Irritant Effect of Disinfection Byproducts in Swimming Pool Water

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Abstract [original English]

Compounds which can occur as disinfection by-products (DBP's) in swimming pool water were examined for their mucous membrane irritating potential. Previous studies using the rabbit eye test (Draizé [1] test) have shown that the irritating potential of typical concentrations of free and combined chlorine are insufficient to explain the degree of eye irritation that can result from exposure to swimming pool water. Other DBP's which may be responsible for eye irritation include halogenated carboxyl compounds (HCC's) which act as precursors during the formation of chloroform. In this study, a modified HET-CAM Test (Hens Egg Test at the Chorion Allantois Membrane) has been used to investigate the mucous membrane irritating effects of HCC's. Some of the compounds tested were found to have a significantly increased irritating effect when compared to a chlorine/chloramine mixture of the same concentration, several mixtures of HCC's where [sic] even more active at lower concentrations than single compounds. However, the irritating effects of individual compounds as well as of mixtures of HCC'S were not sufficiently intense to allow the identification of those compounds specifically responsible for the overall observed increase in irritation. HCC's were therefore tested in the presence of aqueous chlorine solution. When combined with aqueous chlorine, a number of compounds exhibited significantly enhanced effects. Our results show that the eye irritating effects of low concentrations of DBP's can be investigated using a modified HET-CAM assay. Moreover, results obtained using this assay suggest that the mucous membrane irritating potential of swimming pool water is a consequence of the effects and synergistic action of a number of DBP's in the presence of chlorine. Further work should be carried out in order to establish an indicator for eye irritating effects of swimming pool water.

Abstract [translated from German]

The mucous membrane irritation potential was studied for substances that can appear as disinfection byproducts in swimming pool water disinfected with chlorine. Previous studies conducted using the Draize rabbit eye test show that the irritant effect of free and combined chlorine is too low to explain the eye irritation occurring in swimming pools. Possible sources of the irritation are α-halogenated carbonyl compounds, which can appear in swimming pool water as precursors of chloroform. A modified HET-CAM test (*H*en's *E*gg *T*est on the *C*horio*a*llantoic

[1] Translator's Note: The name is generally written as "Draize" in English, not "Draizé". John Henry Draize was an American born in Wisconsin. His father was Belgian, so perhaps Europeans sometimes assume a possible original accent that was dropped here in the US by the first generation. The translation uses "Draize" everywhere except when reproducing the original English abstract and English references by German authors.

*M*embrane) was used to study the irritant effect of such compounds. The exposure time for the compounds was one hour. The test was conducted in an incubator at 37°C. Some of the studied halogenated organic compounds exhibited an irritant effect that clearly was greater than the effect of a chlorine/chloramine mixture of the same concentration. But the irritant effect of the individual compounds is not so great that a particular compound of those studied so far could be identified as responsible by itself. So the effect of the individual compounds was tested in the presence of chlorine. A more severe irritant effect was exhibited in this case than would have to occur from the sum of the individual effects. Therefore it was concluded that the modified HET-CAM test can be used to study the irritant effect of substances in low concentrations. The precursors of certain disinfection byproducts appearing in swimming pool water exhibit an effect in the HET-CAM test in lower concentrations than for free and combined chlorine. Therefore according to the results available so far, the irritant effect is attributed to a synergistic effect of a number of disinfection byproducts contained in the water together with the effect of chlorine. The studies should be continued with the aim of determining a practical indicator parameter for the irritant effect.

Introduction

Chlorine is used in swimming pools as a disinfectant and, because of its reactivity, is able to rapidly and effectively kill microorganisms. Its reactivity is a disadvantage, in view of the fact that it can enter into secondary reactions with organic matter contained in the water. The best known substance that can be formed in such side reactions is chloroform, which in the past has been increasingly the subject of public debate. But little attention has been paid to a problem that affects nearly every user of (public) swimming pools: the irritant effect that the water or certain constituents of the water have on the eyes and sometimes the skin of the bathers.

The eye irritation experienced by bathers in swimming pools has been generally attributed to chlorine concentrations in the water that are too high. However, it has been shown that chlorine by itself cannot be considered solely responsible for the eye irritation. At the same time, not much is known about skin irritation in swimming pools.

However, personal experience and reports from Heidelberg University Dermatology Clinic confirm that skin irritation can also occur after visiting swimming pools. Such irritation can become apparent even up to several hours after visiting the pool, as itching and slight reddening of the skin. Occasionally formation of pustules or blisters can be observed The term "chlorine cold" applies to a phenomenon that can occur when swimming underwater in chlorinated indoor or outdoor pools, where swelling of the mucous membranes can occur along with cold-like symptoms. But the reasons for this phenomenon are not yet known.

Regarding eye irritation, studies by *Mood* [12] back in 1951 showed a connection between the irritant effect of chlorine and the pH value. Later *Eichelsdörfer* [7] was able to show that chloramines have a much stronger irritant effect than chlorine by itself. For chlorine, the irritant effect began at a concentration of about 8 mg/L; chloramines exhibited a corresponding effect at much lower concentrations, starting at about 2 mg/L. In both cases, the visible effects on sensitive rabbit conjunctiva were described as an "uncertain reaction". Nevertheless, these statements were interpreted (even in specialist circles) to mean that combined chlorine is the actual species responsible for the irritant effect, even though this did not emerge in this form from any of the available study results.

Figure 1 shows the average chlorine concentrations occurring in Heidelberg swimming pools (with no ozone stage) in the year 1993. All the values were collected during routine tests by our laboratory.

The median value for free chlorine was 0.53 mg/L; for combined chlorine, 0.10 mg/L. These concentrations are far below the threshold concentration for eye irritation established in the Draize test, both for free and combined chlorine.

Occasionally during pool operation, even higher concentrations can be reached. Even so, it is unlikely that the observed eye irritation is caused by free or combined chlorine alone, since even in this case the concentrations for both free and combined chlorine are well below the irritation threshold established in the Draize test.

But the explanation of the irritant effect might come from other compounds and classes of substances that can be formed as water disinfection byproducts. Based on one of the possible chemical mechanisms for chloroform formation, we might therefore expect that α-haloketo compounds are present in the water as intermediates (that is, before formation of chloroform itself occurs).

It has been known for a long time from toxicology that compounds with a chlorine atom in an α position relative to a double bond can have an irritant effect.

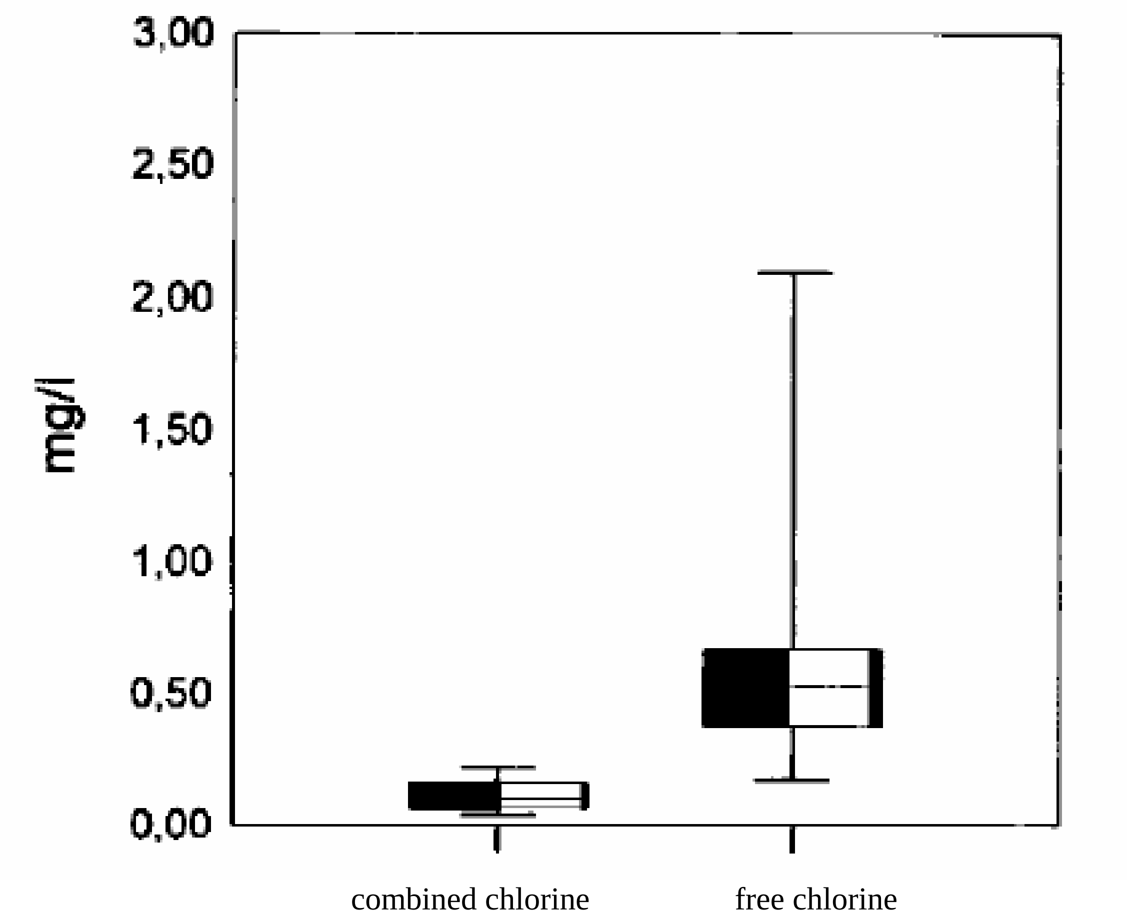

Fig. 1. Average concentration of free and combined chlorine in pools.

So our goal was to determine the effect threshold for certain compounds in aqueous solution. In this study, we therefore aimed to test compounds that can be formed by chlorination of swimming pool water, and that because of their chemical structure can contribute to the observed effects.

Materials and methods

Chemicals used

Suppliers and purities of the tested halogenated organic compounds are given in Table 1.

Table 1. Suppliers and purities of the tested compounds

Substance	Supplier	Purity
Chloroacetone	Aldrich	95%
1,1-dichloroacetone	Aldrich	98%
Tribromoacetic acid	Aldrich	99%
Chloroacetic acid	Riedel-de Haën	99%; Pestanal® standard
Dichloroacetic acid	Riedel-de Haën	99%; Pestanal® standard
2,2-Dichloropropionic acid	Aldrich	90%; technical grade
2,2-Dichloropropionic acid (sodium salt)	Aldrich	94%
1,1,1-Trichloroacetone	Aldrich	97%
ω-Chloroacetophenone	Aldrich	99%
Dibromoacetonitrile	Aldrich	95%
Chloroacetonitrile	Aldrich	99%
Dichloroacetonitrile	Aldrich	98%
Chloral hydrate	Riedel-de Haën	99.8%; DAB (German Pharmacopoeia) grade
1,3-Dichloroacetone	Aldrich	95+%
1,1,3-Trichloroacetone	Aldrich	85%; technical grade
Trichloroacetonitrile	Aldrich	98%
Dibromoacetic acid	Aldrich	90% (remainder is bromoacetic acid)
Chloropicrin	Riedel-de Haën	99%; Pestanal® standard
Trichloroacetic acid	Riedel-de Haën	99%; Pestanal® standard
Bromoacetic acid	Aldrich	99+%

Establishing a method for determination of the irritant effect of a chemical substance in low concentrations

HET-CAM test: The most commonly used model for testing eye irritant effects is the "Draize test", which is preformed (*in vivo*) on rabbit eyes. This test was ruled out for studying the problem addressed here, mainly on ethical grounds.

For some time methods have been available that have been increasingly discussed as alternatives to the Draize test, or by now are even in use [1, 13]. In principle, several suitable *in vitro* methods can be used, but the standard protocols for such methods possibly must be adapted in each case to the special problems arising in testing compounds present in low concentrations. For the studies conducted here, the HET-CAM test (*H*en's *E*gg *T*est or *H*en's *E*gg *T*est on the *C*horio*a*llantoic *M*embrane) was used.

The HET-CAM test is regarded as a particularly promising alternative [10] to the Draize test, even though in a recently published study it was shown that (at least in the cosmetics industry) so far it does not sufficiently correlate with the Draize test [17]. This test is also used outside the cosmetics industry for various purposes [19].

The hen's egg allantoic membrane used for the test is a tissue with the consistency of mucous membrane and well supplied with blood vessels, permitting gas exchange through the egg shell during the chick embryo stage of development.

For the problem to be studied in our case, the following procedure was developed for testing low-concentration solutions.

Eggs and incubation: Fertilized White Leghorn eggs were obtained from the LSL company, Schaafheim. Incubation was carried out in an incubator with a small motor and automatic turning device (Bruja Brutmaschinen, Model 168/EM, Janaschütz, Hammelburg) under standardized conditions of 37°C and 65% relative air humidity. The day on which the fertile eggs were placed in the incubator was counted as Day 0. On Day 9, the eggs were used for the test.

Preparation for test: The margin of the air cell, located above the CAM, was sawed at a distance of about 2 mm from the mucous membrane. Just above the membrane, a small hole was drilled in the shell, through which the test substance can flow directly onto the CAM using a pipet tip inserted through the opening. Immediately before the start of the test, the egg membrane was carefully removed with tweezers, The test substances were predissolved in DMSO. In the concentrations used, DMSO had no effect and was used as a negative control. The dilution series was first prepared with twice-distilled water at room temperature. The different concentrations of test substances were prepared fresh on each test day.

Performing the test: All tests were carried out in the incubator at 37°C. This let us prolong the test time to one hour without the eggs cooling off and the blood flow through the membrane decreasing.

The test substances were placed into infusion bottles and carefully directed onto the CAM with a drip rate of about 1.25 mL/minute, in order to avoid mechanical irritation.

Each test was done in parallel on three hen's eggs. For the lowest dilution step used, the concentration actually reaching the CAM at the outlet of the drip system was tested by gas chromatography (results not shown).

Assessment of HET-CAM test results: The CAM was assessed after a test time of 60 minutes.

The following factors were rated:

1. Hyperemia (vasodilation)
2. Lysis (vessels no longer macroscopically visible)
3. Hemorrhage (discharge of blood from the vessels) or hyperemic reactions, which are apparent as injection or more delineated capillaries
4. Protein coagulation
5. Blood coagulation (thrombosis or clotting)

Most often lysis occurs, followed by protein coagulation. The reactions were divided into the categories mild, moderate, and severe irritant effect according to a subjective scoring scale: "Mild irritant effect" indicated a substance concentration that caused only slight changes such as, for example, slight hyperemia. "Moderate irritant effect" means a substance concentration that in addition occasionally exhibited more severe reactions such as hemorrhage, lysis, or coagulation. "Severe irritant effect" means that one or more of these reactions were generalized.

Results

Chlorine, hypochlorites, and combined chlorine

Chlorine disproportionates in aqueous solution to form chloride (Cl^-) and hypochlorite (OCl^-). The hypochlorous acid fraction is called "free chlorine". The various chloramines are collectively described by the generic term "combined chlorine". These chloramines are formed from reaction of chlorine with nitrogen-containing organic and inorganic compounds. The range of products is therefore strongly pH-dependent, where under the conditions normally prevailing in swimming pools we see almost exclusively monochloramine.

The monochloramine solutions were prepared by slowly adding dilute chlorine water to an ammonium chloride solution, in the mole ratios chlorine:nitrogen = 1:2, 1:10, and 1:50. The solutions were neutralized before performing the HET-CAM test, in order to rule out reactions caused by a change in pH. Before each experiment, the concentration of combined and free chlorine was determined by the DPD method. Table 2 shows the test results for the chlorine/chloramine mixtures.

Table 2. Irritant effect of mixtures of free and combined chlorine

Cl:N	free Cl (mg/L)	combined Cl (mg/L)	Hemorrhage	Lysis	Coagulation	
					Blood	Protein
1:2	9.8	6.3	—	++	—	++
	4.9	3.15	—	+	—	+
	2.0	1.26	—	+	—	+
1.:10	3.0	12	—	+	—	+
	1.5	6	—	—	—	+
	0.6	2.4	—	—	—	—
1:50	1.5	7.8	—	+	—	+
	0.75	3.9	—	+	—	+
	0.3	1.5	—	—	—	—
	0.18	0.72	—	—	—	—

Because an equilibrium is established between the free and the combined chlorine, it is not possible to prepare solutions that contain no free chlorine. By varying the mole ratio between chlorine and nitrogen in preparation of the test solutions, however, the equilibrium can be shifted in favor of free or combined chlorine. Note that in the presence of 2 mg free chlorine per liter, a concentration of 1.26 mg/L combined chlorine is still sufficient to have an effect on the CAM, while this is no longer the case for 1.5 mg combined chlorine and 0.3 mg free chlorine per liter. This result is of particular importance, since it indicates a synergistic effect between chloramine and free chlorine.

The results show that the HET-CAM test is somewhat more sensitive than the Draize test under the test conditions selected here. Also in the already cited paper, studying the effect of chlorine and hypochlorites on rabbit eyes, the test duration was prolonged and the test solution was fed to the eye through an infusion bottle, so the results are comparable from this standpoint.

Halogenated organic compounds

Of the total 19 individual halogenated organic compounds tested, trichloroacetic acid and tribromoacetic acid (both previously detected in swimming pool water [4]) exhibited the greatest irritant effect. Pure trichloroacetic acid vapor has a severe irritant effect on eyes and the respiratory tract.

As individual compounds, these substances in aqueous solution exhibit an irritant effect on the CAM starting from a concentration of 1 mg/L. So these compounds have a clearly higher irritant activity than free or combined chlorine. Table 3 shows the test results for the 19 model compounds.

Table 3. Irritant effect of halogenated organic carbonyl compounds

Substance	lowest still active concentration (mg/L)
Chloroacetone	>100
1,1-Dichloroacetone	>100
Tribromoacetic acid	100
Chloroacetic acid	100
Dichloroacetic acid	100
2,2-Dichloropropionic acid	100
2,2-Dichloropropionic acid (Na salt)	100
1,1,1-Trichloroacetone	100
ω-Chloroacetophenone	100
Dibromoacetonitrile	100
Chloroacetonitrile	100
Dichloroacetonitrile	100
Chloral hydrate	100
1,3-Dichloroacetone	10
1,1,3-Trichloroacetone	10
Trichloroacetonitrile	10
Dibromoacetic acid	10
Trichloroacetic acid	1
Bromoacetic acid	1

Trichloroacetic acid and tribromoacetic acid can be regarded as precursors of haloforms; trichloroacetic acid can be converted readily to chloroform by cleavage of CO_2. Compounds are called haloforms if they basically correspond to the chemical structure of chloroform but bromine is also included in their molecules besides chlorine.

Nevertheless, even for trichloroacetic acid or tribromoacetic acid, the irritation threshold determined in the HET-CAM test (1 mg/L) is not sufficiently low for these compounds alone to be responsible for the observable effects.

After we were able to observe clear synergistic effects in the study of the irritant effect of free and combined chlorine, we also tested the halogenated organic compounds with addition of different free chlorine concentrations.

As the blank, chlorine water was first tested alone and then with the corresponding DMSO concentration (in duplicate in each case). No irritant effect could be detected in these tests.

For some of the studied compounds, the irritant effect of the compounds acting on the CAM could clearly be enhanced by addition of chlorine (Table 4). This enhanced effect is most clearly shown for 1,3-dichloroacetone, where after preparing the dilution with chlorine water, the irritant effect begins by 0.01 mg/L, i.e., the effect is enhanced by a factor of 10^3.

Table 4. Increase in irritant effect of halogenated organic compounds in the presence of free chlorine

Compound	Irritant effect without chlorine (mg/L)	Irritant effect with chlorine (mg/L)
Chloroacetonitrile	100	10
2,2-Dichloropropionic acid	100	10
1,3-Dichloroacetone	10	0.01
Bromoacetic acid	1	0.1
2,2-Dichloropropionic acid	100	10
Chloroacetone	>100	100

At the moment, we can only speculate about the mechanisms or reasons for this synergy. So, for example, we cannot rule out the possibility that the studied compounds react further when free chlorine is added, and that consequently the observed effects do not come just from the starting material itself.

Because of the synergistic effect between chlorine and individual halogenated organic compounds, the effect threshold for these compounds drops down to a concentration range which can be found in swimming pool water. Even though the compounds used here as model compounds (where 1,3-dichloroacetone shows the greatest synergistic effect with chlorine) could not be isolated from swimming pool water, a comparable mechanism of action can also be assumed in principle for other substances. Further studies (whose results are not shown here) also have shown a synergistic effect for various halogenated organic compounds. In this case also, the effect of the mixtures can be additionally enhanced by the presence of free chlorine in the water used for dilution.

Discussion

In one study conducted on the effect of swimming pool water on the cornea, 68% of the test subjects reported that after swimming they saw rainbows and/or halos around light sources, and 94% exhibited epithelial erosion [9]. These events were at least partially attributed to the hypotonic effect of swimming pool water. In other studies, it could also be shown that in pools with 0.5% NaCl content, less damage to the cornea occurred [14] than in pools operated without salt added. In addition, chloramines have been suspected for a long time to be responsible for the irritant effects [5]. In one study back in 1951, it was found that different water treatment methods had different impacts on the irritant effect [15]. But for the most part, chlorine (the most commonly used disinfectant) was held responsible for the effects.

But chlorine is currently the only water disinfectant that meets the requirements necessary from a hygiene standpoint for safe use in swimming pools.

The mechanism by which chlorine inactivates microorganisms is to a large extent unknown [6]. It stands to reason that we should look for a fundamental cause in the chemical reactivity of chlorine. This high reactivity, however, also leads to byproducts, where organic matter contained in the water is chemically altered by the disinfectant. In addition to chloroform, many other byproducts are formed which have been only partially identified. For drinking water applications, in the USA detailed studies were conducted back in the beginning of the 1980s, where various compounds were quantitated.

Chloroform and other byproducts are inevitably also formed in swimming pool water. But since chloroform represents the end product of a multistep chain of reactions, more byproducts must be contained in the water.

As has already been mentioned, the most important disinfection byproduct for swimming pool water is chloroform, which can affect the swimmer through the water and through the air [11, 3]. The best known reaction mechanism by which chloroform can be formed using chlorine from organic compounds is the haloform reaction. This reaction occurs via several steps, where carbonyl compounds with methyl groups in the α position are converted first to α-chlorinated ketones and ultimately to chloroform and an organic acid.

While the toxicological properties of chloroform have been relatively well studied [2] and sufficient data are also available concerning its uptake in swimming pools [16], not much information is available concerning the type and concentration of its immediate precursors. However, in principle, compounds with a halogen atom in an α position relative to a double bond are regarded as having irritant effects on mucous membranes.

Chloroacetone was used as a chemical warfare agent for this reason, because of its severe irritant effect in the gaseous state.

Within this study, tests were conducted on the irritant activity relative to mucous membranes for substances that sometimes already had been detected in swimming pool water. Other compounds were taken as model compounds in the study, in order to be able to take into account their effects. The goal was to determine the threshold concentration for the effect of these substances, so we could demonstrate their fundamental significance in the origin of eye irritation in swimming pools while taking into account realistic exposure times.

We were able to show that under the indicated test conditions, the reaction in the HET-CAM test is somewhat more sensitive than in the Draize test. From this we conclude that this model is suitable in principle for detecting the effect of low material concentrations of compounds with an irritant effect.

In the test, the studied individual halogenated organic compounds proved to be irritants only in concentrations that are not usually found in swimming pools. The concentrations found so far in swimming pools are considerably lower. However, higher concentrations could be measured in outdoor swimming pools than in indoor pools, where concentrations of up to 100 µg/L could be detected.

Based on our results, it is therefore likely that the irritant effect occurring in swimming pools is not due to one individual compound, but rather many compounds present in the water contribute to this effect. At the same time, the concentrations of these compounds are dependent on pool load and can fluctuate considerably over the day [8]. From personal experience, eye irritation is also dependent on pool load. Since the chlorine concentration in modern pools is automatically regulated and generally is not subject to large fluctuations, this can also be considered as evidence that the irritant effect is primarily due to other compounds.

Concerning the question of the extent to which other classes of compounds (besides halogenated organic keto compounds) also contribute to the irritant effect, at the moment we can only speculate. Since the oxidizing agents used in water treatment (chlorine and if necessary ozone) are very reactive, byproducts possibly having activity can also be formed by an oxidative route [18].

In continuing investigations, the model for studying irritant effects should be developed further so that it can be used for directly testing swimming pool water.

In order to develop simple prevention strategies, the mechanism for chloroform formation in swimming pools and especially the resulting intermediates should be studied. Ultimately, effective countermeasures can be further developed only if we know these interrelationships.

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[2] Translator's Note: Corrections to references: (Ref. 3) "I" is deleted. (Ref 10). Editors are C. K. Atterwill (not Atterwil) and C. E. Steele. (Ref. 13) B. M. (not N. M.) Patten, Foundations of Embryology [not embryopogy). (Ref. 14) Vol. 71 (not 592).

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