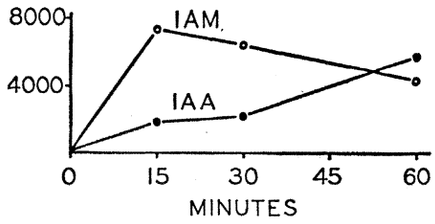

Fig. 1. Rate of formation of IAA and IAM in reaction mixture. The ordinate is count/min.

specific activity of both samples remained constant through three crystallizations.

These experiments indicated that unknown A was indoleacetic acid and unknown B was indoleacetamide.

Because it is possible that IAM was formed as an artifact by ammonolysis of an ester of IAA (*5*) when the chromatogram was developed in the isopropanol, ammonia, and water mixture, other chromatograms of the reaction products were developed in 70-percent ethanol or in deionized water. Only IAM and IAA were detected. These were in amounts similar to those on chromatograms developed in the mixture of isopropanol, ammonia, and water. Apparently, therefore, ammonolysis was not involved in IAM production. Because good separation of IAA and IAM is obtained with this mixture as solvent, it was used to develop all subsequent chromatograms of the two compounds.

Figure 1 illustrates the rate at which IAM and IAA appeared in the reaction mixture. IAM increased rapidly during the first few minutes of incubation, reached a maximum within 15 minutes, and decreased slowly thereafter. On the other hand, IAA increased slowly throughout the incubation period and appeared to accumulate as the end product. The amount of tryptophan in the reaction mixture decreased to approximately 50 percent within 15 minutes and remained relatively constant thereafter. When $10^{-3}M$ D-tryptophan was used as the substrate, neither IAM nor IAA was detected in the reaction mixture. Both products were detected, however, when either $10^{-3}M$ DL- or L-tryptophan was used as the substrate. Therefore, it appears that the L-isomer is utilized preferentially.

Enzyme preparations aged by storage at 4°C retain the capacity to convert tryptophan to IAM for several weeks, but rapidly lose the capacity to convert IAM to IAA, suggesting that at least two enzymes are involved in the reactions.

A number of compounds ($7 \times 10^{-4}M$ NaN_3, $7 \times 10^{-4}M$ KCN, $7 \times 10^{-5}M$ chlorogenic acid, and catalase) inhibited the conversion of tryptophan to IAM. Potassium cyanide at $7 \times 10^{-4}M$ was the most effective, inhibiting the reaction 73 percent. Incubation of the reaction mixture under an atmosphere of nitrogen reduced the conversion of tryptophan to IAM by 77 percent and indicated that oxygen is required for the reaction.

Several pathways have been suggested for the conversion of tryptophan to IAA (*2*, *6*). These are represented by the following reactions or their modifications.

1. tryptophan→tryptamine→ indoleacetaldehyde→IAA.
2. tryptophan→indolepyruvate→ indoleacetaldehyde→IAA.
3. tryptophan→indoleacetonitrile→IAM→IAA.

Only the last scheme includes IAM as an intermediate. This scheme suggests that IAM arises from indoleacetonitrile (*6*). There is very little evidence from studies on enzymes to confirm the occurrence of the last pathway. Earlier reports of the natural occurrence of IAM were open to question when it was shown that IAM may be formed by ammonolysis if NH_4OH were used in the developing solvent (*5*).

The evidence presented in our studies indicates that IAM is an intermediate in the synthesis of IAA from tryptophan in *P. savastanoi* and that the sequence of reactions is: tryptophan→IAM→IAA. Recently, Riddle and Mazelis (*7*) showed that preparations from cabbage convert tryptophan to IAM, thus providing evidence of a similar pathway in higher plants.

Because no radioactive products other than IAM and IAA could be detected in the reaction mixtures in the present studies, it appears that the conversion of tryptophan to IAM might be catalyzed by a single enzyme. A reaction of this type has been discovered and studied by Mazelis (*8*) who showed that methionine is converted to 3-methylthiopropionamide by peroxidase from horseradish. The aforementioned compounds which inhibited the conversion of tryptophan to IAM according to Mazelis also inhibited the conversion of methionine to 3-methylthiopropionamide. Moreover, enzyme preparations used in our studies showed high peroxidase activity when assayed by the method of Willstätter and Stoll (*9*). The second step, the hydrolysis of indoleacetamide to indoleacetic acid, could be catalyzed by an amidase. Reactions of this type are well known (*10; 11*).

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Experimental Differentiation between Phototaxis and Motility in Chlamydomonas snowiae

Abstract. *A reversible inhibition of phototaxis by acetic acid is demonstrated in* Chlamydomonas snowiae. *The inhibition is dependent on the* pH *of the medium and on the concentration of the inhibitor and does not act by affecting motility. Some substances closely related to fatty acids are also shown to be inhibitors of phototaxis, while certain metabolites have no effect. The possible mechanism by which the phototactic response is prevented is discussed.*

The phototactic response of *Chlamydomonas snowiae* is extremely variable (*1–3*). Sachs and Mayer (*2*) found that the cells in a culture might be fully motile yet would not respond to a light stimulus. Although a number of compounds that inhibit phototaxis are known, all of them appear

to exert their effect by inhibiting motility itself, rather than by inhibiting the directed movement caused by a light stimulus (*3, 4*). Any means of differentiating experimentally between motility and phototaxis would therefore be of great importance in understanding the mechanism of the phototactic response. We have found that acetic acid fulfills this requirement.

Cultures of *Chlamydomonas snowiae* were grown in the medium described by Hutner and Provasoli (*4*). Motility was observed directly under the microscope and phototaxis was examined by a method (*1*) that includes visual scoring, which we tested for reproducibility by asking independent observers to grade the responses obtained in critical experiments. Agreement was always good.

The addition of acetic acid to cultures of *C. snowiae* inhibited the phototactic response without affecting motility in any way. From Table 1 it is evident that the inhibition of phototaxis by acetic acid is dependent on the *p*H of the medium and on the concentration of the acid, and that it is the nonionized form of the acid which is effective (the *p*K of acetic acid is 4.6). The effect of the acid at lower *p*H values could not be studied because the motility of the algae used as controls was affected at *p*H values lower than 5.0, apparently because of loss of flagella (*1*).

In cultures treated continuously with acetic acid, inhibition of phototaxis persisted even after 14 hours. The inhibition by acetic acid was also reversible. If the cells were removed from acetic and resuspended in fresh culture medium without acetic acid, the phototactic response was fully restored. The inhibition could also be reversed by changing the *p*H of the medium. Thus, at *p*H 5.6, phototaxis was inhibited by the addition of acetic acid to a final concentration of $50 \times 10^{-4} M$. If the *p*H was then adjusted to 6.5, phototaxis was again restored. This procedure was repeated a number of times on the same culture and in each instance phototaxis was inhibited by the lower *p*H but could be readily observed at the higher *p*H.

A number of substances closely related to acetic acid were also tested for their effect on the phototactic response of *C. snowiae*. Fluoroacetic acid was ineffective at *p*H 5.4, but since its *p*K is lower than that of acetic acid, its inability to cause inhibition might be due to the greater degree of ionization at the *p*H values studied. Both butyric acid and propionic acid were effective in inhibiting phototaxis without affecting the motility of the organisms (Table 2). As with acetic acid, the effects of these substances could not be studied at *p*H values lower than 5.0, and to achieve the same degree of inhibition as acetic acid, higher concentrations of butyric and propionic acids were required. The effect of these two acids was also dependent on the *p*H of the medium.

Table 1. The effects of different concentrations of acetic acid and of changes in the *p*H of the medium on the phototactic response of *Chlamydomonas snowiae*. Degree of response indicated by: +++, very strong; ++, strong; +, fair; ±, weak; −, absent.

Concentration of acetic acid ($10^{-4} M$)	Degree of response				
	*p*H 6.3	*p*H 6.1	*p*H 5.8	*p*H 5.6	*p*H 5.4
0	+++	+++	+++	+++	+++
50	−	−	−		
12.5	+	+	−		
7.5		+	+	−	
3	++	++	++	−	

Since higher fatty acids are practically insoluble in water we tested the effects of detergents, which may be regarded as derivatives of fatty acids. We chose Tween 20 and Tween 80, which contain laurate and oleate residues, respectively. Both compounds were extremely effective in inhibiting phototaxis in the *p*H range tested, 5.8 to 6.7, at a concentration of 0.05 percent (approximately $4 \times 10^{-4} M$). However, this effect has not been studied in greater detail. The differences in the effectiveness between fatty acids and their derivatives could be due to differences of permeability. A number of metabolites were also tested for their effect on phototaxis. Glucose, fructose, sucrose, malic acid, succinic acid, and pyruvic acid all failed to affect the phototactic response of *C. snowiae* in the concentration range in which acetic acid was effective. Substances which block choline esterase, such as parathione, did not inhibit the phototactic response, but this may indicate only that the mechanism of stimulus transmission in algae is different from that in higher organisms.

There are three possible explanations which might account for the inhibition of phototaxis by acetic acid and other agents: (i) the perception of the light stimulus might be affected in the presence of acetic acid; (ii) the transmission of the stimulus from the receptor site to the responding site might be prevented; or (iii) the normal orienting response of the flagella might be prevented.

It seems unlikely that perception of the light stimulus is affected, since the effect of acetic acid is readily and rapidly reversible, and the inhibition of the phototactic response is not influenced by the intensity of the light stimulus, in the range 550 to 33,000 lu/m^2 (50 to 300 ft-ca), white light. The possibility that acetic acid interferes with the light-absorbing pigment system seems unlikely, but cannot be excluded, since the light, though absorbed, might not evoke a response at the receptor

Table 2. The effects of different concentrations of propionic acid (a) and butyric acid (b), and of changes in the *p*H of the medium on the phototactic response of *Chlamydomonas snowiae* (symbols as in Table 1).

Acid concentration ($10^{-3} M$)	Degree of response							
	*p*H 6.6		*p*H 6.4		*p*H 6.1		*p*H 5.8	
	a	b	a	b	a	b	a	b
0	+++	+++	+++	+++	+++	+++	+++	+++
10	+	+	−	−	−	−	−	−
5	+	+	+	±	−	−	−	−
2.5	++		++	++	+	+	+	±
1			++	++	+	++	±	±

site. At present, the second and third explanations, which may be termed transmission and translation of the stimulus, respectively, seem the most probable, but it is impossible to differentiate between them; we need to know more about the mechanism of flagellar contraction and the way in which it results in directed movement of the organism. However, the fact that it is possible to differentiate between motility in general and directed movement caused by a light stimulus should help to further investigations into the mechanism of the phototactic response (*5*).

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Angiotensin II: Its Effects on Corticoid Production by Chicken Adrenals in vitro

Abstract. *Mammalian angiotensin II, added to the incubation medium, failed to increase corticoid production by chicken adrenal tissue in vitro. Mammalian corticotropic hormone (ACTH) stimulated both aldosterone and corticosterone production.*

Angiotensin II, in addition to its known pressor activity (*1*), has been implicated in the regulation of aldosterone secretion by the adrenal cortex of the human (*2*) and dog (*3*). Kaplan and Bartter (*4*) recently demonstrated that angiotensin II, like ACTH (corticotropin), acts directly on bovine adrenal cortex tissue in vitro, stimulating the production of aldosterone, as well as corticosterone and cortisol. However, angiotensin II failed to stimulate significantly the production of aldosterone and corticosterone by the adrenal cortex of the laboratory rat.

Angiotensinogen, the inactive precursor of angiotensin, has been demonstrated in chicken plasma. Renin, the enzyme responsible for the liberation of angiotensin from angiotensinogen, has been extracted from chicken kidney tissue. The concentration of angiotensinogen and renin in the chicken approximate the concentration determined for the dog, cow, and rat (*5*). Accordingly, it was of interest to examine the possibility that the avian renin-angiotensin system is involved directly in the biosynthesis of aldosterone and corticosterone by the chicken adrenal (interrenal) glands.

Table 1. Effects of angiotensin II and ACTH on steroidogenesis by chicken adrenal tissue. All data presented per 100 mg of incubated tissue.

Angiotensin II (μg)	ACTH (units)	Number of determinations	Steroid production* (μg)	
			Corticosterone	Aldosterone
None	None	4	0.23 ± 0.08	0.33 ± 0.11
5	None	2	0.13 to 0.62	0.13 to 0.55
25	None	4	0.16 ± 0.07	0.15 ± 0.06
50	None	3	0.00 to 0.18	0.00 to 0.18
25	1.0	1	4.90	0.73
None	1.0	4	6.64 ± 0.66†	1.39 ± 0.19†

* Either the mean $\pm$ the standard error, or the range is given. † Significant increase in corticosterone production ($p < .05$) and aldosterone production ($p < .05$) with added ACTH.

One hundred and forty-four, inbred, white leghorn cockerels were obtained as one-day-old chicks and raised in an incubator until they were 32 to 38 days old. Tissue was obtained from the adrenal glands, and after a preincubation period of 30 minutes, the incubation medium was changed and discarded. The tissue was then incubated for an additional 3 hours with a change of medium after 1½ hours. Further details of the procedures used for incubation, extraction, chromatography, and quantitative evaluation, are described by deRoos (*6*). The angiotensin II (*7*) and ACTH (*8*) were added to the incubation medium after the preincubation period and were renewed at the second change of the medium. Different concentrations of angiotensin II were used in three series of experiments. Additional experiments with ACTH, or angiotensin and added ACTH, and control experiments, were performed. The results are presented in Table 1.

The addition of angiotensin II to the incubation medium failed to result in an increase in corticoid production. In contrast, the addition of mammalian ACTH resulted in a significant increase in both aldosterone and corticosterone production.

The failure of mammalian angiotensin II to stimulate corticoid production by the chicken adrenal would not appear to be due to the dose levels employed. A similar experimental procedure, in which 4 μg of angiotensin II per 100 mg of incubated tissue was used, resulted in a significant stimulation of corticoid secretion by the bovine adrenal cortex (*4*). Our data suggest that the renin-angiotensin system is not involved directly in the biosynthesis of aldosterone or corticosterone by the chicken adrenal. The possibility of species specificity within the renin-angiotensin system represents an alternative explanation. Mammalian renin has been reported to cause a pressor response only among mammals, and chicken renin to be active only with chicken blood—that is, angiotensinogen (*5, 9*). However, chicken angiotensin II, obtained by incubation of chicken renin with homologous serum, resulted in a pressor response when administered to rats (*5*). It remains to be demonstrated whether chicken angiotensin II will stimulate corticoid production in mammals. This would appear to be probable, since mammalian studies suggest that the pressor and aldosterone-stimulating activities of angiotensin II depend on the same functional groups (*10; 11*).

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