THE EFFECTS OF 5-HYDROXYTRYPTAMINE, INDOLE-3-ACETIC ACID, AND SOME OTHER SUBSTANCES, ON PIGMENT EFFUSION, SODIUM UPTAKE, AND POTASSIUM EFFLUX, BY SLICES OF RED BEETROOT *IN VITRO*

by

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INTRODUCTION

Hydroxytryptamine is widely distributed in the animal body, and has been shown by pharmacological methods to have many effects. The physico-chemical basis of these effects however remains unknown. Similarly the mode of action of indole-3-acetic acid, the principal natural regulator of plant growth, is uncertain. It was suggested by VELDSTRA AND BOOIJ[1], partly on the basis of experiments like those to be described, that one such action is to increase plasma-membrane permeability. Although evidence was adduced that this was unlikely alone to account for the growth regulating properties of indole-3-acetic acid and related compounds, the experimental findings are not disputed.

Hydroxytryptamine, and more especially its metabolic product 5-hydroxy indole-3-acetic acid, fulfil some at least of the known structural requirements for plant growth regulating activity (AUDUS[2]). So far as the authors are aware however, no experimental evidence of such activity has been described. It has seemed pertinent to enquire whether 5-hydroxytryptamine and some related substances show the same effect on the permeability of red beet cells, as does indole-3-acetic acid. For this purpose the influence on pigment effusion, sodium influx, and potassium efflux has been examined. The experiments on pigment effusion are described in Part I of this paper, (V.R.P.), and those concerned with sodium uptake and potassium output, which were performed independently in London, are described in Part II, (J.F.S.).

PART I. PIGMENT EFFUSION

EXPERIMENTAL METHODS

The substances to be tested were dissolved in glass-distilled water, with or without the addition of 0.03 *M* KH_2PO_4/Na_2HPO_4 buffer to give solutions at pH 6. The presence of buffer constituents which VELDSTRA AND BOOIJ[1] said "influenced the test in an unfavourable way" caused the effusion of pigment instigated by cutting the tissue into slices to cease after about 5 hours, whereas it continued for much longer in their absence. It seemed desirable to use buffered solutions in at least some experiments to eliminate, not only the possible effects of varying reaction on pigment effusion, but also to mitigate slow changes which may take place in the pigment *in vitro*, especially after effusion into neutral or alkaline solution.

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In the series in which indole-3-acetic acid was tested, the substance was first rapidly dissolved in an amount of ethanol equal to 1% of the final volume, to which it was made up by adding water or buffer solution. A similar percentage of ethanol was added to the other solutions in the same series. In an experiment with indole-3-acetic acid, without the addition of ethanol, essentially the same results were obtained. The 5-hydroxytryptamine was in the form of the creatinine sulphate, and in most experiments in which it was used, creatinine and sodium sulphate were added to a corresponding concentration in the comparison solutions. These substances appeared to have very little effect at the concentrations used.

The concentration of the test substances employed was generally 100 μg/ml, but in some of the experiments, concentrations of 10 μg/ml and 1 μg/ml were also used.

Freshly gathered specimens of *Beta vulgaris* were kept in the laboratory with the roots in water until use. Disks, 6 mm in diameter and about 1 mm thick were cut, and washed for 10–150 min in glass-distilled water or buffer solution, and then placed in batches of about 20 in 5 ml aliquots of the test solutions. In two experiments the disks were washed for 18 h in running tap water, and then rinsed in glass-distilled water, before transfer to the test solutions. In most of the experiments to be described the first experimental media were poured off after a period of about 3 h, and replaced by fresh solutions into which the effusion of pigment was again determined.

The pigment exuded from the tissue during the experiments was assayed spectrophotometrically at 530 mμ. The optical density of 1 cm of each solution is expressed in the results presented below as a multiple of that of a control solution into which exudation occurred for the same period, or in some instances the mean of two controls.

All the pigment exudation experiments were conducted at room temperature, 18 to 22° C. The measurements of oxygen consumption were made with the Warburg apparatus at 21° C.

RESULTS

As a measure of the experimental error of the test, a comparison was made in the first instance between 12 similar batches of tissue in the same solutions, which should ideally have shown equal pigment effusion. The difference between any pair of determinations averaged 9% of the mean; the largest discrepancy found being 23%.

Table I summarises the results of 8 experiments in which the effect of 5-hydroxytryptamine was compared with that of tryptamine, and indole-3-acetic acid, at a concentration of 100 μg/ml. The data show that there were no major differences between the experiments in which buffered solutions were used, and those in which they were not. It is concluded from Table I that hydroxytryptamine caused at least as great an increase in pigment effusion as did indole-3-acetic acid at the same concentration, whereas tryptamine had an insignificant effect.

TABLE I

Initial washing period (min)	*Phosphate buffer*	*1st period of 3 h approx.*			*2nd period of 1–2 h*		
		HT	*T*	*IAA*	*HT*	*T*	*IAA*
10	+	0.91	0.87	1.16	1.32	0.60	1.93
10	+	1.22	0.88	1.00	2.05	0.30	1.66
10	+	1.39	1.22	1.12	3.35	1.39	2.62
30	+	1.75	1.05	1.08	3.22	1.78	1.50
10	+	1.79	1.01	1.07	4.00	1.18	1.94
45	—	1.30	1.00	1.27	1.95	1.04	1.81
45	—	1.86	1.02	1.75	2.30	1.10	2.38
150	—	1.81	—	1.93	3.43	—	3.93
Means of above ratios		1.50	(1.00)	1.30	2.70	(1.06)	2.22
Significance of difference from 1.00:		$P<0.02$	NS	$P<0.05$	$P<0.01$	NS	$P<0.01$

Ratios, in 2 periods in each of 8 experiments, of the optical densities at 530 mμ of solutions containing 100 μg/ml of 5-hydroxytryptamine (HT), tryptamine (T), or indolylacetic acid (IAA), as multiples of that of control solutions, taken as 1.00.

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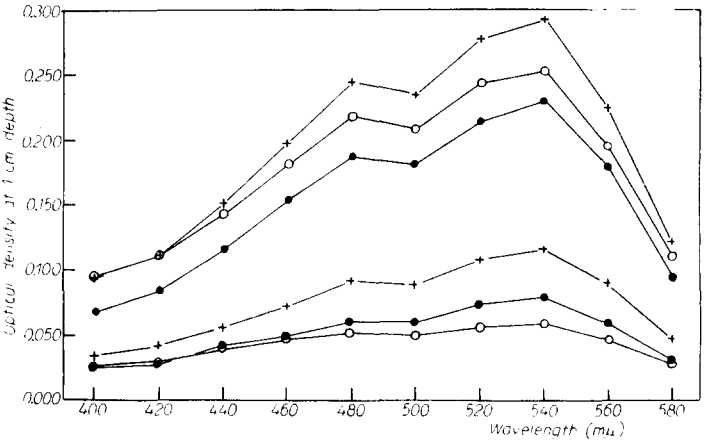

Fig. 1. The absorption characteristics of the pigment effused in a typical experiment (the first in Table I). The relatively high control values in this experiment are unusual. |——+| : indole-3-acetic acid 100 μg/ml; ●——● : 5-hydroxytryptamine 100 μg/ml; O--- O: control. The upper 3 curves show the values for the first 3 h, the lower 3 those for the next 1½ h.

Fig. 1 shows the absorption characteristics of the pigment in one of the experiments listed in Table I. It indicates that although minor numerical differences in the results may be obtained by selecting a different wavelength for the estimation, this is not a major source of error.

Fig. 2 illustrates the fact that in general, the effect of hydroxytryptamine and indole-3-acetic acid continued for several hours after the solutions had been drained off the slices and replaced by distilled water. The figure also shows that hydroxytryptamine at a concentration of 10 μg/ml produced a detectable increase in pigment effusion. At a concentration of 1 μg/ml however, no measurable effect was observed.

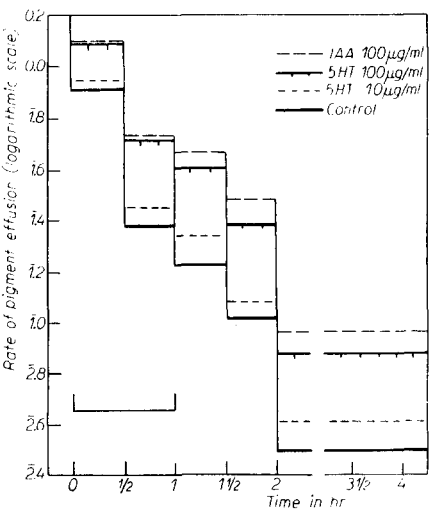

Fig. 2. Experiment to illustrate the time-course of the pigment effusion. The beetroot slices were washed for 2 h before time 0 on the graph, when they were placed in the solutions as shown. From 1 h onwards water only was used for all batches. Ordinate = log rate of increase of optical density at 530 mμ and 1 cm depth in h $^{-1}$.

When batches of disks were washed initially for 18 hours in tap water before transference to the experimental solutions, they showed a much smaller absolute rate of pigment effusion, in both the "test" and "control" media, but the ratio "test effusion": "control" was found to be greater (9:1 and 4:1 in two experiments on hydroxytryptamine 100 μg/ml) than in the series represented by Table I.

Although several attempts were made, no evidence was obtained to indicate that previous or simultaneous treatment of the slices with tryptamine would inhibit the response to hydroxytryptamine, in the way which can be demonstrated with plain-muscle preparations. In the present experiments it was impossible to use very high initial concentrations of tryptamine, since these had an effect like that of hydroxytryptamine at a lower

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concentration. Thus a concentration of 1 mg/ml of tryptamine in two experiments increased the effusion of pigment by factors of 2.13 and 1.90, the effect persisting after the withdrawal of the tryptamine.

Other substances cursorily examined at concentrations of approximately 100 μg/ml included: creatinine, tryptophane, bufotenine, histamine, mepyramine maleate, oestrone, oestradiol, (solution-suspension in 2% ethanol) and the detergent "Lissapol"*. The only one of these found to have a marked effect was "Lissapol" which is known to be a powerful haemolytic agent.

The effect of 5-hydroxytryptamine on oxygen consumption

Previous experiments (unpublished) had suggested that 5-hydroxytryptamine creatinine sulphate, at a concentration of approximately 300 μg/ml, temporarily diminished the rate of oxygen consumption by suspensions of yeast in buffered glucose solutions. One experiment on the beetroot preparation indicated that the substance at a concentration of 100 μg/ml diminished oxygen uptake by about 15%. That such an influence can be dissociated from the pigment effusion effect was shown by three experiments in which lower concentrations (20, 40 and 80 μg/ml) of hydroxytryptamine were used. The results of Table II show that this treatment caused no significant change

TABLE II

Time (h)	*Ratio of "experimental" to control values of:*	
	Oxygen consumption	*Pigment effusion*
12.12 to 3.0 p.m. (both batches of beet slices in water only)	1.04	1.22
3.10 to 5.5 p.m. (hydroxytryptamine 40 μg/ml added to "experimental" flask)	1.07	1.31
5.20 to 8.40 p.m. (both batches in water only)	1.28	1.30

The effect of 5-hydroxytryptamine 40 μg/ml on the rate of oxygen consumption and the rate of pigment effusion of beetroot slices *in vitro*. The figures show the values for the "experimental" batch of slices divided by the corresponding value for the "control" batch.

in the rate of oxygen consumption although pigment effusion was increased. Upon subsequently transferring the tissue to distilled water, oxygen absorption by the hydroxytryptamine treated slices was higher that in the control, and the rate of pigment effusion continued at the increased level.

PART II. SODIUM UPTAKE AND POTASSIUM EFFLUX

EXPERIMENTAL METHODS

In general, the experimental methods were the same as those employed in other investigations of salt uptake by red beet root tissue (SUTCLIFFE[3]).

A number of disks were cut, 7.5 mm in diameter and 0.75 mm thick, with a hand microtome, and washed in several changes of glass-distilled water for either 30 min or 24 h at room temperature. Some of the disks were then set aside for determination of the initial sodium and potassium content

* Imperial Chemical Industries, Ltd.

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of the tissue. The analyses were made by flame photometry, upon water extracts prepared as has been described in the citation above.

The remaining disks were weighed in batches of 30 (fresh wt about 0.9 g) and transferred to 50 ml wide-mouthed conical flasks each containing 4 ml of 0.02 *M* NaCl, in the absence, and presence of indole-3-acetic acid or hydroxytryptamine at a concentration of 50 μg/ml. The flasks were maintained in a water bath at 25° C, and shaken to facilitate aeration for 6 hours. The influx of sodium and efflux of potassium by the tissue during this time was determined by an analysis of the external medium with the flame photometer.

Each group of disks was then washed free from the remaining medium in several changes of glass-distilled water, blotted to remove adhering liquid, and placed in 4 ml of 0.02 *M* NaCl for a further period of 90 h under the same conditions of temperature and aeration as before. Determinations of sodium uptake and potassium output were made after 18 h and thereafter at intervals of 24 h until the end of the experimental period. For each analysis, 3 ml of the medium was withdrawn, and replaced by 3 ml of fresh 0.02 *M* NaCl solution, so that the concentration of sodium in the medium throughout the experiment never decreased to below 0.01 *M*.

Three replicate flasks were set up for each treatment, and each experiment was repeated twice, so that the results presented below represent the mean of 6 separate determinations. Disagreement between replicates was always less than 10% of the mean value, and usually less than 5%.

The respiration determinations were made by measuring oxygen uptake with a Warburg apparatus during the first 4 hours and last 2 hours of each experiment.

RESULTS

Determinations of the initial potassium and sodium content of the two types of tissue showed that disks which had been washed in water for 30 min contained about 53 μeq. of potassium and 19 μeq. of sodium per g fresh-weight. Disks which had been washed for 24 h contained rather less (49 μeq. of K and 17 μeq. of Na per g fresh weight).

The uptake of sodium and output of potassium by the material under the different experimental conditions is shown in Tables III and IV. The data demonstrate that

TABLE III

Influx of sodium and efflux of potassium (μeq/g fresh wt.) with beet disks, washed in distilled water for 30 min and transferred to 0.02 *M* NaCl in the absence or presence of indole-3-acetic acid (I.A.A.) or 5-hydroxytryptamine (H.T.). All disks transferred to 0.02 *M* NaCl alone, at the end of 6 h.

Time (h)	*Na Influx*			*K efflux*		
	0	*I.A.A.*	*H.T.*	*0*	*I.A.A.*	*H.T.*
6	15.1	12.5	9.2	3.2	2.8	4.1
24	10.3	7.0	8.3	1.3	2.4	0.9
48	19.9	9.9	20.4	2.9	1.8	2.4
72	38.7	17.4	43.7	1.6	1.3	1.9
96	40.2	27.3	44.7	1.9	1.4	1.6
Total	124.2	74.1	126.3	10.9	9.7	10.9

TABLE IV

Influx of sodium and efflux of potassium (μeq/g fresh wt) with beet disks, washed in distilled water for 24 h. Other details as for Table III.

Time (h)	*Na Influx*			*K efflux*		
	0	*I.A.A.*	*H.T.*	*0*	*I.A.A.*	*H.T.*
6	18.8	15.1	16.4	2.1	1.7	1.7
24	15.3	11.4	11.9	1.6	1.5	1.2
48	25.2	17.4	24.0	1.5	0.9	1.6
72	46.6	29.6	46.2	1.5	1.1	1.3
96	46.8	33.8	46.1	1.3	1.0	1.7
Total	152.7	107.3	144.6	8.0	6.2	7.5

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although sodium was absorbed with all treatments, uptake was markedly inhibited when either 5-hydroxytryptamine or indole-3-acetic acid was present in the medium. This effect persisted during 18 h following transfer of the tissue to a 0.02 *M* NaCl solution. Subsequently the absorption of sodium by the 5-hydroxytryptamine-treated disks occurred at a rate which was about the same as the controls at first, and later became higher, so that the final amount of sodium absorbed by this tissue during the whole of the experiment was about the same. On the other hand, the indole-3-acetic acid treated material continued to absorb ions more slowly until the end of the experimental period, and the total sodium absorbed was therefore considerably less than with the control or hydroxytryptamine treated disks.

It should be noted that the progressive increase in the rate of absorption of sodium with time observed in these experiments is a characteristic feature of salt uptake by dormant tissue such as beet. The possible causes of it will not be discussed here since they are irrelevant to the present problem.

The results of Tables III and IV also show that despite the considerable concentration gradient existing, the efflux of potassium occurred very slowly under all conditions; and it was somewhat reduced following the treatment with either 5-hydroxytryptamine or indole-3-acetic acid.

A comparison between the data of Table III and IV shows that rather more sodium was absorbed during the experimental period by the disks which were given the longer preliminary washing. This is attributable to the development of some absorptive capacity during the washing period. Conversely, the loss of potassium by this material was somewhat less, as a consequence perhaps of leaching of readily loseable potassium during the preliminary treatment. Otherwise the results obtained with the two tissues were entirely comparable.

In order to examine the possible metabolic effect of these substances, determinations of oxygen absorption were made, with each tissue, during the first 4 hours, and last 2 hours of the experimental period. In the first period the disks were in contact with the experimental solutions, and in the second they had already been in simple

TABLE V

Rates of oxygen absorption by beet disks relative to controls in 0.02 *M* NaCl, (= 100) during and after treatment with indole-3-acetic acid (I.A.A.) and 5-hydroxytryptamine (H.T.) at a concentration of 50 μg/ml, at 25° C. Tissue A was washed for 30 min and Tissue B for 24 h in distilled water before transference to the experimental media.

Tissue	*Time during experiment*	*Control*	*I.A.A.*	*H.T.*
A	0–4 h	100	100	118
A	94–96 h	100	93	101
B	0–4 h	100	93	104
B	94–96 h	100	91	100

salt solution for many hours. The results presented in Table V, which represent rates of oxygen uptake relative to the controls (100), indicate that the effects of treating the tissue with either indole-3-acetic acid or 5-hydroxytryptamine are slight.

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GENERAL DISCUSSION (PARTS I AND II)

The word "effusion" rather than "diffusion" has been used in the description of the results in Part I above, because it is not intended to imply that the effects observed are necessarily to be interpreted in terms which apply to diffusion through membranes. VELDSTRA AND BOOIJ[1] however in describing similar experiments with plant growth-regulators, seem to accept such an interpretation.

There is no evidence that diffusion of ions plays any important part in the movement of inorganic salts across plant cell membranes, in either direction.

It has seemed possible from the results of Part I, that the additional pigment is liberated from cells in a grossly abnormal condition as a result of the "toxic" effect of 5-hydroxytryptamine or indole-3-acetic acid on them. Against this hypothesis however must be set the fact that the pigment effect can be demonstrated without any great change in the rate of respiration being observed, such as would occur if a proportion of the cells were injured. Moreover the depression of sodium uptake which hydroxytryptamine causes is completely reversible by washing the disks for about 18 h in an hydroxytryptamine-free solution. It has also been found, although the results are not presented here, that the effect of indole-3-acetic acid is likewise reversible, after more prolonged washing. Finally there is no evidence that either hydroxytryptamine or indole-3-acetic acid causes the increased output of potassium from the tissue expected if a number of cells are killed.

It is suggested that the experimental findings may best be interpreted in terms of an effect on membrane permeability, which may however be not as simple as that proposed by VELDSTRA AND BOOIJ[1]. Clearly the substances cause an increase in the permeability of the protoplasm to pigment, without having any corresponding effect on the permeability to cations. This may perhaps be associated with the fact that the resistance of the cell membranes in beet to diffusion and exchange of ions is very high (SUTCLIFFE[3]), and uptake therefore depends entirely upon an active transport mechanism. The inhibition of sodium uptake by indole-3-acetic acid and 5-hydroxytryptamine probably results from an influence on this mechanism, which is independent of their effect in promoting the effusion of pigment. The mechanism of this inhibition, which apparently occurs in the absence of marked respiratory effects, is to be the subject of further investigation.

The fact that both the effects on pigment effusion and ion uptake persist after the withdrawal of the active substances from the bathing medium suggests that hydroxytryptamine and indole-3-acetic acid become incorporated in the plant tissue. The much greater activity of hydroxytryptamine than tryptamine in the pigment tests is suprising, since the 5-OH substitution of indole derivatives has not been known to have any significance in plant physiology. At present two such compounds have been reported as being isolated from plant sources: 5-hydroxytryptamine (BOWDEN, BROWN AND BATTY[4]); and bufotenine or N.N dimethyl-5-hydroxytryptamine (WIELAND AND MOTZEL[5]; STROMBERG[6]). It is possible that this series of compounds may prove of more importance in plant physiology than has so far been realised.

The relevance of these findings to animal physiology is a matter of conjecture, but it may be suggested that a change in the permeability of membranes to water-soluble substances might accord teleologically with the predominant occurrence of 5-hydroxytryptamine in glandular and nervous tissues.

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The 5-hydroxytryptamine (serotonin) creatinine sulphate used in these experiments was kindly supplied by Dr. K. RICHARDS, Abbott Laboratories, Chicago.

SUMMARY

1. Hydroxytryptamine shows approximately the same power of increasing the effusion of pigment from beetroot slices as does indole-3-acetic acid, and a much greater power than is shown by tryptamine.

2. Both 5-hydroxytryptamine and indole-3-acetic acid are found to be strong inhibitors of sodium uptake by this material, although they have little effect on the rate of potassium efflux.

3. These effects are not necessarily associated with any appreciable change in the rate of oxygen consumption by the tissue.

4. The effects are generally irreversible in experiments lasting only a few hours, but may be completely reversible over longer periods of time.

5. Tryptamine is shown not to inhibit the effect of hydroxytryptamine in promoting pigment effusion.

6. The implication that 5-hydroxytryptamine and indole-3-acetic acid affect the permeability of cell membranes to water-soluble substances is briefly considered in relation to animal and plant physiology.

RÉSUMÉ

1. L'hydroxytryptamine provoque la sortie des pigments de coupes de betterave presqu'aussi efficacement que l'acide indole-3-acétique et beaucoup plus que la tryptamine.

2. La 5-hydroxytryptamine et l'acide indole-3-acétique sont tous les deux des inhibiteurs puissants de l'absorption du sodium, quoiqu'ils n'aient que peu d'effet sur la vitesse de transfert du potassium.

3. Ces actions ne sont pas nécessairement associées à un changement appréciable de la vitesse de consommation de l'oxygène par le tissu.

4. Ces effets sont généralement irréversibles, dans des expériences qui durent seulement quelques heures, mais peuvent être complètement réversibles en des temps plus longs.

5. La tryptamine n'inhibe pas l'action de l'hydroxytryptamine sur la sortie du pigment.

6. La conclusion selon laquelle la 5-hydroxytryptamine et l'acide indole-3-acétique modifient la perméabilité des membranes cellulaires aux substances hydrosolubles est rapidement examinée en relation avec la physiologie animale et végétale.

ZUSAMMENFASSUNG

1. Hydroxytryptamin besitzt, ungefähr in gleichem Masse wie Indol-3-Essigsäure, und weit mehr als Tryptamin, das Vermögen, die Farbstoffeffusion aus Zuckerrübenwurzelschnitten zu erhöhen.

2. Es wurde festgestellt, dass 5-Hydroxytryptamin, sowie Indol-3-Essigsäure, die Natriumaufnahme des obigen Materials stark hemmen, obgleich dieselben wenig Einfluss auf die Geschwindigkeit der Kaliumausscheidung ausüben.

3. Es besteht kein unbedingter Zusammenhang zwischen diesen Einwirkungen und dem Sauerstoffverbrauch der Gewebe.

4. Diese Einwirkungen sind im allgemeinen irreversibel, wenn es sich um Versuche handelt, welche nur einige Stunden dauern. Im Falle von längeren Zeitspannen können sich dieselben jedoch als vollkommen reversibel erweisen.

5. Es wird bewiesen, dass Tryptamin die durch Hydroxytryptamin hervorgerufene erhöhte Farbstoffausscheidung nicht hemmt.

6. Im Bezug auf Tier- und Pflanzenphysiologie wird kurz die Möglichkeit erörtert, dass 5-Hydroxytryptamin und Indol-3-Essigsäure die Permeabilität der Zellmembran wasserlöslichen Substanzen gegenüber beeinflussen.

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