

with that of *I*, 11 cm above the patellar articular surface, shows a beginning lateral displacement of bone substance, which implies a beginning shifting of the back part of the vastus medialis muscle.

Femur *VI*, in this way, appears to be really a fossil remain of a *pithecanthropus erectus*.

This fossil femur is certainly not from Trinil but from another part of the Kendeng region. As the two last ones of the four removals of my collection have been effectuated without my direct supervision, they caused much disarrangement of such small and seemingly less valuable specimens. There is, however, some probability that this fossil was found at Kedung Brubus.

The question, why again and again thigh bones and no other limb-bones of *Pithecanthropus erectus* turn up in my collection, may find an answer in the consideration that the plurality of the bones were broken by crocodiles, mostly so the weaker ones, and that in the same proportion the latter were destroyed in the digestion process of those voracious animals.

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**Botany.** — *A physiological analysis of the growth substance.* By A. J. HAAGEN SMIT and F. W. WENT.

(Communicated at the meeting of September 28, 1935).

The investigation of the growth substance of plants in the last few years has passed through a series of phases, of which we find an analogue in the work on sex hormones. Originally "auxin" was isolated from urin, and this substance was regarded as the plant growth hormone (1). After some time it was shown, that two auxins exist, *a* and *b*, which have the same activity as growth substance (2). Serious objections against the specificity of the universally employed avena test were raised, when it was found, that also  $\beta$  indole-acetic acid showed the same bending reaction in comparable dilutions (3). It was then shown, that it is highly probable, that in the top of the avena coleoptile auxin-*a* is formed, whilst  $\beta$  indole-acetic acid is formed by yeast, fungi and bacteria. The easy synthesis of  $\beta$  indole-acetic acid and related substances led to an investigation as to how the growth activity was related to a definite structure. Derivatives and homologues of  $\beta$  indole-acetic acid were synthesized and the activity determined by F. KÖGL and D. KOSTERMANS. Great differences in activity were found (4).

The problem acquired an added interest, when K. V. THIMANN showed that the activity of an active substance in the avena test is based on two independent properties: the polar transport in the coleoptile, necessary for the substance to arrive at the growing cells, and its property to stimulate cell elongation (5). Only those substances, which possess both properties are active in the avena test. THIMANN has shown, that  $\beta$  coumaryl-acetic acid did not cause a curvature in the avena test, but still stimulated the growth of small cylinders of avena coleoptiles, when they are put in a

dilute solution of this substance (cylinder test). An easy method for the estimation of the growth stimulating effect has been found in the pisum test (6). Here also the polar transport of the active substance is not necessary to obtain a reaction.

To get an idea of the specificity of the different tests and the effect of more drastic alterations in the molecular structure of  $\beta$  indole-acetic acid, it was necessary to determine quantitatively the activity of a large number of organic substances. With the help of these three tests, we could study the effect of changes in the chemical structure on the transport capacity and at the same time the effect of these changes on the cell elongation activity.

#### *Method.*

The activity of the solutions of a great number of organic substances has been tested with the pea test (6), the cylinder test (5) and the avena bending test (7). The peas used in the pea test are grown on saw dust in a dark room with a constant temperature of 22° C. After 8 days the zone of 5—35 mm below the terminal bud is cut and split lengthwise. Upon subsequent immersion in water the two halves will bend outwards. If they are put in solutions containing substances inducing growth, the free ends of the two halves will start to bend inwards. After 12 hours the pea shoots are photographed and the inward bending is compared with the result of a series of solutions from  $\beta$  indole-acetic acid as standard.

At the same time in the same dark room the growth of avena coleoptile cylinders is measured. From 4 day old avena coleoptiles the top is removed, the primary leaf is drawn out and after 1½ hours 3 cylinders of 5 mm length are cut from each plant. They are slipped on thin glass rods and the length is measured by means of a microscope with a low magnification. The cylinders are then put in the solutions and the increase in length is measured after 12 hours.

#### *Results.*

In table I the results of the pea test, cylinder test and avena bending test are compared, when different substances are used. The activities of the substances are expressed in terms of the activity of  $\beta$  indole-acetic acid. This substance induced 2 % growth in the cylinder test and a curvature of 10° at a concentration of 1 mg. in 20.000 cm<sup>3</sup> and a curvature of 180° in the pea test at a concentration of 1 mg. in 1000 cm<sup>3</sup>. (When avenas are used in this test this concentration is 1 mg. in 3000 cm<sup>3</sup>.)

The solutions used in the pea test and cylinder test were neutralised; the highest concentrations used contained 1 mg. in 5 cm<sup>3</sup>; if not active in this concentration, they were considered as inactive.

Through the kindness of Prof. KÖGL we could test the derivatives of  $\beta$  indole-acetic acid synthesized by D. KOSTERMANS.

From table I it is evident, that many substances show an activity in the

TABLE I.  
Comparison of growth activity of organic substances with  $\beta$  indole-acetic acid.

Substance	Pea test	Cylinder test	Avena test	Type of bending
$\beta$ Indole-acetic acid . . . . .	1	1	1	normal
Isopropyl-indole-acetic ester . . . . .	1	0.1	0.005	..
5-methyl-indole-acetic acid . . . . .	1	0.2	0.1	..
$\beta$ indole-pyruvic acid . . . . .	0.5		0.2	..
1-methyl-indole-3-acetic acid . . . . .	0.3	0.2	0.002	in the top
2-5 dimethyl-indole-3 acetic acid . . . . .	0.2	0.05	0.002	..
2-methyl-indole-3-acetic acid . . . . .	0.2	0.05	0.001	..
2-methyl-indole-3-methyl acetate . . . . .	0.5	0.1	inactive	
2-ethyl-indole-3-acetic acid . . . . .	inactive	inactive	..	
Phenylacetic acid . . . . .	0.1	0.01	0.0002	..
Phenylpropionic acid . . . . .	0.02		inactive	
Methyl-phenylacetate . . . . .	0.05			
Isobutyl-phenylacetate . . . . .	0.05			
Ethyl-phenylpropionate . . . . .	0.01			
Cinnamic acid . . . . .	inactive	inactive	inactive	
Allo-cinnamic acid . . . . .	1	0.2	0.0006	in the top
Cis-o-methoxy-cinnamic acid . . . . .	0.05	0.002	inactive	
Isatinic acid . . . . .	0.05	0.02	0.002	
Atrolactic acid . . . . .	0.005	0.005	inactive	
m. nitro-cinnamic acid . . . . .	0.02			
m. nitro-phenylacetic acid . . . . .	0.05			
p. methylcinnamic acid . . . . .	0.0005			
m. Phenylene diacetic-ethyl ester . . . . .	0.05			
Vulpinic acid . . . . .	0.002		inactive	
Pulvinic acid . . . . .	0.002			

Inactive in the pea test are also: coumarin, o-coumaric acid, cinnamic acid,  $\alpha$ -methyl-cinnamic acid,  $\alpha$ -phenylcinnamic acid, trans-o-methoxy-cinnamic acid, p-methoxy-cinnamic acid, cinnamic-ethyl ester, benzilic acid, phenylglyoxylic acid, benzoic acid, benzalmalonic acid, r-mandelic acid, phenylacetamid, diphenyl-acetic acid, phenylvalerianic acid, p-nitro-phenylacetic acid, 2-4-dinitro-phenylacetic acid, phenylalanin, indolcarbonic acid, phenylamino-acetic acid, acetic acid, propionic acid, butyric acid, chloracetic acid, jodacetic acid, malonic acid, maleic acid, adipinic acid, serin, cysteine, isatin, angelic acid, tiglic acid, crotonic acid, acetophenone, tyrosine, phenylacetate, eosin.

pisum test, which are also active in the cylinder test. Only a few substances which are closely related to indole-acetic acid show an activity with the ordinary avena test. (\*)

Of these derivatives only the 5 methyl-indole-3-acetic acid, the esters of  $\beta$  indole-acetic acid and indole-3-pyruvic acid show normal curvatures in the avena test. In the other substitution products a distinct influence of the diminished velocity of transport can be observed. The curvature then is localised in the upper end of the coleoptile. In the pisum test the difficulties with the transport do not exist and these derivatives and also the esters show an activity of the same order as  $\beta$  indole-acetic acid.

It is obvious that the pyrrol nucleus in indole-acetic acid plays an important part in the polar transport of a substance. Substitution or opening of this nucleus therefore reduces the activity in the avena bending test.

To further study the minimum configuration of the molecule necessary for the growth promoting action, more substances were investigated, which did not contain the pyrrol ring, but where only the phenyl nucleus of indole-acetic acid did remain intact.

Of the phenyl substituted fatty acids only phenylacetic acid and propionic acid were active in the pisum test and cylinder test and showed resp.  $1/10$  and  $1/100$  of the activity of  $\beta$  indole-acetic acid. Benzoic acid and phenylvalerianic acid were inactive, which was also the case in the unsaturated cinnamic acid. The remarkable fact was observed, that the steric isomer, allo-cinnamic acid had the highest activity of these growth promoting substances. This activity was as high as that of  $\beta$  indole-acetic acid in the pea test. That steric conditions play an important part in the activity of these substances was confirmed by the activity of cis-o-methoxy-cinnamic acid, whilst the trans compound was inactive. In the mean time the substitution with the methoxy group has greatly diminished the activity.

In different cases we could obtain a rise in the activity by substitution with methyl, amino and nitro groups. The place of the substituent whether ortho, meta or para is there of great importance, for example: the table on the following page.

Further substitution products have to be tested before general rules concerning the effect of these changes can be established. Then it will be interesting to compare the physical and colloidal chemical behaviour of these substances, which might throw some light on the mechanism of the

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(\*) A. E. HITCHCOCK and coll. (Contr. Boyce-Thompson Inst. 7, 87 and 209) have tested a series of substances as regards their growth stimulating properties by applying them, mixed with lanolin, on one side of the stems of different plants. However the results of STRUGGER and BONNER have not been considered in their experiments, so that the question remains open, whether their substances have a direct effect on cell elongation or whether they change e.g. the *PH* of the cells, influencing the activity of the auxin inside the tissues.

		Activity in pea test	
cinnamic acid, (inactive).....	}	o-nitro-cinnamic acid .....	inactive
		m-nitro-cinnamic acid .....	0.02
		p-nitro-cinnamic acid .....	inactive
		p-methyl-cinnamic acid .....	0.0005
phenylglyoxylic acid, (inactive).....	o-amino-phenylglyoxylic acid ...	0.05	
phenylacetic acid, (0,1).....	}	p-nitro-phenylacetic acid .....	inactive
		m-nitro-phenylacetic acid .....	0.05
		2-4 dinitro-phenylacetic acid ....	inactive

activity of the growth substance in plants. There is no doubt, that the transport property of the auxins and  $\beta$  indole-acetic acid is of fundamental importance for the role as hormone. We could show, that a substance like allo-cinnamic acid with a high growth promoting capacity has lost the ability to inhibit the growth of the side buds, when applied to the top of a *pisum* stem.

Summarizing we may say, that the physiological activity of any substance is based upon the following combination of properties:

1<sup>o</sup>. Its direct effect on cell elongation, once present inside the cell.

2<sup>o</sup>. Its secondary properties, influencing its concentration at the place of action.

a. by affecting the polar transport.

b. by changing the permeation constant.

c. by inactivation before reaching the reacting cells.

It is of interest, that a few of these substances, which are only active in the pea test, occur in nature in different plants. Special attention is drawn to the fact, that lichens contain large quantities of a substance, that shows this growth stimulating properties, namely vulpinic acid. It remains to be seen whether there growth is limited by other factors necessary for elongation of the cells.

### Summary.

An analysis of the cell elongation and polar transport properties of various substances is made.

If activity differences of such substances occur, it has been shown, that primarily they have to be attributed to their secondary properties as transport, permeation etc., whereas their cell elongation properties may

be left unchanged. Both properties can be changed independently by altering different parts of the molecule.

A number of substances is found only having growth stimulating properties, but not showing polar transport in the plant. These substances do not act in the avena bending test, nor do they show the regulative power of the auxins for example in bud inhibition.

A substance showing the highest cell elongation capacity is found in allo-cinnamic acid, which can be compared with that of  $\beta$  indole-acetic acid.

The steric isomer cinnamic acid has no activity.

A preliminary study was made of the effect of substitution on the cell elongation property.

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**Astronomy.** — *The theorem of minimum loss of energy due to viscosity in steady motion and the origin of the planetary system from a rotating gaseous disc.* By H. P. BERLAGE Jr. (Royal Magnetical and Meteorological Observatory Batavia).

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Recent papers by GUSTAF STRÖMBERG<sup>1)</sup> and G. DEDEBANT, PH. SCHERESCHEWSKY and PH. WEHRLÉ<sup>2)</sup> suggested to me that with the aid of a hydrodynamical theorem due to HELMHOLTZ and KORTEWEG, the solution could be found of a problem, which has occupied me ever since I became convinced that the planetary system has evolved from a gaseous disc surrounding the sun<sup>3)</sup>.

As was previously shown<sup>4)</sup>, the equilibrium of a rotating gaseous

<sup>1)</sup> G. STRÖMBERG, The origin of the galactic rotation and of the connection between physical properties of the stars and their motion, *Astroph. J.* **79**, 460 (1934); Formation of galaxies, stars and planets, *Astroph. J.* **80**, 327 (1934).

<sup>2)</sup> G. DEDEBANT, PH. SCHERESCHEWSKY and PH. WEHRLÉ, Sur une classe de mouvements naturels de fluides visqueux caractérisée par un minimum de la puissance dissipée, *C.R.* **199**, 1287 (1934).

<sup>3)</sup> These Proceedings **33**, 614 (1930); **33**, 719 (1930).

<sup>4)</sup> These Proceedings **35**, 554 (1932).