium large incubator of another trout hatchery, whose water altogether was daily mixed with malachite green for one hour, absorbed 420 l. water per minute. In one hour this is around 25,000 l. For the manufacture of a concentration of 1 mg/l, therefore, 25g. of malachite green was necessary daily. This was dissolved in about 80 l. of water, and within one hour was brought to the distributory channel through a siphoning tube from a supply vessel. At a price of around 28, -DM/kg for malachite green, the daily treatment costs dependent on the substance amounted to 0.70 DM.

The incubation temperature varies in all the incubators, in which we carried out our experiments, between 3 and 9°C. The pH-value lies between 7 and 8. The SBV amounts in one case to 1.6ml/l 1N HCl, in others, however to 5.0 ml/l 1N HCl.

A daily treatment of one hour and a concentration of 1 mg/l proved to be sufficient. In one operation there nevertheless appeared some negligible fungoid growth. Here 2 mg/l proved to be better. Eventually it must be assumed from these considerations that the original solutions were prepared with normal water for a longer period of time, and possible through standing lost some of their effectiveness.

Generally the eggs did not need to be sorted before hatching. In practice, nevertheless, this is done at least once up to the instant they eye points appear. Treated eggs are substantially better and more rapidly sorted, as threads of fungus, which join the eggs with each other or with the bottom of the strainer, are lacking.

The advantages in operating efficiency are available for the use of malachite green. We were able to show by our investigations that eggs which

had been treated without sorting up to hatching sustained damage somewhat less important than those which had not been treated and thus constantly being sorted. Through this it is clear that mechanical injuries to the eggs, which could arise from sorting, drop and the general development of the eggs can proceed without further mechanical disturbances. If, out of a deficiency of work capacity, unskilled workers are put to sorting the eggs, the danger of increased losses through careless and inexpert work is particularly high.

An incubator in which a maximum of several million rainbow trout eggs are being hatched needs during the incubation period at least one worker who is exclusively employed on sorting the eggs. Should there arise a delay on the removal of the deceased eggs just once, it can come about that in many cases the remainder simply cannot be recovered without supplementary help. If we take the wage bill for this person during a three-month incubation period at around 900DM and set against it the costs for the malachite green (in the above-mentioned incubator these were 0.70 DM daily, ie. about 63 DM in three months), then it is seen that the expenses for the treatment of the eggs with the dye amount to less than 10% of the otherwise normal costs. It is worth mentioning that this percentage should then also not be exceeded, if the expenses for the malachite green receptacles and service are taken into consideration.

In spite of the widespread use which malachite green already has today in the prevention of fungus on fish eggs, still many questions are unanswered. It is still not known which concentration and which period of action are most appropriate. It is on the other hand safe to say the treatment in running water is quite possible and that this technique has great advantages over the method whereby the water is cut off. Furthermore, temperature must receive greater attention than hitherto. Plants which work with comparatively cold and scarcely polluted water have often suffered little from fungus attacks, and hence were able for a time to dispense with the use of malachite green. Further investigations into the action of different salts of malachite green and the interrelationships in the chemical composition

of the incubator water should not also be left to the end. It is possible that in the future, in case further work is done with malachite green, that in general the nature of the incubation which we practised will be altered. If a constant selection becomes superfluous, larger amounts of eggs could be hatched in a smaller space (vertical cabinets, cf. VIBERT (34)).

The toxic effects of malachite green on fish.

For precise and exhaustive data on the toxicity of malachite green in the form of an oxalate salt we are grateful to CLEMENS and SNEED (4). They tested the action of this dye on the fry of Ictalurus punctatus of 5-7.5 cm. long and 4-6 months old. Their results are reproduced in Table 3.

Table 3

Toxicity of malachite green oxalate (Fisher Scientific Company, New York) in mg/l on the fry of Ictalurus punctatus at 23°C (after CLEMENS and SNEED (4)).

Hours. *DL_{100}: lethal dose, with which 100% of the experiment animals die.

Tabelle 3

Toxizität von Malachitgrünoxalat (Fisher Scientific Company, New York) in mg/l gegenüber Setzlingen von *Ictalurus punctatus* bei 23° C (nach CLEMENS und SNEED [4])

Stunden	1	2	4	8	24	48	72	96
DL_{100}*	1,00	0,50	0,25	0,25	0,19	0,19	0,19	0,19
DL_{50}	0,79	0,31	0,20	0,17	0,14	0,14	0,14	0,14
DL_{0}	0,40	0,25	0,16	0,10	0,10	0,10	0,10	0,10

* DL_{100} : Letaldosis, bei der 100 % der Versuchstiere sterben.

Da die Wirksamkeit chemischer Substanzen in vielen Fällen von der Wasserqualität abhängig ist, haben die Autoren gleichzeitig eine Wasseranalyse durchgeführt und deren Befund mitgeteilt (Tab. 4).

As the action of chemical substances is in many cases dependent on the quality of the water, the authors at the same time conducted an analysis of the water, and given the results (Tab.4).

Table 4

The chemical composition of the water, in which the toxicity of malachite green for Ictalurus punctatus was determined (cf.Tab.3).

Tabelle 4

Die chemische Zusammensetzung des Wassers, in dem die Toxizität von Malachitgrün für *Ictalurus punctatus* festgestellt wurde (vgl. Tab. 3)

SiO_2 mg/l	Fe mg/l	Ca mg/l	Mg mg/l	Na mg/l	K mg/l	Karbonat ($CaCO_3$) mg/l	Bikarbonat ($CaCO_3$) mg/l	SO_4 mg/l	Cl mg/l	F mg/l	NO_3 mg/l	pH
12	0,02	4,0	2,8	127,0	1,1	16,0	310	15	4,8	0,1	1,4	8,7

HUBLOU (16) arbeitete mit Salmoniden, und zwar Setzlingen von *Salmo gairdneri* (Gewicht 0,5 g), *Oncorhynchus kisutch* (Gewicht 15,1 g) und *O. tsha*

HUBLOU (16) worked with Salmonidae, and also fry of Salmo gairdneri (weight 0.5 g), Oncorhynchus kisutch (weight 15.1 g) and O.tshawytscha (weight 11.4 g). He determined the mortality within 24 hours from a one-hour period of treatment in running water and at a temperature of 7.2°C (Tab.5).

Table 5

Mortality of Salmonidae fry after 1-hour action of malachite green and temperatures of 7.2°C (after HUBLOU (16)).

Concentration — Percentage mortality 24 hours after treatment.

Tabelle 5

Sterblichkeit von Salmonidensetzlingen bei 1stündiger Einwirkung von Malachitgrün und Temperaturen von 7,2° C (nach HUBLOU [16])

Konzentration (1 Std.)	Prozentuale Sterblichkeit 24 Stunden nach der Behandlung		
	S. gairdneri (0,5 g)	*O. kisutch* (15,1 g)	*O. tshawytscha* (11,4 g)
Kontrolle	2	0	0
Malachitgrün 6,7 mg/l	85	0	0
Malachitgrün 2,7 mg/l	10	0	0

The concentrations used were 1:150,000 (6.7 mg/l) and 1:375,000 (2.7 mg/l). As can be seen, the larger salmon fry withstood the action of the malachite green without losses, while 85 and 10% respectively of the rainbow trout fry perished. It should at any rate be noted here that these fish were strongly attacked with Trichodina. The author does not reveal what kind of a preparation was used.

AMLACHER (2) determined the action of malachite green on different species of fish. With these experiments the oxalate form was used (private communication). Ten different species of toy fish (Pterophyllum scalare, Aequidens maroni, Hyphessobrycon serpae, Brachydanio rerio, Lebistes reticulatus, Trichopsis spec., Gymnocoymbus ternetzi, Ambassis lala, Platypoecilus maculatus, Corydoras, spec.) underwent in general 0.1 mg/l over several days. Quite small increases of the concentration led to isolated losses. Rainbow trout fry held out for a longer time with 0.2 mg/l, higher values could be fatal. They could however have been tolerated by acclimatisation. The common) ? carp Einsommrige) (Cyprinus carpio) appears to withstand concentration up to o.9 mg/l for a certain time, 1.0 mg/l were fatal.

Similar results were attained with some pilot experiments, which had already been undertaken earlier with malachite green sulphate. Almost fully grown specimens of Lebistes reticulatus died with concentration of 1x and 2 mg/l at the latest after 10 hours. 0.2 mg/l were in part withstood for several days. Here at any rate the solution was not changed. Perch (Perca fluviatilis) 15 cm. long died after 3-4 hours in concentration of 1 and 2 mg/l. 0.5 mg/l were in some cases tolerated for almost 20 hours. Also solutions of 0.2 mg/l were still dangerous. Lower concentrations (0.1 mg/l and lower) were withstood by perch for several days. From other experiments it can be concluded that sticklebacks (Gasterosteusx aculeatus), Bitterlinge (?) (Rhodeus amarus) and groundlings (Gobio fluviatilis) also died after only 10-15 hours in these low concentrations.

In order to determine differences between the different forms of the malachite green, we tested an oxalate[1] and a sulphate. Difference in

1 KG chemical refinery, Sebnitz.

the action of preparations of various origins were observed, as has been mentioned, by DEUFEL (6).

One-year-old rainbow trout (Salmo gairdneri) of a total length of 10 to 15 cm. served as experimental fish. The temperature in the aerated aquaria amounted to 9-12°C. At least 4 fish were used for each test. As can be seen from figures 1 and 2, the dye solutions at the same concentration showed definite distinctions. The original solutions were prepared partly in normal mains water, partly in distilled water. We determined the working of the poison at once or 1-2 days after the start. The toxicity was further tested if the final dilutions were used once, i.e. if trout had already been kept in them for several days. The water for dilution was for the lost part mains water, in some cases however acidified water was also used (pH 6.5).

Fig. 1 Toxicity of malachite green sulphate and oxalate solutions of different ages at the preparation of the solution in mains water and distilled water and manufacture of the final dilution in lightly alkaline and acid (pH 6.5) aquarium water, concentration 1 mg/l. The black columns give the start and the finish of the mortality of Salmo gairdneri in the sulphate, the white ones in the oxalate.

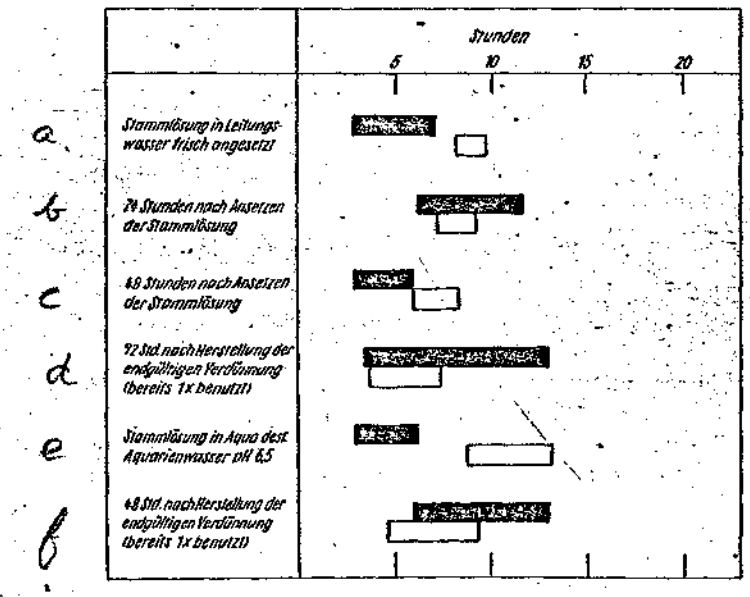


Abb. 1 Toxizität von Malachitgrünsulfat- und -oxalatlösungen verschiedenen Alters bei Ansetzen der Stammlösung in Leitungswasser und Aqua dest. und Herstellung der endgültigen Verdünnung in leicht alkalischem und angesäuertem (pH 6,5) Aquarienwasser. Konzentration 1 mg/l. Die schwarzen Säulen geben Beginn und Ende der Sterblichkeit von *Salmo gairdneri* im Sulfat, die weißen im Oxalat an.

a. Solution freshly added to mains water.
b. 24 hours after addition of solution.
c. 48 hours after addition of solution.
d. 72 hours after manufacture of the final dilution (already used 1x)
e. Solution in distilled water, qauarium water pH 6.5.
f. 48 hours after manufacture of final dilution (already used 1x).

In figure 1 the toxic action of 1 mg/l of malachite green is presented. With the trout kept in the oxalate solution, death set in mostly somewhat later. The oxalate as well as the sulphate did however effect the death of all the fish in less than 15 hours. Figure 2 shows the toxicity of 0.5 mg/l of malachite green. At the latest all trout perished after 2 days in the sulphate. In the oxalate solution there survived after 5 days 25-75%, in many cases even 100% of the fish. It was also shown in lower concentrations that the sulphate has a more toxic effect, which is certainly explained to a certain degree by the higher content of the dye component. Solutions of malachite green sulphate of 0.2 and 0.1 mg/l were still toxic for a large number of the trout, on the other hand only isolated losses appeared in the oxalate.

Following increased pH-values (8.1) the dye solutions were partially discoloured through the transformation of the malachite green into the carbinol form. It is noteworthy that by these experiments now also these discoloured solutions were still toxic, at any rate in a somewhat smaller amount than in the unchanged malachite green solutions. Consequently the action of the poison is not linked with the chinoidal (?) structure of the dye molecule.

Fig. 2 Toxicity of solutions of malachite green sulphate and oxalate of different ages at the addition of the solution to mains water and distilled water, and manufacture of the final dilution in lightly alkaline and acid (pH 6.5) aquarium water. Concentration 0.5 mg/l. The black columns give the start and finish of the mortality of Salmo gardneri in the sulphate, the white ones in the oxalate.

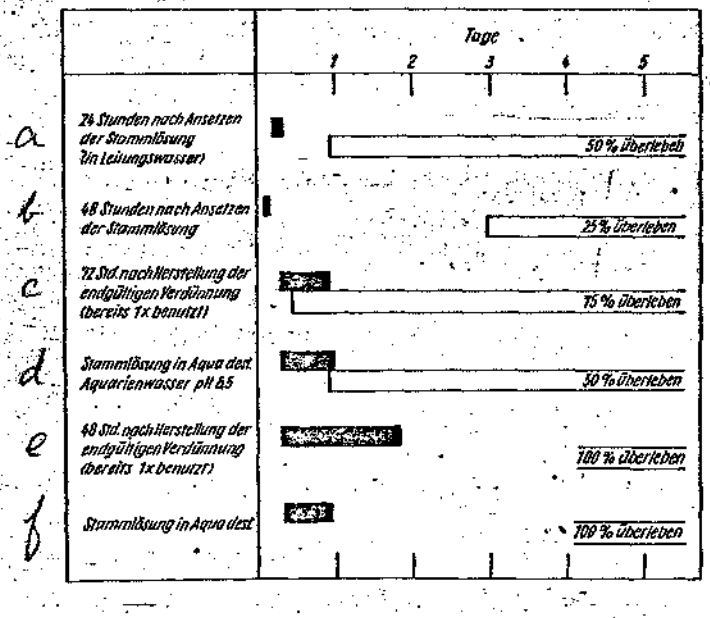


Abb. 2 Toxizität von Malachitgrünsulfat- und -oxalatlösungen verschiedenen Alters bei Ansetzen der Stammlösung in Leitungswasser und Aqua dest. und Herstellung der endgültigen Verdünnung in leicht alkalischem und angesäuertem (pH 6,5) Aquarienwasser. Konzentration 0,5 mg/l. Die schwarzen Säulen geben Beginn und Ende der Sterblichkeit von *Salmo gairdneri* im Sulfat, die weißen im Oxalat an.

a. 24 hours after addition of the solution (in mains water).

b. 48 hours after addition of the solution.

c. 72 hours after addition of the final dilution (already used 1x).

d. The solution in distilled water, qquarium pH 6.5.

e. 48 hours after the manufacture of the final dilution (already used 1x).

f. Solution in distilled water.

Combating the growth of fungus on fish with malachite green.

The growth of fungus on fish should always be of a secondary nature. The fish are attacked by Saprolegniaceae, when they are weakened, e.g. when skin or gills show evidence of injuries. Once the fungus threads have settled in the tissue of the fish, they can spread under suitable temperatures very quickly and accelerate the death of the animal. This is why the growth of fungus occurs frequently with fish which during capture have sustained injuries in the nets and have been kept for a long time. All previous attempts to combat this with common salt, copper sulphate or potassium permanganate could not be completely satisfactory (SCHAPERCLAUS (29)).

FOSTER and WOODBURY (8) were the first to investigate the fungoid action of malachite green on fish. ALLISON (1) quotes a verbal communication from L.E.WOLF, who contrary to general usage treated the fish constantly with the zinc salt of the dye and by this means always obtained good results. Attacks of Saprolegniaceae on fish could, according to O'DONELL (22) be successfully prevented on 18 species of fish (including Salmo gairdneri, S.trutta fario and Salvelinus fontinalis). He used a bath of 10 to 30 seconds and a solution of 1:15,000 (66 mg/l). According to ALLISON (1) a solution of 1:180,000 (5.5 mg/l) was in many cases used regularly in the treatment of fish in the tank. The period of the operation should take 45 minutes. CLEMENS and SNEED (4) consider possible the combating of Saprolegniaceae on Ictalurus punctatus with a solution of 1 mg/l, if the dye is again completely washed away after one hour.

The prevention of attack by saprolegnia can have considerable importance if it is a question of valuable fish spawn. In Poland the salmon (Salmo salar) and sea trout (Salmo trutta) can, following a river blockage, in some cases no longer reach the spawning places which lie beneath cherry-trees (?) in neighbouring rivers. They are thus captured at the start of the ascent at the beginning of September and kept

for several weeks (end of November to the middle of December) until they are ripe for spawning. Injuries during capture, transport and captivity and the comparatively high water temperatures led many times to strong fungoid growth and losses amongst the fish. SAKOWICZ and GOTTWALD (27, 28) therefore used malachite green to combat the Saprolegniaceae (cf. also GOTTWALD (15)). The spawn were kept in concrete holders of 20 to 30 sq m. in size and 0.5 - 0.6 m. deep. The concentration in the tank should amount to 1:200,000 (5 mg/l). After the necessary amount of the dye has been dissolved in a bucket full of water, the water level of the tank is lowered to half and the outflow shut off. The malachite green solution is then gradually poured into the inflowing water. When the water level again reaches a height of 50 cm, the inflow of water is also stopped. The whole bath should last for not more than 20 minutes. In these times however the change of water at the end must be taken into consideration. Shortly before the completion of the term the inflow and outflow are turned on, and there is a strong current going through. The spawn of salmon and sea trout withstand this treatment as a rule. 70-80% of the fish survive during the handling and become mature. The bath is repeated every 3 days. Pilot tests which were undertaken with younger individuals for other species of fish in aquaria yielded negative results. Only carp at puberty (Carassius carassius) tolerated the concentration which was given. All other fish died during or soon after the bath.

Combating external animal parasites of fish with malachite green.

HUBLOU (16) succeeded in effectively combating Trichodina with malachite green. He tested 7 different concentrations of 1:200,000 to 1:8000,000 (5 mg-1.25mg/l) and the same in a further experiment of 1:200,000 (5 mg/l), 1:400,000 (2.5 mg/l) and 1:8000,000 (1.25 mg/l) at 6.1°C, while he placed the fins of rainbow trout which had been attacked by parasites in 90 and 30 minutes respectively in petrischalen(?)

with the corresponding solutions, and then added fresh water. Tables 6 and 7 reproduce part of the results of his experiment.

Table 6

Activity of Trichodina on fins after 90.minute treatment with malachite green (after HUBLOU (16)).

Treatment. Activity of the parasite during the treatment. Percentage of those surviving after 90 minutes' treatment and 60 minutes in clean water.

A = active, M = dull, O = without movement, only the cilia beating, T = dead.

Tabelle 6

Aktivität von *Trichodina* auf Flossen nach 90minutiger Behandlung mit Malachitgrün (nach HUBLOU [16])*

Behandlung	Aktivität der Parasiten während der Behandlung Minuten									Prozentsatz der Überlebenden nach 90minutiger Behandlung und 60 Minuten in reinem Wasser
	10	20	30	40	50	60	70	80	90	
Reines Wasser (Kontrolle)	A	A	A	A	A	A	A	A	A	100
Malachitgrün 1 : 200 000	A	A	M	O	O	O	O	T	T	0
Malachitgrün 1 : 300 000	A	A	M	M	O	O	O	T	T	0
Malachitgrün 1 : 400 000	A	A	M	M	O	O	O	T	T	0
Malachitgrün 1 : 500 000	A	A	M	M	O	O	O	T	T	0
Malachitgrün 1 : 600 000	A	A	M	M	O	O	O	T	T	0
Malachitgrün 1 : 700 000	A	A	M	M	O	O	O	T	T	0
Malachitgrün 1 : 800 000	A	A	A	M	M	O	O	O	T	0

* A = aktiv; M = matt; O = ohne Bewegung, Cilien schlagen noch; T = tot.

Table 7

Activity of Trichodina on fins after 30-minutes treatment with malachite green. (After HUBLOU (16)).

Treatment. Activity of the parasites during the treatment. Percentage of those surviving after 30 minutes' treatment and 60 minutes in clean water.

Tabelle 7

Aktivität von *Trichodina* auf Flossen nach 30minutiger Behandlung mit Malachitgrün (nach HUBLOU [16])*

Behandlung	Aktivität der Parasiten während der Behandlung Minuten			Prozentsatz der Überlebenden nach 30minutiger Behandlung und 60 Minuten in reinem Wasser
	10	20	30	
Reines Wasser (Kontrolle)	A	A	A	100
Malachitgrün 1 : 200 000	A	A	M	0
Malachitgrün 1 : 400 000	A	A	M	0
Malachitgrün 1 : 800 000	A	A	M	5

* A = aktiv; M = matt.

In the covered tank a concentration of almost 1.25 mg/l was produced without a drop in the water level. The treatment lasted one hour. Only small losses appeared on the rainbow trout which had been attacked by Trichodina. The parasites died off.

DEUFEL (7) recommended malachite green for the destruction of Ichthyophthirius on trout. If we count on a tank depth of around 1 m., according to him concentration of 0.1 mg/l are required for combating. The dye should be delivered two or three times in intervals of two days. The positive results which could have been gained by this found repeated confirmation in practice (cf. eg. GOTTBEHUT (13) and ROHDE (26), if also the greatest significance is ascribed from these groups, partly in error, to the water being made turbid by the dye. With similar concentrations as given above, (DEUFEL (7)), GOLLUB (12) was able to bring about a subsidence in Ichthyophthirius diseases in two trout hatcheries, which appeared in July, by treatment in the tank upon one or two occasions. To this end the tanks, which had been slightly run off before the addition of malachite green, were left for two hours without a current. DEUFEL (7) succeeded in addition in further effectively combating Costia, Trichodina and Glossatella with malachite green.

On the other hand RASMUSSEN (23) had no success, when he used malachite green against Costia on trout. He used a short bath lasting one minuted and a concentration of 24 mg/l of malachite green.

AMLACHER'S (2) experiments could confirm the results of DEUFEL (7). He treated trout, which had been attacked by Ichthyophthirius and Trichodium, for several days in a stagnant solution (oxalate combination) and determined that concentrations of 0.15 mg/l bring about a detachment of the parasites after 96 hours. With the death of Ichthyophthirius it can be calculated after 144 hours. Trichodina was not found on the fish after 24 hours with 0.35 mg/l. Here also 0.15 mg/l of malachite green should probably already be sufficient.

X ~~Other experiments with malachite green~~

Other experiments with malachite green

According to American statements, thin-shelled eggs appear in many plants, leading then to losses through premature hatching. In two brief notices RAYNER (24) and TANNER and others (33) report on their experiments, ~~in which RAYNER (24) and TANNER and others (33) report on their experiments~~, in which they compare gentian violet and malachite green as a means against the appearance of this disease. Definite results were not attained.

TACK (32) tested malachite green with regard to its effect on the cause of infectious kidney swelling and degeneration of the liver. It was shown here that the substance had no influence on the course of the disease.

According to some observations different creatures close to fish (Cladocera and Copepoda) died at the latest after several hours, when exposed to concentration of malachite green of 2/mg/l. On the other hand larva of Culicidae were still alive after 10 hours. AMLACHER (2) found that concentrations of up to 0.6 mg/l were tolerated by Daphnidae for several days. Also the dye was not harmful to snails at 0.3 mg/l.

In order to determine the effect of malachite green on algae, several aquarium experiments were set up in the months of June and July. The tests were concerned with colonies of algae which had been taken from ponds and on the whole consisted of Oedogonium and Mougeotia. In the first series we allowed concentrations of 1, 2, 5 and 10 mg/l act on the plants for 24 hours. Afterwards the solutions were drained off and the algae subsequently kept in clean water. In the second series concentrations of 1 and 2 mg/l were used for a period of action of 72 hours. The third series was distinguised from the above-mentioned by shorter periods of treatment. Here the algae were esposed to concentrations of 1, 2 and 5 mg/l for one hour on 6 successive days. As control there served untreated aquaria with the same algae colonies, which otherwise remained under the same conditions. The temperature varied in the course of the day a great deal and reached 9-30°C. The observations took place over 2-3 weeks. Next a strong depression of the algae had to be established in 10 mg/l - basins. They lay at the bottom and did not assimilate. At the end

of the experiments the algae masses were here fairly negligible, and damage was quite obvious. In all other aquaria in which the algae had been treated for 1 and 3 days with concentrations of 1, 2 and 5mg/l, only insignificant differences were discovered compared with the control basin. The algae which had been exposed to the malachite green for one hour on 6 successive days showed light depressions compared with the control, but nevertheless assimilated. A notable increase of the algae mass, however, appeared only on the untreated plants. Rapid destruction of the algae could not be attained in any case, whereby naturally the possibility lies open, that an action with stronger concentrations and longer-lasting could perhaps prevent a yield of larger algae colonies.

Lysimachia mummularia and Hydrocharis morsus ranae showed, according to AMLACHER (2), no damage in any case with concentrations of 0.3 mg/l and an action of 10 days.

Damage through malachite green.

For exhaustive and very interesting investigations on damage through malachite green on rats we are grateful to WERTH (35, 36). In an animal organism the dye produced anoxy in the tissues; rats succumb with the typical symptoms of anoxia, if they are injected i.v. (= intravenously?) with DL_{50} of 3 to 3.5 mg of the dye per 100 g. of body weight. All symptoms and also death can be avoided, if the breath enzyme stimulator (? Atmungsferment) Cytochrom c is delivered in time, likewise i.v. Here as a rule for 1 mg of malachite green 2 mg of Cytochron c are necessary. The experiments of the above-named author covered several years and were practised on rats inbred with an albino strain, which had already been held for some time under conditions of the institute and by which no characteristics of disease and especially no kind of tumour had appeared. The volume of the work shows that up to 2500 rats were held and supervised at the same time. The females of one experimental group received intermittent oral doses of malachite green. The daily dose per animal lay under 1 mg (about 0.5-0.9 mg). This treatment had no recognisable damage as a consequence.

The rats' cycle, their reactions as well as their eagerness for feeding and playing remained normal. Various disturbances were shown in the untreated descendants of this parent generation, which extended to the eighth or ninth filial generation, according to the record of the work. All the damage reproduced here affects only the following generations of the rats which had been fed with malachite green. The descendants of the control animals (and also the animals which had been receiving malachite green together with cytochrom c) remained without data.

Next there appeared in the (untreated) F_1 -generation completely unexpected alterations of the shape of the head. There were further shown the same irregularities in the electrocardiogram, which had been caused directly through toxic or protracted intermittent doses. A deficiency of cytochrom c was chemically determined in the tissue of the heart muscle. With all these phenomena it is a question of disturbances in the inheritied structure, which are detectable in the entire breed of the 7th to 9th generation. To this there then comes a further group of symptoms, which in a varying manner were observed now in one, now in another family. Thus there were found differences in the size of the eyes, partial Protrusio bulbi with cataract, temporally differing eye openings and weakening of the eyes. Defects in the lungs could be established especially frequently in the second to fourth and fifth generations. From the third generation stunted and dwarf forms appeared. Characteristically this was noticeable first in the second week of life to a halt in development. "Proportioned dwarfs without other damage" and "dwarfs with eye damage, pointed skulls, bone anomalies. etc." can be distinguished. Moreover damage to the hide attracted attention in the third generation, comprising all transitions up to complete loss of hair. In other generations appeared bone defects constantly. Damage to the teeth was also not infrequent. Finally deformities on the extremities (absence of a hind leg, double hind leg) and damage to the organs were determined. Earlier experiments had shown that malachite green has no carcinogenic effect in experiments of a year's duration. After $1\frac{1}{2}$ years there now appeared the first tumours on the untreated filial generations. Tumours of the lungs, ovaries and breasts, as well as growths on the kidneys were especially frequent.

Certainly malignant growths are present amongst other kinds of tumours, which, as it was possible to show in the case of some breast tumours, are infectious. WERTH's experiments have here, as is stressed, revealed a novel mechanism for the development of inherited damage and tumours. It is possible with the help of malachite green to cause damage in the parent generation, which is first manifested in the untreated filial generations.

KEYL and WERTH (19) treated larvae of Chironomus thummi with a solution of malachite green, whose concentration amounted to 2×10^{-6} g/ml (= mg/l). Larvae which at 24 hours old had been treated for 7 hours with the dye solution showed at the pre-pupal stage structural alterations to the salivary gland chromosomes, which were shown in disturbances in development, fractures and recombinations of breaks. The cells and nuclei of a salivary gland could be afflicted quite differently with it. No alterations were noticed on newly-hatched larvae and embryos. The dye solution had a toxic effect on older larvae.

The occurrences of fracture which were encountered on the chromosomes of salivary glands were of the same type as those which could be brought about by X-raying larvae of 24 hours old. KEYL and WERTH (19) draw the conclusion from comparing with the results on X-ray treatment, that the partial fractures during or immediately after the treatment with malachite green take place, and are to be regarded, as genuine mutations. As specific structural alterations induced through the treatment with malachite green there appears frequently severe entanglements amongst the chromosomes of the salivary glands, which can be interpreted as a special kind of disturbance to development.

All these facts occasioned us to test the malachite green effect on fish eggs and fish cytologically. The results can be summarised as follows:

1. Eggs of the rainbow trout (Salmo gardneri) which had been treated in mains water with malachite green either daily for one hour with a weak solution (1 mg/l) or for one minute for each of five days

with a stronger solution (500 mg/1), showed disturbances in mitosis soon after the start of the treatment (in the early dome-stage of the development of the embryo).

The kind of malachite green used (oxalate or sulphate) appeared to be unimportant. What is important, however, is that the use of the strong solution led to a more rapid and larger number of eggs being disturbed, than the weak solution, with which damage was somewhat later in appearing and affected the eggs more disproportionately (the first disturbances in mitosis with 500 mg/1 after twice repeated, with 1 mg/1 after 5 to 6 times repeated treatment in the previously-mentioned way).

2. With fish - tests were carried out on the species which are especially suited to cytological research, roach (Rutilus rutilus), groundling (Gobio fluviatilis), Bitterling (?) (Rhodeus ararus), stickleback (Gasterosteus aculeatus) - under the influence of a solution of malachite green (oxalate or sulphate) of concentration 0.1 mg/1, which can be used as a means of combating external parasites, there su succumbed at the latest after five hours all cell divisions in the reproductive tissue at the roots of the tail fins. The picture of the disturbances (the same moreover underneath) was the same as with the trout eggs which had been treated, except that the scale of the disturbances was unequally larger. Probably the eggshell prevents the malachite green from penetrating further, so that on treating the eggs smaller concentrations act on the embryo cells than on the cells of treated fish whose reproductive tissue had been immersed in malachite green solution.

3. The cytological disturbance syndrome of malachite green comprises fractures in the chromosomes and their consequences (formation of di-central (?) chromosomes, breakages in the anaphase, akinetic fragments, eliminations etc.) as well as - especially as soon as

stronger concentrations become effective - intense "stickiness" phenomena, hence the chromatin substance becoming viscous, when as a result of this very severe disturbances in division appear. While with trout eggs the breakage and recombination occurrences seem to be prominent (Figs. 3-8), and stronger stickiness is outstanding only by a relatively small number of cell divisions (Figs. 9 and 10), it is the most important disturbance of the mitoses in the reproductive tissues of the fish resulting from the effect of malachite green.

The viscosity of the chromatin is so strongly pronounced here, that frequently complete inability to divide results amongst the chromosomes, and these sustain more or fewer losses with striking phenomena of swelling in their shape, and join up into chromatin complexes (lumps, strings, and other forms). In the anaphases the chromatin complexes which correspond to the daughter sheets are mostly unequally large and joined to each other with massive links, (Figs. 11 and 12).

Because of the treatment the cells of the reproductive tissue practically lose the capacity for further division. It is remarkable that the tissuesdoes not however become worn away or quiescent (? nekrotisch), but is preserved. A check-up carried out after 25 days on the treated fish showed that almost no cell divisions were encountered (ie. only very few mitoses and quite isolated, very strongly disturbed, mitoses), and that the tissue persists in a condition where it is incapable of division, in which it is displaced by treatment with malachite green. There is nothing more to be said at this moment in the record, about the further fate of the reproductive tissue and about the question, when and in what way the regenerative processes recommence and carry on. Perhaps the regeneration (in case it can generally still take place) of deep-lying cell parts of the stem of the tail, which perhaps were not exposed to the action of malachite green, can still take place.

WERTH is inclined to see the causes of the genetic damage in rats after the administering of malachite green in the anoxia which arises from the blockage of the breathing enzyme cytochrome c. Obviously anoxia is represented as a cause of damage of quite prominent significance; the prevention of damage to treated rats and the issue which have been similarly afflicted through treatment with cytochrome c, shows sufficiently that the teratogenic and carcinogenic effect of malachite green takes place with damage to the respiratory enzyme system. According to our opinion, however, there exist yet other possibilities to explain the injuries. We draw this conclusion from having established that hypoxia experiments, which have been carried out on fish eggs (LIEDER (20)), led primarily to disturbances in the helical mechanism of the mitosis. This effect, however, did not emerge in a particularly striking quantity in the experiments with malachite green. Conversely, the enormous stickiness of the chromatin, on which in the first place the cytostatic effect of the malachite green appears to be based, is not encountered on experimental hypoxia.

Both disturbance syndromes, therefore, differ from each other so fundamentally, that we believe it is necessary to take into consideration yet another mechanical effect of malachite green. With reference to this is given the conclusion of KEUL and WERTH (19), that the accumulation of malachite green in nucleic acid (without both being fluorescent) gives a deep red, fluorescent combination. Stickiness of the chromosomes is today interpreted as the result of depolymerisation processes in the DNA. It seems to us from the results we have, that the action of malachite green on the cell nucleus very probably leads to chemical alterations of the substance of the chromosomes, and that above all through this action there is accomplished severe damage to mitosis.[2] Further extensive experiments

[2] H.H.Pfeiffer (Exper. Cell res. 22, 1961), likewise brings into consideration the "chromosome poisoning " through malachite green, with its chemical affinity with DNA.

were necessary to a definite clarification of this question. It now, however, appears sensible to us not merely to consider summarily the damaging effects on the organism, or to subdivide into direct and secondary effects (WERTH), but to take into consideration whether or not the damage through malachite green is brought about through several different mechanisms, partly predominantly carcinogenic, partly mutagenic.

MAZURANICH and NIELSON (21) report on the possible damage to fish through malachite green. According to their statements the disease of white spots appeared particularly severe after treatment with malachite green. Yjey therefore recommend, as has been mentioned previously, that the treatment be halted (5 mg/l one hour for two days) on the hatching of a breed of salmon. Their conclusions are remarkable insofar as in many cases the brood was also exposed to malachite green, without any negative result being noticed. The white spots disease, which can also be designated as a coagulation of the yol, can appear on the spawn of the most divergent species of Salmonidae. The first symptoms, small white spots in the yol sac, show themselves some days after hatching. The coagulation can increase with the growth of the young fish. Under circumstances there are high losses, and the resorption of the yolk sac does not make any progress. The white spots disease can naturally appear in a dangerous quantity also somewhere where malachite green has still not been applied. That was, for example, the case on the incubation of the eggs of sea trout (Salmo trutta) in January 1960, which could be controlled by us. Here the mortality was exceptionally high, less that 1% of the hatched spawn grew up into fry. The white spots disease could, moreover, be observed by us in rainbow trout in a trout hatchery, in which only a few incubators had been supplied with malachite green. Diseased spawn was available in treated as well as untreated plants. Differences in the frequency were not established. The conclusions of MAZURANICH and NIELSON should still also find attention in the future.

Discussion and conclusions.

If we finally survey the previous operations, we can establish that malachite green has proportionately spread very rapidly in fishery practice. Its effect on Saprolegiaceae and the most divergent external parasites is plain, and the application quite simple, as small variations in concentration or temporary infringements do not influence the results of the treatment. In addition Ichthyophthirius multifiliis, which as is know parasiteses not on the skin but in it, can definitely be combated with the dye, and indeed also in tanks.

Special mention is needed in this connection of the obviously detrimental influences of malachite green on the trout stock itself in a fish hatchery, which hitherto have not been observed. Here the entire eggs and also the freshly-hatched spawn have been in contact with the dye in the above-mentioned manner for no more than 6 years.

The exhaustive damage that the malachite green caused, entitled us however to the question of whether the use of this combination in pond management and pisciculture is on the whole advisable. In a discussion of this problem we must consider separately two subjects, first the possible dangers for humans, next the eventual consequences for the fish stock.

The employees of the fish hatchery plant whose duty it is to provide the eggs regularly with solutions of malachite green, and who under the circumstances administer the solutions themselves, are the first who are exposed to the danger. An express caution in relation to the substance could already here afford a definite protection. Otherwise the situation arises where fish treated with malachite green come into the market and are consumed. The amounts which are thus absorbed could however be so negligible as to escape attention. Furthermore (WERTH, in a letter) it appears that malachite green bound in egg-white has on the whole no effects. If the dye is mixed with food for rats and mice, dried and then delivered in a mould (? Pressling) in the usual manner, there appears no damage in the

animals. It could also well explain the unobjectionableness of malachite green bound in egg-white, that according to FOTT (9) that in Asiatic lands (Japan, China) the laminates serving as food are dyed in boiling water with malachite green on account of its improved appearance in the fabric after drying. Nevertheless, however, treatment of fish which are meant for food, and later coming up for sale, should be avoided on the grounds of safety.

The results attained by us on fish eggs and fish also bring us to consider carefully the dangers which are threatening in this regard. If also, as mentioned, outwardly recognisable damage on trout can itself after a year's treatment of the eggs not be established, then a final decision can still by no means be taken. WERTH (noted in a letter)[3] considers doses which lie under 1/5 - 1/8 of the DL_{50} relatively safe, and those of $\frac{1}{3}$ critical. With the exalate of malachite green this vale (DL_{50}) appears to lie at about o.5 mg/l for the fry of rainbow trout, (cf. Fig.2). Consequently, even o.2 mg/l, as is recommended and used for combating parasites, would not be objectionable if applied again. In the reproductive test solutions of o.1 mg/l showed prominent cytostaic effects; disturbances in cell division must therefore also be assumed in still weaker concentrations. Theoretically it could be thought from this that eggs from which later fish for breeding are to be raised should be incubated in the normal manner, while the amounts of eggs designated for the production of fish for eating should eventually be treated with malachite green subject to the necessary caution. Such a procedure, however, would in practise encounter great difficulties. There is moreover the consideration that the danger of misuse is constantly there, so that the use of the dye must remain a problem according to the present state of knowledge.

[3]Our most sincere thanks may be expressed here for the wife of Dr WERTH for her valuable references.

Illustrations. [not reproduced]

3. Rainbow trout; undisturbed embryonic post-metaphase.
4. Rainbow trout; undisturbed embryonic anaphase, the daughter elements lie in different levels of contrast and are excluded somewhat obliquely.
5. Rainbow trout; undisturbed embryonic telophase.
6. Rainbow trout; embryonic mitosis with numerous fragments, adhesions and very obvious disturbances in orientation of the chromosomes; after treatment with malachite green oxalate, 1 mg/l.
7. Rainbow trout; embryonic mitosis with numerous fractures in the anaphase; after treatment with malachite green oxalate, 1 mg/l.
8. Rainbow trout; embryonic mitosis with elimination and formation of links; the chromatin substance shows striking deformities. Treatment with malachite green oxalate, 1 mg/l.
9. Rainbow trout: embryonic mitosis with formation of links and strong deformity of the chromatin substance. Treatment with malachite green oxalate, 1 mg/l.
10. Rainbow trout; embryonic mitosis with very strong formation of links following stickiness and warping of the chromatin. Treatment w 500 mg/l of malachite green oxalate.
11. Roach; regeneration mitosis with strong formation of links, unequal division of the chromatin substance on both daughter elements and very strong evidence of deformity. Treatment with 0.1 mg/l of malachite green oxalate.
12. Roach; very strongly disturbed regeneration mitosis with unequal division of the chromatin substance and massive formation of links. The chromosomes have joined up inot almost homogenous complexes. Treatment of the fish with 0.1 mg/l of malachite green oxalate.

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Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.