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The Effect of Indole-3-Acetic Acid on the Ageing Male Rat*

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The plant growth hormone, indole-3-acetic acid (IAA), has been isolated from human urine (*Wieland* et al., 1954). However, the origin and significance of this indole derivative in the mammalian organism is not yet clear. It is reported that IAA increases the blood white cell count in the guinea pig (*De Landsheere*, 1958), and that it decreases the blood sugar level in man (*Mirsky and Diengott*, 1956) and in the rat (*Mirsky* et al., 1956).

The effect of IAA on the growth of the young rat has been studied by various workers with conflicting results. *Kodicek* et al. (1946) reported that IAA had a growth inhibiting action in the rat, but this could not be confirmed in a later study by these workers (*Kodicek* et al., 1947), nor by *Rosen and Perlzweig* (1947). On the other hand, studies by *Henderson* et al. (1947) and *Raoul and Marnay* (1948) suggest that IAA actually has a growth promoting action in the young rat.

The purpose of this investigation is to determine the effect of indole-3-acetic acid on the growth of the middle aged rat and to study its action on the ageing process in middle aged and old rats.

Materials and Methods

Eighty-six male rats of a non-inbred Wistar strain were housed in a small air-conditioned room (temperature $25 \pm 1°$ C, relative humidity $55 \pm 10\%$) and fed a pelleted diet as described previously (*Everitt*, 1958).

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Animals were weighed on an Ohaus sliding weight animal balance at weekly intervals from weaning until natural death. The relative weight increment from 300 to 400 days, $\frac{W_{400}-W_{300}}{W_{400}}$, was determined and the 10 rats with the lowest values were discarded, because of their reduced life expectancy (*Everitt and Webb*, 1957). Of the remaining 76 rats, 30 were used for studying the effect of small daily doses of IAA on the course of ageing, and 46 for studying the effect of large doses.

In the small dosage experiment, 15 rats were treated with IAA and 15 control rats with the saline vehicle. Rats were carefully matched on the basis of their relative weight increments before being assigned at random to these two treatment groups. Indole-3-acetic acid was injected intraperitoneally, at the dosage level of 0.5 mg once a day for 6 days per week, between the ages of 400 and 600 days. The required weight of β-indolyl acetic acid (British Drug Houses) was dissolved in a minimum volume of 95% ethyl alcohol and diluted with 0.9% NaCl of pH 9. Solutions were prepared twice weekly and stored at −10° C when not in use. All solutions were warmed to 37° C before injection. The controls received daily 0.5 ml intraperitoneal injections of the vehicle, 5% ethyl alcohol in 0.9% NaCl of pH 9, at the same times as the experimental animals.

In the large dosage experiment IAA was administered in the diet at the rate of 5 mg per day. A batch of pelleted rat food containing 25 grams of indole acetic acid (*L. Light & Co.*) was eaten by the 23 experimental rats in about 220 days. The 23 control rats were supplied with ordinary rat cubes.

Metabolic, excretory, haematological and electrocardiographic studies were carried out on these rats in young adult life, in middle age and in old age. Details of the methods employed have been described previously (*Everitt*, 1959). Determinations in middle age were carried out during the period of treatment, and in old age about 150 days after the cessation of treatment.

Statistical analysis of the data was performed using the methods described by *Snedecor* (1953). The significance of the over-all effects of IAA on the course of ageing was determined from the analysis of variance. Values for the variance ratio (F) between ages are for 2 and 54 degrees of freedom, and for F between treatments are for 1 and 54 degrees of freedom.

Results

Body Weight

In table I the mean body weights of IAA treated rats before treatment (at 300 and 400 days), during treatment (at 500 and 600 days) and after treatment at 700 days are compared with the corresponding weights in the control rats.

The natural age change in the body weight of the male rat was not affected by prolonged treatment with either small (table I) or large (table I, fig. 1) doses of IAA in middle age. It therefore follows, that while IAA may stimulate growth in plants it has no growth promoting properties in the middle aged male rat.

Life Duration

No significant change occurred in the life duration of the male rat as the result of prolonged treatment with IAA, in either small or large doses. The life durations of individual rats are given in table II.

Table I

The Effect of the Daily Administration of Indole-3-Acetic Acid at 2 Dosage Levels, during the Period from 400 to 600 Days of Age, on the Mean Body Weight of Male Rats (Living + Dead) at Various Ages

Experiment	Group	Body weight (grams) ± S.E. at 300 days	400 days	500 days	600 days	700 days	Death	Maximum weight
Small dose	Control (15)*	376 ± 9.4	391 ± 9.3	389 ±12.7	375 ±14	344 ±19	259 ±12.4	411 ± 9.0
	Indole acetic acid (15)	378 ±10.7	394 ±11.4	390 ±16	344 ±20	307 ±22	250 ±14.7	412 ±12.8
	Difference	+ 2	+ 3	+ 1	−31	−37	− 9	+ 1
Large dose	Control (23)	404 ± 5.3	416 ± 5.3	403 ± 9.6	370 ±16	331 ±17	244 ± 5.4	435 ± 5.9
	Indole acetic acid (23)	392 ± 5.4	405 ± 6.2	388 ±10.6	357 ±15	314 ±17	244 ± 5.5	421 ± 6.8
	Difference	−12	−11	−15	−13	−17	0	−14

* Number of rats.

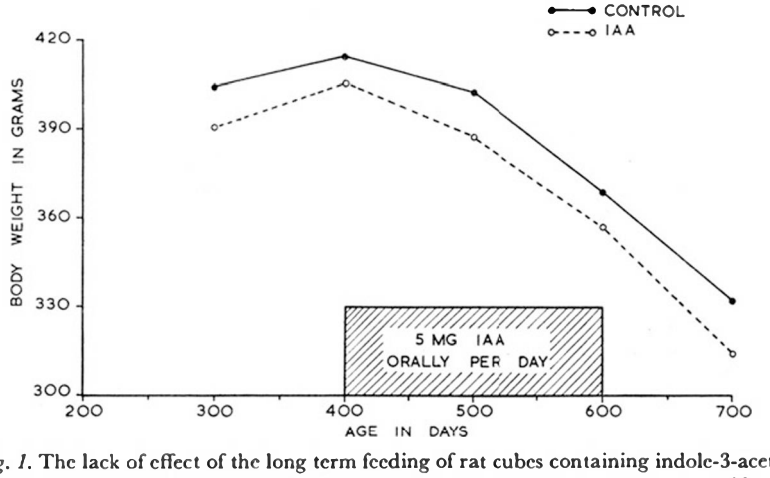

Fig. 1. The lack of effect of the long term feeding of rat cubes containing indole-3-acetic acid on the age change in the body weight of 23 male rats. The control group of 23 rats were supplied with ordinary rat cubes.

Metabolic and Excretory Changes

The F between ages values given in table III show that the consumption of food decreased significantly with age, while the production of urine increased significantly. In a similar way the F between ages values given in table IV show that the excretion of

Table II

The Effect of the Daily Administration of Indole-3-Acetic Acid at 2 Dosage Levels, during Middle Age from 400 to 600 Days of Age, on the Life Duration in Days of Individual Rats

Small dose				Large dose			
Controls		IAA		Controls		IAA	
504	834	591	715	543	779	529	744
526	846	619	739	548	805	544	760
584	859	633	770	604	863	551	838
655	872	633	868	627	869	563	859
663	894	644	875	637	877	574	896
697	900	646	916	637	890	631	924
752	1011	657	981	649	960	661	926
828		680		665	968	675	980
				682	989	687	1000
				708	1010	689	1052
				729	1025	691	1057
				748		694	
Mean ± S.E.		Mean ± S.E.		Mean ± S.E.		Mean ± S.E.	
762 ± 39		731 ± 32		774 ± 36		762 ± 36	

Table III

The Effect of the Daily Administration of 5 mg of Indole-3-Acetic Acid for 200 Days during Middle Age, on the Age Changes in Food and Water Consumption, and in Faeces and Urine Production. Each Group Contained 10 Rats all of which Survived until Old Age

Quantity	Units	Control			Indole acetic acid			F between ages	F between treatments
		Youth	Middle age	Old age	Youth	Middle age	Old age		
Age	days	311	506	743	310	520	745		
	± S.E.	± 9.1	± 8.5	± 3.1	± 6.2	± 0.6	± 2.9		
Body weight	grams	410	418	363	403	405	368		
	± S.E.	±11.8	± 8.5	±18	± 9.0	± 7.5	±13.5		
Food consumption	g/day	19.6	20.4	16.8	19.5	20.8	18.7	5.18**	< 1
	± S.E.	± 0.9	± 1.1	± 0.9	± 0.7	± 0.6	± 1.0		
Faeces production	g/day	11.9	11.7	10.3	12.4	11.3	10.3	2.93	< 1
	± S.E.	± 0.7	± 1.0	± 0.9	± 0.5	± 0.5	± 0.7		
Water consumption	ml/day	27.4	26.6	29.0	26.9	30.6	30.3	1.22	1.49
	± S.E.	± 1.5	± 1.2	± 1.0	± 1.8	± 1.6	± 2.3		
Urine production	ml/day	10.6	10.9	12.9	10.3	9.6	13.0	4.57*	< 1
	± S.E.	± 0.7	± 0.9	± 1.4	± 0.6	± 0.5	± 1.4		

* Significant at the 5% level.
** Significant at the 1% level.

Table IV

The Effect of the Daily Administration of 5 mg of Indole-3-Acetic Acid for 200 Days during Middle Age, on the Age Changes in the Urinary Excretion of Various Constituents. Each Group Contained 10 Rats all of which Survived until Old Age

Quantity	Units	Control			Indole acetic acid			F between ages	F between treatments
		Youth	Middle age	Old age	Youth	Middle age	Old age		
Age	days	311	506	743	310	520	745		
	± S.E.	± 9.1	± 8.5	± 3.1	± 6.2	± 0.6	± 2.9		
Body weight	grams	410	418	363	403	405	368		
	± S.E.	±11.8	± 8.5	±18	± 9.0	± 7.5	±13.5		
Protein excretion	mg N/day	3.5	5.9	9.9	2.7	5.8	13.7	5.07**	< 1
	± S.E.	± 0.9	± 1.9	± 2.9	± 0.4	± 2.1	± 5.4		
Non-protein nitrogen excretion	mg N/day	422	431	409	409	396	423	< 1	< 1
	± S.E.	±15	±22	±24	±18	±15	±21		
Uric acid excretion	mg/day	3.8	4.0	3.9	3.9	4.4	4.7	1.23	3.66
	± S.E.	± 0.19	± 0.25	± 0.18	± 0.34	± 0.34	± 0.35		
Creatinine excretion	mg/day	22.1	20.0	16.2	22.0	20.0	17.7	10.9 **	< 1
	± S.E.	± 0.7	± 1.0	± 1.0	± 0.9	± 1.4	± 1.4		
Phosphate excretion	mg P/day	15.7	14.4	13.9	16.8	12.7	15.2	2.35	< 1
	± S.E.	± 0.8	± 1.8	± 1.4	± 0.9	± 0.7	± 1.5		
Chloride excretion	mg NaCl/day	180	171	140	177	164	175	3.09	1.47
	± S.E.	± 6	± 8	±12	± 8	± 7	±10		

** Significant at the 1% level.

protein increased significantly with age, while the excretion of creatinine decreased significantly.

From the F between treatment values given in tables III and IV, it is clear that treatment with large doses of IAA in middle age had no significant effect on the age changes in any of these physiological variables. In no case was the interaction between ages and treatments significant.

Blood Picture

The F between ages values given in table V indicate that significant age changes occurred in the blood haemoglobin level, in the red cell count and in the eosinophil count. The corresponding F between treatments values were not significant. However, the interaction between ages and treatments was significant in both the

Table V

The Effect of the Daily Administration of 5 mg of Indole-3-Acetic Acid for 200 Days during Middle Age on the Age Changes in the Blood Picture. Each Group Contained 10 Rats all of which Survived until Old Age

Quantity	Units	Control			Indole acetic acid			F between ages	F between treatments
		Youth	Middle age	Old age	Youth	Middle age	Old age		
Age	days	327	543	753	325	540	755		
	± S.E.	± 4.6	± 3.3	± 3.2	± 9.5	± 2.1	± 3.2		
Body weight	grams	414	420	366	408	411	360		
	± S.E.	±12.4	± 8.2	±16	± 8.6	± 8.2	±12.9		
Haemoglobin	g/100 ml	16.4	16.4	16.7	16.1	16.1	18.0	7.77**	< 1
	± S.E.	± 0.22	± 0.24	± 0.52	± 0.23	± 0.23	± 0.46		
Red cell count	10^6 cells/mm^3	8.7	8.0	8.3	8.6	7.8	9.1	11.2**	1.09
	± S.E.	± 0.09	± 0.17	± 0.25	± 0.16	± 0.20	± 0.23		
White cell count	10^3 cells/mm^3	12.9	11.9	13.5	13.9	15.0	15.2	< 1	3.45
	± S.E.	± 1.3	± 0.6	± 1.5	± 1.2	± 1.3	± 1.7		
Eosinophil count	cells/mm^3	546	346	339	580	361	362	3.52*	< 1
	± S.E.	±77	±68	±94	±100	±52	±136		

* Significant at the 5% level.
** Significant at the 1% level.

haemoglobin level ($F = 3.81$, $P<0.05$) and in the red cell count ($F = 4.25$, $P<0.05$). These significant interactions were due to the significant elevations of the haemoglobin level ($t = 2.69$, $P<0.05$) and the red cell count ($t = 2.96$, $P<0.01$) in old age in IAA treated rats, compared with the controls.

The white cell count was increased during the period of treatment with large doses of IAA (analysis of covariance $F_{1,17} = 4.43$, $P<0.05$). This effect of IAA in raising the white cell count has previously been observed by *De Landsheere* (1958) in acute experiments on the guinea pig.

Electrocardiogram

The F between ages values given in table VI show that heart rate decreased significantly with age and that the mean QRS vector in the frontal plane rotated to the left.

The significant F between treatments value for the direction of the mean QRS vector, indicates that the heart was more horizontal (smaller angle made with the horizontal) in the IAA group than in

Table VI

The Effect of the Daily Administration of 5 mg of Indole-3-Acetic Acid for 200 Days during Middle Age on the Age Changes in Heart Rate and the QRS Vector in the Frontal Plane. Each Group Contained 10 Rats all of which Survived until Old Age

Quantity	Units	Control			Indole acetic acid			F between ages	F between treatments
		Youth	Middle age	Old age	Youth	Middle age	Old age		
Age	days	316	548	711	314	545	717		
	± S.E.	± 3.3	± 1.3	± 8.2	± 3.6	± 1.7	± 4.4		
Body weight	grams	410	424	379	402	414	382		
	± S.E.	±11.8	± 8.5	±15	± 9.3	± 8.7	±13		
Heart rate	beats/min	406	368	348	391	361	344	10.4**	< 1
	± S.E.	±11	±13	±14	± 7.3	±14	± 7.7		
QRS vector	μV	304	280	335	276	315	347	< 1	< 1
magnitude	± S.E.	±22	±21	±52	±29	±45	±48		
QRS vector	degrees	60	49	24	47	27	17	14.5**	7.98**
direction	± S.E.	± 5	± 6	± 8	± 5	± 6	± 6		

** Significant at the 1% level.

the controls. Since this difference was present in the young animals before treatment was commenced, it could not be attributed to the action of IAA.

Pathological Changes

The mean postmortem weights of the heart ventricles, liver, kidneys, testes and adrenals in the 23 rats which consumed 5 mg of IAA per day in the diet in middle age, were not significantly different from those in the 23 control rats (table VII).

Table VII

The Effect of the Daily Administration of 5 mg of Indole-3-Acetic Acid for 200 Days during Middle Age on the Weights of Various Organs at Autopsy

Group	Number of rats	Body (g)	Lungs combined (g)	Heart ventricles (g)	Liver (g)	Kidneys combined (g)	Testes combined (g)	Adrenals combined (mg)
Control	23	244	8.4	1.12	9.8	2.91	1.17	75
		± 5.4	± 0.76	± 0.05	± 0.79	± 0.11	± 0.12	± 4.3
Indole-3-	23	244	11.3	1.10	8.7	2.70	1.10	69
acetic acid		± 5.5	± 1.14	± 0.05	± 0.48	± 0.08	± 0.13	± 2.0
t		0.05	2.10*	0.30	1.25	1.42	0.39	1.34

* Significant at the 5% level.

The weight of the lungs in these rats, however, was significantly greater than in the controls ($t = 2.10$, $P < 0.05$). This was due to the greater frequency and severity of lung disease in IAA treated rats compared with the controls (fig. 2). The severity of lung disease was graded according to the external area of the lung involved in lesions as described previously (*Everitt*, 1959).

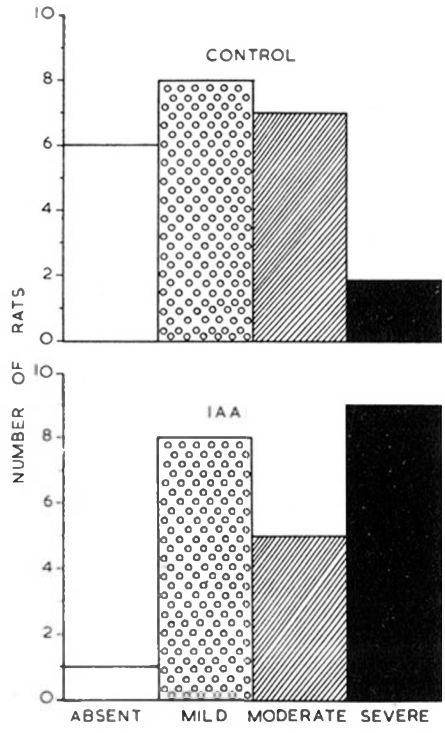

Fig. 2. The effect of the daily addition of 5 mg of indole-3-acetic acid to the diet for 200 days in middle age on the incidence and severity of lung disease at autopsy in the male rat.

The incidence of other diseases studied was not affected by treatment with large doses of IAA. There was only one tumour in the IAA group (4.1 g testis) compared with 2 tumours in the control group (2.5 g adrenal, 5 g skin tumour). Two rats in the IAA group had ventricular hypertrophy (ventricular weights 1.50 and 1.75 grams) compared with 3 in the control group (1.39, 1.57 and 1.80 grams). Severe nephrosis developed in old age in 2 rats in the

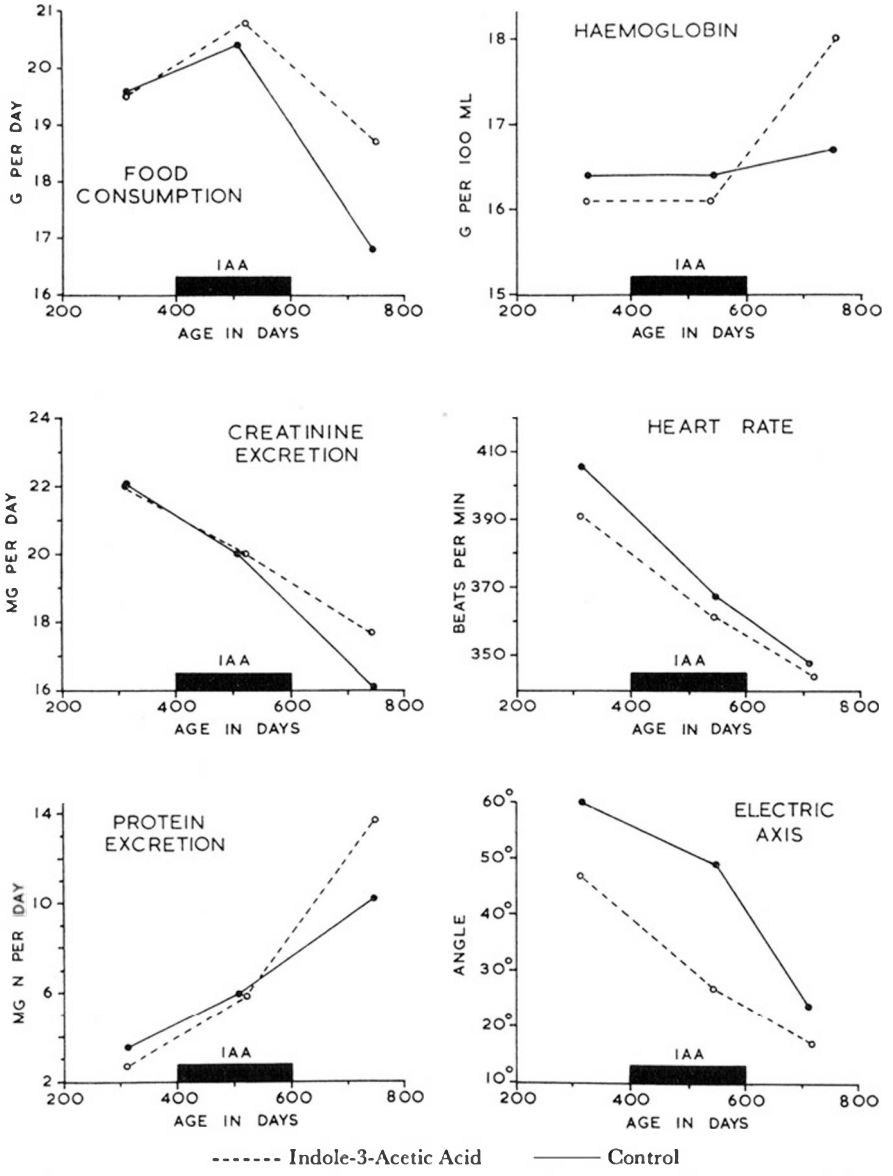

Fig. 3. The effect of the daily addition of 5 mg of indole-3-acetic acid to the diet for 200 days in middle age, on six indices of ageing. Both the experimental and control groups consisted of 10 male rats, which all survived until old age.

IAA group (urinary protein excretion 35.4 and 53.8 mg N per day) and in 2 controls (24.2 and 28.6 mg N per day).

Discussion

The duration of life has often been correlated with the rate of growth (*Osborne* et al., 1917; *McCay* et al., 1935; *Sherman and Campbell*, 1935; *Backman*, 1940; *Comfort*, 1956), and with the rate of metabolism (*Northrop*, 1926; *Pearl*, 1928). In the present study the long term treatment of middle aged rats with IAA did not significantly alter the body weight (fig. 1) or any of the metabolic processes studied (tables III, IV, VI; fig. 3). Therefore it was not surprising to find that IAA had no effect on life duration.

Indole-3-acetic acid, however, produced two changes in the course of ageing in the post-treatment period: 1. it augmented the natural increases in the haemoglobin level (table V and fig. 3) and the red cell count (table V), and 2. it increased the frequency and severity of lung disease at autopsy (fig. 2). It is likely that these two changes are related. Probably IAA accelerates the development of lung disease, causing anoxia, which then increases the red cell count and the haemoglobin level. This effect of lung disease in stimulating erythropoiesis in the rat has, however, been questioned (*Everitt and Webb*, 1958).

Summary

1. The oral administration of one gram of indole-3-acetic acid, in 5 mg daily doses over a period of 200 days in middle age, had no significant effect on either body weight or life duration in the male rat.

2. There were no significant metabolic, excretory, haematological or electrocardiographic changes during the period of treatment. However, in the post-treatment period in old age significant increases were observed in the haemoglobin level and the red cell count. Indole-3-acetic acid also increased the frequency and severity of lung disease at autopsy.

Zusammenfassung

1. Per-os-Gaben von 1 g Indol-3-Essigsäure in 5 mg Tagesdosen während einer Periode von 200 Tagen hat bei männlichen Ratten mittleren Alters keine signifikante Wirkung auf das Körpergewicht oder die Lebensdauer gehabt.

2. Es treten keine signifikanten Stoffwechsel-, Ausscheidungs-, Blut- oder elektrokardiographischen Veränderungen während der Behandlung auf. Dagegen sind in der Periode nach der Behandlung bei alten Tieren signifikante Zunahmen des Hämoglobingehaltes und der Erythrozytenzahl beobachtet worden. Auch die Häufigkeit und Schwere der Lungenerkrankungen nahm bei diesen zu.

Résumé

1° L'administration orale d'un gramme d'acide indole-3-acétique, réparti en doses quotidiennes de 5 mg pendant 200 jours, chez des rats mâles d'âge moyen n'a pas eu d'effet net sur leur poids corporel ni sur leur durée de vie.

2° On n'a constaté aucune modification métabolique, excrétoire, hématologique ou électrocardiographique nette pendant la durée du traitement. Pendant la période qui a suivi, on a cependant observé chez les vieux animaux une augmentation significative du taux d'hémoglobine et du nombre d'hématies. L'acide indole-3-acétique a augmenté également la fréquence et la gravité des lésions pulmonaires.

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