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〔Review〕

Occurrence of Phytohormone "Auxin" in Human and Animal Organs

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Introduction

The most important phytohormone "auxin" (indole–3–acetic acid) was first isolated from human urine in 1934 by Dutch biochemists. This phytohormone has also been found to be synthesized in human tissues and a variety of animal organs. However, the physiological significance of the plant hormone in humans and animals is not well understood.

On the other hand, steroid hormones (brassinosteroids) have recently been found to be produced in plant bodies and to play a regulatory role in plant growth. These facts suggest that phytohormones may play some regulatory role in humans and animals and steroid hormones in plants. This review will discuss the occurrence of auxin (IAA, indole–3–acetic acid) in human and animal bodies and its possible physiological role in animals as well as in plants.

The definition of "hormone" in the human and animal kingdom is a chemical substance produced in a gland and transported through the bloodstream to a target organ to function there in a minute amount. Plants, in contrast, have no specific glands nor target organs. However, the so-called phytohormones are produced in different organs such as meristems, leaves or roots and are transported to other organs where they act in minute amounts. Therefore, plant scientists conventionally use the term "hormone" for plant growth regulators biosynthesized in plant bodies.

A. Phytohormones

It was who first used the term "hormone" in botany was the German botanist Hans Fitting to refer to a substance present in the ether extract of pollen which promoted growth of unfertilized ovary (Fitting, 1910). Laibach (1932, 1933) found that the ether-extract not only from plant pollen but also from urine and other animal tissues caused growth of unfertilized ovary and of coleoptiles of graminaceous plant seedlings.

As reviewed previously (Masuda, 1998), the existence of phytohormones was suggested early in the middle of the 19th century by Julius Sachs as organ-forming substances and by Charles Darwin as the substance carrying the light stimulus to cause phototropism. Later, Boysen-Jensen (1910) offered experimental evidence that the light stimulus was transported in plant body as a substance passing through wounds and even through a layer of gelatin or agar. Finally, the Dutch botanist F. W. Went isolated a substance from the tip of oat coleoptiles in an agar block in 1928. He named the substance "Wuchsstoff " (growth substance) and said "Ohne Wuchsstoff, kein Wachstum" (without growth substance there is no growth) (Takahashi and Masuda, 1994).

In 1933 Kögl and his co-workers claimed to have isolated and crystallized IAA from human urine. Kögl must have obtained a hint from Laibach's study. The pollen hormone extracted earlier by Fitting and Laibach was later

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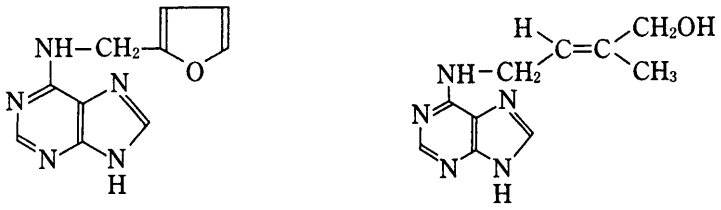


Kinetin　　Zeatin

Fig. 1　Chemical structures of a synthetic cytokinin, kinetin (left) and a natural cytokinin, zeatin (right)

found to be IAA.

The IAA isolated from human urine showed a growth-promoting effect in plants, but it took some time for plant scientists to realize that IAA was a "phyto"-hormone. Haagen-Smit and his co-workers finally isolated IAA from plant materials after World War II in 1946, and many botanists then isolated IAA from a variety of plants and plant organs. Curiously, long before the discovery of Haagen-Smit et al. (1946), the term "phyotohormones" to include IAA had been in use, since F. W. Went and K. V. Thimann published a book entitled "Phytohormones" in 1937.

The second oldest plant hormone is a group of gibberellins which were discovered and first isolated by Japanese agricultural chemists at the Imperial University of Tokyo at about the same time as auxin. After World War II, more hormones were discovered, such as cytokinins, ethylene, abscisic acid, and most recently brassinosteroids. Although those substances are called phytohormones, the suggestion has been made that they be called "plant regulators" or "plant growth substances" (A Committee of the American Society of Plant Physiolgists. Plant Physiol. 29 : 307, 1954).

Among the six groups of phytohormones, auxin was first isolated from human urine and the first non-naturally-occurring cytokinin, kinetin (Fig. 1) was isolated from fish (herring) sperm DNA which had been stored for a long time (Miller et al., 1955). Skoog and his co-workers, during their studies on tissue culture, tried to find naturally-occurring substances which might enhance cell division in vitro. They found a conspicuous promoting effect of stored herring DNA on tobacco tissue culture growth but not with fresh DNA. When they autoclaved the fresh DNA at 120 C° at pH 4.3 for 30 minutes, the treated DNA showed cell multiplication activity on the tobacco tissue culture. The herring DNA was hydrolyzed and then neutralized, extracted with *n*-butanol, subjected to column-chromatography through Dowex 50, and eluted with 1.5 N HCl. The active fraction was re-chromatographed and eluted with 0.1 N ammonia, and the eluate formed a crystal which was identified as kinetin (6–furfuryl adenine). Later, in 1963 a naturally-occurring substance, zeatin (Fig. 1), was discovered (Letham, 1963) and kinetin was listed, not as a naturally-occurring cytokinin but as a synthetic cytokinin.

B. Isolation of IAA from urine

Kögl and his co-workers in Utrecht, the Netherlands, first extracted substances from urine and identified two curious substances, namely, "auxin a" and "auxin b" by a complicated chemical procedure (1931, 1933). Starting with 150 liters of human urine, which was concentrated and extracted with ethyl ether and purified by several steps with different solvents, they finally obtained 0.04 g of active principle. The extent of purification from evaporated urine was reported to be about 20,000 to 50,000 times.

Their finding aroused tremendous attention : Botanists around the world became extremely interested in these two substances which regulated plant growth and asked for samples. Others tried to repeat the extraction procedures to isolate the same substances but in vain. Unfortunately, World War II broke out and research activity in the Netherlands came to a halt. The extractions and purification experiments had been mainly carried out by one of

Kögl's co-workers, H. Erxleben, a woman assistant, who during the war was said to have returned to her home in Germany, also Professor Kögl's home country. After the war, it was said that she wanted to return to the laboratory but was not allowed to. In 1950 at the International Botanical Congress held in Stockholm there was a special committee meeting of scientists studying hormones and they tried to come to a decision of whether or not auxins a and b really existed. All votes except one, by F. W. Went, were negative, which led to the conclusion that auxins a and b did not exist unless new, reliable evidence appeared. In 1967 when the laboratory of organic chemistry at Utrecht University, where Kögl and his co-workers isolated auxins a and b from urine, was re-built, glass vials with the labels of those substances were discovered in the old laboratory. After being subjected to modern chemical analysis including GC–MS, the substances in the vials were found to be entirely different from what they had been claimed to be (Vliegenthart and Vliegenthart, 1967). Many opinions have been expressed on this "scandal", most being severely critical. Professor Hans Burström of Lund University, Sweden, the present author's former senior co-worker, once said "Kögl was factually and morally guilty of fraud. We should perhaps blame the nearly hectic competition in biochemistry," suggesting that the fraud was due to excessive competition in the scientific world.

Kögl and his co-workers (1934), however, also isolated and identified a third substance from urine, termed "heteroauxin" which was entirely different in chemical structure from the "genuine" auxins. After those auxins were found to be non-existent, the name "heteroauxin" disappeared, and this substance was found to be the actual naturally-occurring auxin, and therefore called "auxin" (Fig. 2).

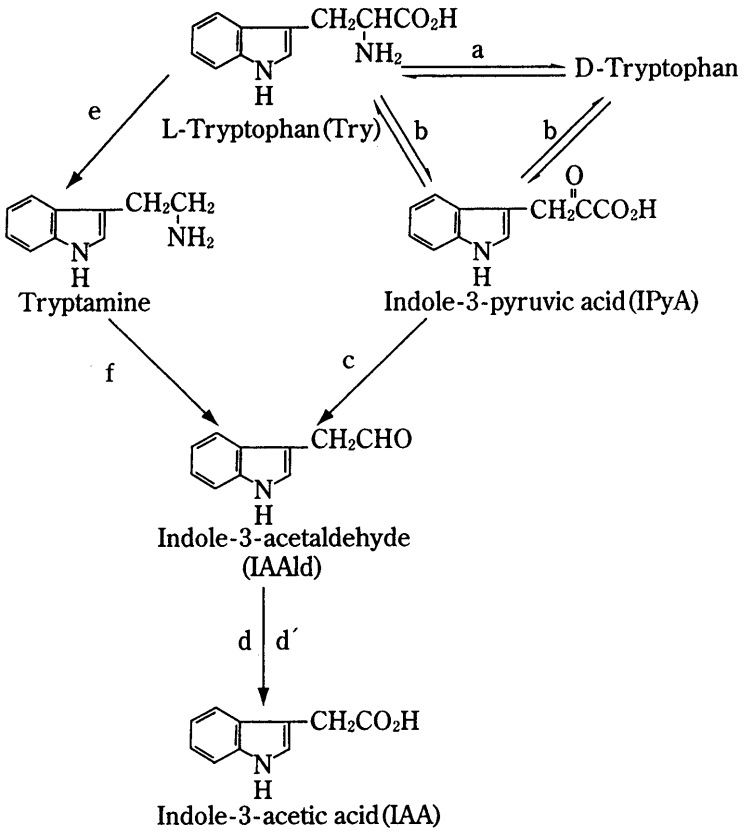
The precursor of auxin (IAA) was found to be an amino acid tryptophan which is converted to IAA via different pathways (Schneider and Wightman, 1974; Fig. 2). The other phytohormones have different precursors, namely, gibberellins and abscisic acid are from mevalonic acid; cytokinins from adenine; and ethylene from methionine.

C. IAA biosynthesis in plant and animal organs

IAA is usually produced in buds, or young, growing organs in plants (e. g. Wightman, 1977). The IAA production in the tip and young leaves is high and decreases as the leaf ages. However, senescent tissues often produce large amounts of IAA, as indicated in Table 1 (Wheeler, 1968) as there is a "second increase in IAA content in senescent tissues." These tissues display high catabolic activity, leading to degradation of macromolecules including proteins, resulting in an increase in the amount of free amino acids. Thus, in senescent tissues, the amount of substrate for IAA biosynthesis, i. e. tryptophan, is abundant, resulting in active production of IAA.

As shown in Fig. 2, two major pathways from tryptophan (Try) to IAA are known: (1) the indole–3–pyruvic acid (PyA) pathway and (2) the tryptamine pathway. The first is the main pathway in plants. IAA biosynthesis involves several enzymes as shown in Fig. 2; tryptophan aminotransferase (b, from tryptophan to PyA), indole-3–pyruvic acid decarboxylase (c, from PyA to indole–3–acetaldehyde, IAAld), indole–3–acetaldehyde oxidase (d, IAAld to IAA) and indole–3–acetaldehyde dehydrogenase (d', IAAld to AA), tryptophan decarboxylase (e, Try to tryptamine) and tryptamin oxidase (f, tryptamine to IAAld). As shown in Table 2, the Km values for some of the enzymes involved in IAA biosynthesis are relatively high, as compared with those involved in protein synthesis. This suggests that amino acids including tryptophan are preferentially used for protein synthesis when the amount of free amino acids is limited, but may be consumed for IAA synthesis if an excess amount of tryptophan in senescent tissues is available by protein degradation. This may be the reason for the secondary increase in IAA synthesis in senescent tissues.

As mentioned above, Kögl and his co-workers first isolated IAA from human urine. To explain the origin of IAA in human urine, Weisbach et al. (1959) hypothesized that IAA is produced in the liver by metabolism from the proteinous tryptophan contained in food. It was also supposed (Went and Thimann, 1937) that the relatively high concentration of auxin in urine is attributed to the ingestion of foods containing the hormone and to the metabolic



a . tryptophan racemase
b . tryptophan aminotranspherase
c . indole–3–pyruvic acid decarboxylase
d . indole–3–acetaldehyde oxidase
d'. indole–3–acetaldehyde dehydrogenase
e . tryptophan–decarboxylase
f . tryptamin oxidase.

Fig. 2 Biosynthetic pathways, 1) indole–3–pyruvic acid pathway and 2) tryptamine pathway, from tryptophan to indole–3–acetic acid (IAA)

Table 1 Amount of free IAA in *Phaseolus* (bean) leaves (from Wheeler, 1968)

Age of leaves, days	IAA content, ng/g
3	143
7	15
19	9
40	196

Table 2 Km values of the enzymes involved in IAA biosynthesis

Enzyme	plant organ	Km	Reference
Tryptophan aminotranspherase (EC 2. 6.1)	*Phaseolus* shoot	0.33 mM	Truelsen, 1972
〃 〃	*Lycopersicon* shoot	5.0 mM	Gibson et al., 1972
Indole–3–acetaldehyde Oxidase	*Avena* coleoptile	0.35 mM	Rajagopal, 1971
〃 〃	*Pisum* epicotyl	1.4 mM	Miyata et al., 1972
Tryptophan Decarboxylase (EC 4. 1. 1. 28)	*Lycopersicon* shoot	3.0 mM	Percival & Purves, 1974

byproducts of intestinal bacteria. However, it turned out that the major fraction of urinary and perhaps fecal IAA of humans and animals derives from activity of endogenous enzymes intrinsic to the animal (Gordon et al., 1972). Also, Shimojo (1972) reported that the amount of Salkowski's reagent* positive substances (IAA) in human urine was higher in cancer patients than in healthy people. These studies suggest that human organs are capable of synthesizing IAA from proteinous tryptophan and the activity could be higher when cells are actively dividing. Yamaki et al. (1979) extensively studied IAA by a bioassay and GC–MS assay and found it to be synthesized in dividing human cells and cancer organs. Thus, human organs and possibly animal cells in general may have an enzyme system for IAA biosynthesis from the amino acid tryptophan.

(* Salkowski reaction : a chemical assay for IAA)

D. IAA biosynthesis in animal

Gordon and Buess (1967) determined biosynthesis in a variety of animal organs and obtained the following results. First, IAA and the enzymes that convert tryptophan to IAA are found in many animals though not in all of their organs. Organs relatively rich in the enzyme are the kidney, liver and testis. They also found that the enzyme is similar to that of plant origin in that it responds to exposure of the organism to X–ray radiation. However, there is a qualitative difference in response. The activity rises in mouse liver after total-body doses on the order of 100 R and is depressed by doses in the kR range. In plants, inhibition of the enzyme follows single doses of X–radiation. They suggested that the response of the liver enzyme to X–rays may be interpreted as an indirect endocrine phenomenon. Finally, they suggested that IAA biosynthesis from tryptophan and its responses to X–ray may reflect control of tryptophan transaminase activity.

Gordon and Buess (1967) interpreted the IAA synthesis in animal organs as an indirect endocrine phenomenon because (a) there is a small stress-induced stimulation by low doses of X–ray radiation, (b) the activity in fetal liver (and kidney) is high ; it drops at birth and slowly climbs to high titer again in the mature animal ; (c) the enzyme appears to be subject to substrate adaptation in vivo ; and (d) adrenalectomy virtually abolishes the radiation response.

Some of the results reported by Gordon and Buess (1967) are introduced in Table 3. Their results show no apparent correlation between the measured contents of free auxin and enzyme in the various organs examined. Weinsbach et al. (1959) previously reported similar results, showing that among the animal organs tested guinea pig kidney had the highest activity in IAA biosynthesis..

However, results reported by Gordon and Buess (1967) on the auxin-forming activity in the liver of newborn rats are interesting (Fig. 3). The enzyme titer is high in fetal rat liver, drops precipitously after birth and then rises to fetal levels as the animal matures. They reported a similar phenomenon occurring in rat kidney. Based on their

Table 3 Enzyme and free acid–auxin activities in the kidney and testis of various young adult animals (from Gordon and Buess, 1967)

	Enzyme activity		Free auxin
	Micrograms IAA/hr/tissue	micrograms IAA/hr/mgN	
Kidney			
Guinea pig	218	15	*
Hamster	120	3.5	—
Mouse	11	0.63	—
Dog	4.0	0.29	4
Testis			
Hamster	22	2.0	—
Dog	12	1.6	2
Guinea pig	10	1.0	55
Cat	3.3	0.5	—

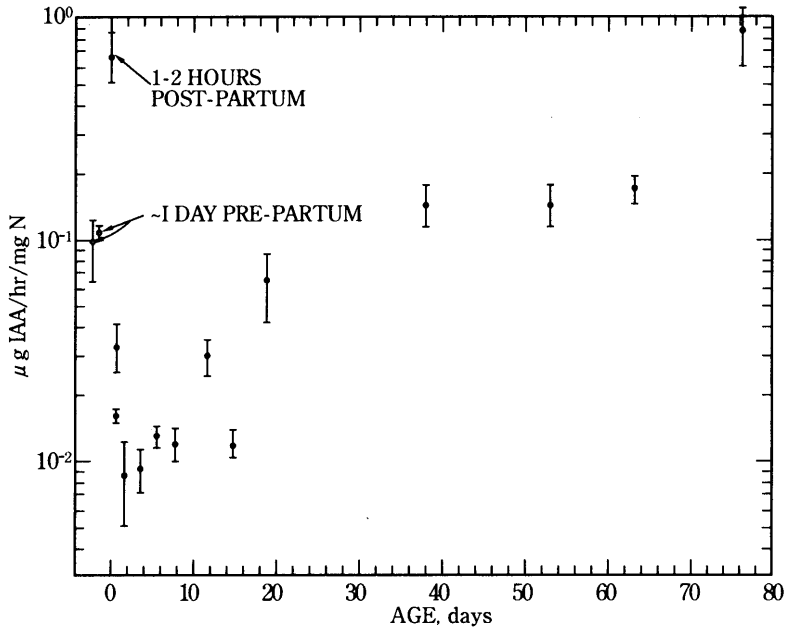


Fig. 3 Influence of age on the auxin-forming capacity in rat liver (from Gordon and Buess, 1967)

results, Gordon and Buess (1967) proposed a possibility that an activator of the enzyme is maternally supplied to the fetus ; and self-activation slowly develops as the animal matures.

They also reported that, when X–ray irradiated animals were compared with the corresponding sham-irradiated controls, the activity of IAA-synthesis (micrograms IAA produced per milligram N per hour) was affected by relatively low levels of ionizing radiation, but exposure of the mice to 50 R resulted in a five-fold increase in enzyme activity, then the radiation-induced enhancement dropped with increasing exposure ; the reductions in titer occur in the range of 1 kR and higher.

It is also interesting that adrenalectomy essentially abolishes both the enhancement and reduction in enzyme titer caused by X–ray irradiation. Thus, they concluded that the effect of X–ray irradiation on IAA biosynthesis in animals is an indirect endocrine phenomenon.

Gordon et al. (1972) also reported that intraperitoneally injected ^{14}C–tryptophan was secreted into urine as ^{14}C–IAA, and that when ^{14}C–IAA was introduced directly into the stomach, it was absorbed and excreted into urine.

E. IAA biosynthesis in cancer cells

As described above, Shimojo (1972) reported that the amount of auxin (Salkowski positive substances, auxin) in human urine was higher in cancer patients than in healthy people. Yamaki et al. (1979) extensively studied the biosynthesis of IAA in cancer cells and the effect of auxin on animal cell division. They found that the amount of IAA in the resected tissues was highest in cancerous regions, followed by that in the surrounding areas and lowest in normal areas, based on examination of human gastric, esophaged, jejunum, colon, rectal and mammary cancers (Fig. 4). They also found that the cell division (first and second) of sea urchin eggs was accelerated by IAA and retardation of embryo development due to anti-auxin (synthetic substances similar to active auxins structurally but with no auxin activity ; they can competitively inhibit the auxin effect) was neutralized by adding IAA. They interpreted their results as suggesting that plant hormone IAA may play a regulatory role in cell division in animals and in cancer multiplication. They also hoped that the determination of IAA in human urine or organs could be used for early diagnosis of cancer.

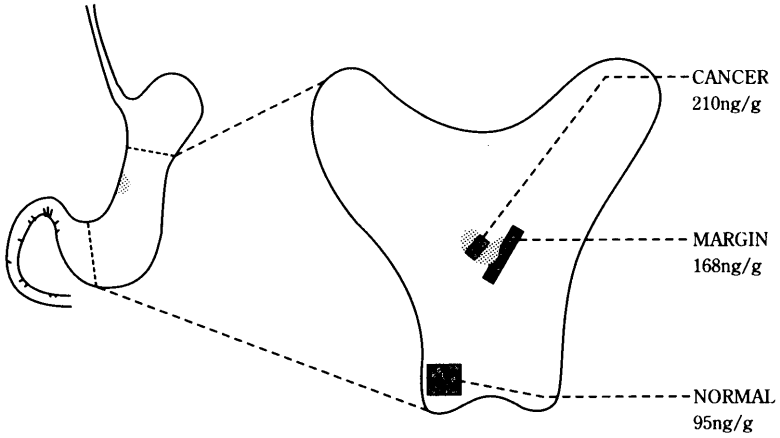


Fig. 4 Distribution of auxin in a stomach with incipient cancer. Dotted part indicates cancer. The excised tissue was cut open and the sections corresponding to the black parts were isolated, and the auxin (ng IAA eq./g FW) was determined using a plant bioassay system (Avena curvature test*) (from Yamaki et al., 1972) (*Avena curvature test : a bioassay for auxin, using young etiolated oat coleoptiles)

Conclusion

Why plant growth substances, auxins, were first discovered in human urine by Kögl and his co-workers has long been puzzling. IAA was subsequently found in microorganisms including fungi and other materials and finally in higher plants. If IAA is biosynthesized in human and animal tissues, it is natural to assume that this plant hormone might have some regulatory significance in human and animals. As introduced in the present review, there have been studies suggesting the role of IAA in human and animal cells, cell division, stress reaction or cancer growth. As suggested by Gordon and Buess (1967), the role of IAA in animal cells may be an indirect one, possibly through some endocrine phenomenon. It would be extremely interesting if auxin does indeed play some regulatory role in animals such as in cell division or cancer growth. Auxin thus may be worthy of further study in relation to humans and animals.

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