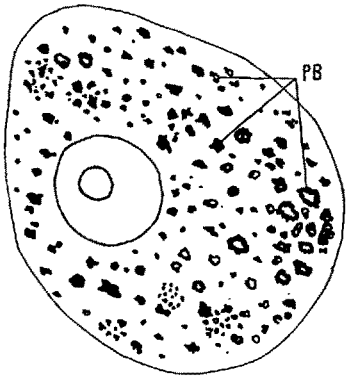

Diagrammatic representation of a reptilian spinal neurone showing the general distribution, and the various sizes and shapes of the pigment bodies (PB).

In formaldehyde-calcium/gelatin and Zenker-, Helly-, Bouin/paraffin sections of the ganglia coloured with ethanolic Sudan black B[4], some pigment bodies appear fairly coloured, while others even in the same site are only partly positive. These bodies give a faint red coloration in periodic acid-Schiff[5] and methyl green/pyronin G[6], react almost negatively to Sudan IV[2], and give a strong positive reaction with chrome alum haematoxylin[7]. The performic acid-Schiff[8] gives capricious results with these bodies, while the plasmal reaction[9,10] responds negatively.

The pigment bodies are, therefore, in all probability, some lipofuscins[2].

Besides these intracellular pigment bodies heaps of brownish-black, barrel- or spindle-shaped pigment bodies also occur surrounding the entire ganglion in the sections. It is noteworthy that unlike the above-mentioned intracellular pigment bodies these bodies defy all the histochemical reactions referred above.

In author's opinion the pigment bodies described above are not the products of secretion from the classical Golgi apparatus as claimed by MOUSSA and BANHAWY[11], since the author[12,13] has not come across any such apparatus or even a remotely comparable structure either in the living or in the processed young neurones. THOMAS[14,15], BAKER[16,17], NATH[18], and MALHOTRA[19,20], also hold identical views. The pigment bodies, on the other hand, appear to have originated as a result of partial or total oxidation of some of the lipid bodies. Lipid participation in the synthesis of pigment has also been advocated by COHN[21].

What, however, is the significance of this oxidation resulting in the accumulation of the pigment, in the economy of neurones, is a matter of speculation at this stage.

NAYAR[22] in the neurones of *Iphita limbata* Stal. (Hemiptera) considers the neurosecretory product as lipofuscins. Whether or not the above mentioned pigment bodies could be correlated with the neurosecretory product of Nayar in *I. limbata* Stal. is a matter of comprehensive investigation, but that the former bear a close histochemical relationship with the latter, inasmuch as both contain in them lipofuscins, is absolutely beyond doubt.

The refractile, pale-yellow pigment observed in the cores of certain duplex lipid bodies (*vide supra*) seems to have originated within the interna of these lipid particulates (Golgi bodies of HIRSCH) in much the same way as described by BAKER[4], HIRSCH[23], LACY[24,25], and KANWAR[26,27].

Résumé. Des lipofuscines, corps pigments jaune-sale à brun-sombre, ont été observées dans les neurones vivants et fixés de quelques reptiles. L'auteur y a aussi constaté la présence de corps pigmentés extracellulaires, noir-brunâtre, de nature histochimique inconnue.

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The Lateral Transport of Indoleacetic Acid-C^{14} in Geotropism[1]

The concept that the upward curvature of geotropically stimulated shoots results from the directed migration of auxin to the lower side has for over 30 years been a prominent feature of the theory of the tropisms. It was DOLK[2] who, by collecting auxin in agar blocks applied to the upper and lower parts of the basal surfaces of horizontal *Avena* coleoptile sections, and assaying the blocks by the standard *Avena* test, first provided clear-cut experimental support for this idea. He found that about 62% of the transported auxin was recovered from the block in contact with the lower side and 38% from that in contact with the upper side. This was true whether the source of auxin was the coleoptile tip, still attached, or, instead, agar containing exogenous auxin applied at the apical cut surface. Some recent workers, however, have suggested that DOLK's data need reinterpretation. They have applied C^{14}-labeled indoleacetic acid (IAA) to horizontally placed plant organs and, after bisecting these organs in the horizontal plane, have been unable to find any difference in radioactivity between the two sides. The results of BÜNNING et al.[3], CHING and FANG[4], REISENER[5], and especially of REISENER and SIMON[6] seem to show clearly that the total amount of auxin in the upper half of

[1] This work was supported by a National Science Foundation predoctoral fellowship to BARBARA GILLESPIE and by a grant from the National Science Foundation, No. G-9084, to Professor K. V. THIMANN.
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a horizontally placed oat coleoptile or seedling root, to which exogenous auxin has been applied, is approximately the same as that in the lower half. On the other hand, DOLK's experiments were successfully repeated on *Avena* by NAVEZ and ROBINSON[7], and on other plants by DIJKMAN[8], BOYSEN-JENSEN[9] and GILLESPIE and BRIGGS[10]. In most of these cases the positive results were obtained by analyzing the agar blocks into which the auxin had diffused. Thus, experiments of this type suggest that it is auxin moving in the polar transport system which mediates the geotropic response[11]. The lateral transport hypothesis ('Cholodny-Went theory') might still be correct, therefore, for the auxin which is being actively transported, but would have to be discarded if it were proved that labeled indoleacetic acid actually carried by the polar transport system undergoes no sidewise displacement. In the present paper experiments are described which, to some extent, distinguish between transported and non-transported auxin and thus allow a direct test of this alternative.

The coleoptiles used were those of *Avena*, var. Segerhavre ('Victory oats'); the methods of purifying the carboxyl-labeled indoleacetic acid ($ICH_2C^{14}OOH$)[12], and of measuring low radioactivity both in agar blocks and in tissue were those of GOLDSMITH and THIMANN[13]. The purity of the indoleacetic acid was checked chromatographically, using 8:1:1 isopropyl alcohol : 28% ammonia : water as solvent. At least 98% of the total radioactivity of the paper chromatograph strip was associated with a single spot which contained all of the detectable Salkowski-positive compounds to be found in the solution of labeled hormone; it was checked that 2% of the Salkowski-positive material could have been detected under the chromatographic conditions employed, had it occurred elsewhere on the paper. The spot moved with the same Rf value as did a control spot of unlabeled pure indoleacetic acid.

The first series of experiments were modeled after the classical investigation of DOLK[2]. A razor blade, coated with stopcock grease, was mounted in a lucite holder. On either side of the blade, agar blocks of washed 1.5% agar were set in such a way that the cutting edge of the blade protruded 1 mm above the surface of the blocks. Next, 20 sections were prepared from *Avena* coleoptiles by removing their apical 1.5 mm and trimming them to a length of 7 mm. The sections were pressed on to the razor blade so that the basal ends were bisected in a plane perpendicular to that of the vascular bundles and rested on the agar. Finally, agar blocks containing 0.4 mg/l labeled indoleacetic acid were set on the apical ends of the coleoptile sections; these blocks were held in place by the lid of the plastic apparatus. After being placed in a small humid chamber, the assembly was set either so that the *Avena* sections were held horizontally, or else so that they were held upright as a control. After 165 min, the coleoptile sections, the 'donor' agar block, and the upper and lower (or right and left) 'receiver' agar blocks were placed in separate planchets. Unused duplicates of the 'donor' blocks were similarly treated. After drying the samples, their radioactivity was measured as described by GOLDSMITH and THIMANN[13]. The results are presented in Tables I and II. Each measurement is corrected for absorption of radiation by the agar or by the tissue in which the labeled auxin was assayed, so that the total amount of activity in each assembly may be computed and compared with the amount known to be present initially in the donor block. The correction factors for agar in Tables I and II are 1.29 and 1.36, respectively, while the correction factors for tissue are 1.84 and 1.90 respectively. The data

Tab. I. Distribution of IAA-C^{14} transported out of *vertical* coleoptile sections into receiver blocks in contact with the basal surfaces. Time of transport, 165 min; initial donor blocks contained 510 cpm[a], average

Figures represent cpm[a], corrected for self-absorption of agar or tissue

A Donor block	B Tissue	C Right receiver block	D Left receiver block	% Recovery $\left[\frac{100(A+B+C+D)}{510}\right]$	% of transported auxin in right block $\left[\frac{100\,C}{C+D}\right]$
286	172	28.6	24.2	101	56.7
288	135	26.4	27.9	94	48.6
306	135	27.4	29.4	98	48.2
306	128	27.0	32.2	97	45.7
296	151	28.5	30.6	98	48.3
305	158	23.6	24.9	101	47.8
302	123	28.2	24.8	94	53.1
322	142	28.8	22.3	101	56.3
268	127	23.6	28.8	88	45.1
303	157	30.2	24.6	101	55.0
289	136	23.2	28.4	94.0	45.0
302	151	24.5	37.8	101	39.4
Average 298	143	26.7	28.0	97	49.4

[a] Counts per min

of Table I, concerning vertical sections, show that the results obtained by this method have good consistency and that the recovery of applied radioactivity is very high. Table II shows that, after applying the same auxin concentrations to horizontal sections, there appears an asymmetry in the distribution amounting to *40.5:59.5*%. The ratio for a duplicate series similar to that in Table II, and also comprising eleven experiments, was found to be *39.1:60.9*. Six other preliminary experiments gave similar results. It will be recalled that DOLK[2] by bioassay found a ratio of 37.5:62.5. GILLESPIE and BRIGGS[10], using *Zea mays* coleoptiles, found 34.0:66.0. Thus *the isotope method confirms the bioassay*, within narrow limits.

The discrepant results reported by other workers have without exception been obtained by halving coleoptiles supplied with IAA-C^{14} and determining the radioactivity in the tissue. This procedure measures not only the IAA in transit, but also any additional IAA which is not being transported. Considerable amounts of such non-transported IAA occur at the uppermost part of the section where IAA enters also by pure diffusion; further, in this part of the section the asymmetric redistribution is only just beginning. A new group of experiments was therefore carried out in which the upper and lower halves of cole-

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[11] See discussion in WENT and THIMANN, *Phytohormones* (Macmillan, New York 1937), p. 160.
[12] We wish to express our thanks to Dr. B. B. STOWE, who worked out the method of synthesis via gramine methosulfate.
[13] M. H. GOLDSMITH and K. V. THIMANN, in preparation. We wish to thank Dr. GOLDSMITH for her cooperation and help.

Tab. II. Distribution of IAA-C^{14} transported out of *horizontal* coleoptile sections into receiver blocks in contact with the basal surfaces. Time of transport, 165 min; initial donor blocks contained 511 cpm, average
Figures represent cpm, corrected for self-absorption of agar or tissue

A Donor block	B Tissue	C Upper receiver block	D Lower receiver block	% Recovery $\left[\frac{100(A+B+C+D)}{511}\right]$	% of transported auxin in upper block $\left[\frac{100\,C}{C+D}\right]$
244	109	30.0	38.1	83	44.0
261	105	27.0	41.4	85	39.8
297	105	28.7	33.2	91	46.4
292	143	30.4	33.8	98	47.3
292	138	25.8	34.4	96	43.0
303	109	20.3	30.2	91	40.2
248	150	27.4	45.6	93	37.6
236	104	19.9	34.2	77	36.7
287	121	24.9	39.0	92	39.0
260	133	23.1	37.5	89	38.1
245	150	21.7	40.2	90	35.0
Average 269	124	25.4	37.1	90	40.5[b]

[b] Duplicate series of 11 experiments averaged 39.1%.

Tab. III. Distribution of IAA-C^{14} within upper and lower halves of horizontal coleoptile sections
Time of transport, 165 min; initial donor blocks contained 511 cpm, average
Figures represent cpm corrected for self-absorption of agar or tissue

A Donor block	B Upper tissue	C Lower tissue	D Receiver	% Recovery $\left[\frac{100(A+B+C+D)}{511}\right]$	% of tissue auxin found in upper half $\left[\frac{100\,B}{B+C}\right]$
250	61.6	70.6	61	88	47.8
316	63.5	72.8	62	101	46.6
310	58.5	77.3	67	101	43.5
284	68.3	77.8	70	99	46.7
276	70.0	72.1	62	95	49.4
296	74.9	72.6	73	102	50.7
319	70.0	66.5	61	102	51.4
296	63.6	65.4	55	95	49.3
286	68.6	68.8	64	96	50.0
296	69.9	82.7	60	101	45.4
306	58.8	71.1	53	97	45.4
Average 294	66.4	72.5	62.5	98	47.8[c]

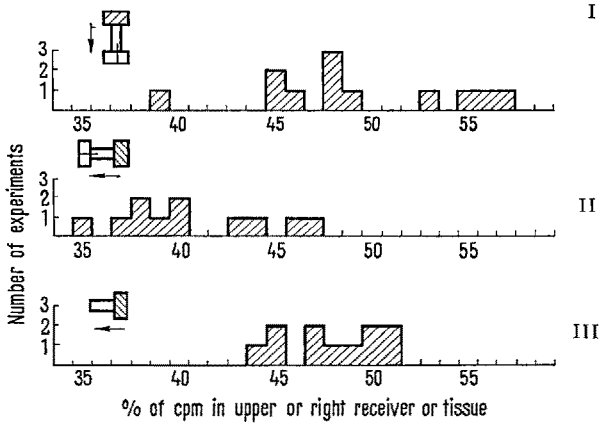
[c] Duplicate series of 6 experiments averaged 47.3%.

optile sections were distinguished by means of carefully placed spots of India ink, and after a 165-minute diffusion period, the sections were placed on the stage of a dissecting microscope in a cold room at 3°C and bisected under red light in the plane which had previously lain horizontal. When the coleoptile sections are removed from the assembly they begin to wilt, and unless frozen quickly their radioactivity begins to disappear. For this reason, the lots of halved coleoptiles were immediately placed in a freezer to await measurement of the radioactivity, without waiting to determine their weights. However, 5 other sets of coleoptile sections were bisected and weighed; the average difference between 20 upper and 20 lower halves was found to be 2.2% with a maximum difference of 5.3%, so that any variation in the precision with which the sections are bisected is not large enough to invalidate comparisons between the radioactivity of the upper and lower halves.

The results of this series are shown in Table III. The corrections were applied as before, the factors being, for agar 1.37, and for tissue 1.76. The total radioactivity found in the tissue agrees very well with that in Tables I and II. The figures do show a small difference between the amounts of auxin on the two sides, but it is barely significant.

The data of all the experiments are summarized in the Figure. It must be concluded that the geotropic response of *Avena* coleoptiles is indeed mediated by lateral redistribution of auxin.

The absence of any clear lateral redistribution in the tissue itself also agrees with the results of other workers[3–6] and the data are compatible with the accumulating body of evidence that not all the auxin present in a tissue is capable of exerting immediate physiological effects. This conclusion is indeed supported by the following simple computation. Since the rate of transport has on several occasions been shown to be 10–12 mm/h, since the rate has been found essentially constant during a 3 h diffusion period, and since the sections are 7 mm long, auxin must pass through them in about $^3/_4$ h; that is, the amount of freely-moving auxin which would be expected to be found in the tissue is that which can be transported in $^3/_4$ h. Table II (columns C and D) shows that 63 cpm are received in 165 min, which means that in $^3/_4$ h only about *23 cpm* are transported. But *124 cpm* were found in the tissue (Table II, column B). Thus some 80% of the tissue auxin found in the experiment of Table III (columns B and C) *cannot be in transit.* If much of this material does not become redistributed by gravity its presence will of course mask the influence of gravity on the transported auxin.



Summary of the experiments of Tables I, II, and III. Each small square represents one complete experiment in which the distribution of auxin between 2 blocks of agar (upper two lines) or 2 halves of the coleoptile section (lowest line) is presented as the percentage found in the upper (or right)half. *Top:* vertical coleoptile sections, % of total transported auxin found in right-hand agar block. *Middle:* horizontal coleoptile sections, % of the total transported auxin found in *upper agar block.* (A second group of eleven experiments gave almost identical results.) *Bottom:* horizontal coleoptile sections, % of the total auxin found in *upper half of coleoptile section.*

Zusammenfassung. Die Hypothese, dass eine geotropische Reizung von Hafer-Koleoptilen eine Querverschiebung des Wuchsstoffes zur Folge hat, ist mit radioaktiver β-Indolylessigsäure (Indol-$CH_2C^{14}OOH$) bestätigt worden. Das Verhältnis der aus der oberen und der unteren Flanke von Koleoptil-Zylindern mit Agarblöckchen abgefangenen Indolylessigsäure wurde gleich 40:60 gefunden.

Die Ursache dafür, dass dieser Effekt in längshalbierten Koleoptilen nicht festgestellt worden ist, liegt im grossen Anteil nicht transportierten Auxins in den Koleoptilen.

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Harvard Biological Laboratories, Cambridge (Mass.), December 5, 1960.

Über neue chemotherapeutisch wirksame Sulfonamide

Im Rahmen von Forschungen in der Pyrimidinreihe haben wir neue 2-Sulfanilamido- bzw. 4-Sulfanilamido-5-alkoxypyrimidine und deren Hydroxy-, Methoxy-, Methylmercapto- und Methylderivate hergestellt. Bei einigen von diesen Stoffen haben wir eine ziemlich protrahierte chemotherapeutische Wirkung bei experimenteller Infektion weisser Mäuse mit *Streptococcus pyogenes* festgestellt und deshalb möchten wir über unsere Ergebnisse eine vorläufige Mitteilung veröffentlichen[1].

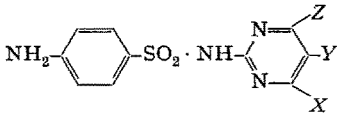
Die chemotherapeutische Wirksamkeit wurde an mit einer 1000fach letalen Dosis von *Streptococcus pyogenes* Tiere, wurde der Wert der relativen chemotherapeutischen Aktivität (R.C.A.) berechnet:

$$\text{R.C.A.} = \frac{1}{n} \cdot \left(\frac{k}{a_1} + \frac{k}{a_2} + \ldots \frac{k}{a_n} \right),$$

wo n die Zahl der Versuchstiere bedeutet, denen die geprüfte Substanz appliziert wurde; a_1, $a_2 \ldots a_n$ geben die Überlebenszeiten der einzelnen Versuchstiere an. Bei den Tieren, die den Versuch (d. h. 10 Tage) überlebt haben, setzen wir $a = \infty$ ein; k bedeutet die durchschnittliche Überlebenszeit der Kontrolltiere.

Von den Monoalkoxyderivaten der Sulfanilamidopyrimidine wiesen die grösste antibakterielle Wirksamkeit das 5-Methoxy- und 5-Äthoxy-2-sulfanilamidopyrimidin (Tab. I; Präp. I-2586, I-1013) auf. Durch Verlängerung der Alkylkette in Stellung 5 sank die antibakterielle Wirksamkeit rasch herab (I-2580, I-2581). Relativ wenig wirksam war auch die 5-Phenoxyverbindung (I-2642). Durch Eintritt einer Hydroxy- oder Methoxygruppe in Stellung 4 ging die Wirksamkeit nahezu verloren (I-2655, I-2583). Von den disubstituierten Derivaten der 2- bzw. 4-Sulfanilamidopyrimidine waren diejenigen am wirksamsten, deren alle drei Substituenten sich gegeneinander in *m*-Stellung befanden (I-890, I-1018), wie es beim 4-Sulfanilamido-2,6-dimethoxypyrimidin (Sulfadimethoxin, Madribon) der Fall ist. Die Lage der Sulfanilamidogruppe in *o*-Stellung zum 5-Methoxyl hat eine starke Verminderung der Aktivität zur Folge (Tab. II; I-2951, I-2654, I-2588). Von Trimethoxyderivaten war das 2-Sulfanilamido-4,5,6-trimethoxypyrimidin (I-2952) beträchtlich wirksamer als das 4-Sulfanilamido-2,5,6-trimethoxypyrimidin (I-2950),

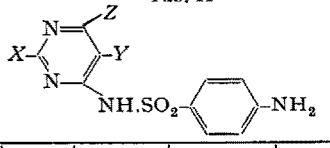
Tab. I



Präparat Nr.	X	Y	Z	Fp. °C	Darstellungsmethode	R.C.A.[a]
I-2586	H	OCH_3	H	214–216	A	0,09±0,07
I-1013	H	OC_2H_5	H	204–205	A	0,14
I-2580	H	OC_3H_7-*n*	H	205–207	A	0,3
I-2581	H	OC_4H_9-*n*	H	224–225	A	0,9
I-2582	H	OC_4H_9-sec	H	206–208	A	0,4
I-2642	H	OC_6H_5	H	237	A	0,48
I-2655	OH	OCH_3	H	262–264	D	0,91
I-2583	OCH_3	OCH_3	H	215	A	0,66
I-1018	OCH_3	H	OCH_3	174–175	A[3]	0,07
I-2952	OCH_3	OCH_3	OCH_3	204–205	A	0,25
I- 890	OH	H	CH_3	251–254	D[4]	0,15

[a] R.C.A. bedeutet die relative chemotherapeutische Aktivität bei experimenteller Infektion der weissen Mäuse unter den im Text beschriebenen Versuchsbedingungen.

Tab. II



Präparat Nr.	X	Y	Z	Fp. °C	Darstellungsmethode	R.C.A.
I-2951	H	OCH_3	H	242	B	0,59
I-2654	CH_3	OCH_3	H	213–214	B	0,48
I-2588	OCH_3	OCH_3	H	263–264	A	0,95
I-2950	OCH_3	OCH_3	OCH_3	201–203	B	0,32
I-2587	SCH_3	OCH_3	H	218–221	C	0,24
I-3063	SCH_3	H	CH_3	210–212	B[5]	0,68
I-3064	SCH_3	H	OCH_3	175–176	A, B	1,0
I-3065	SCH_3	H	OC_2H_5	232–234	A	1,0
I-3066	SCH_3	H	OC_3H_7-i	124–126	A	1,0
I-3068	SCH_3	H	SCH_3	169–171	A	0,95
4-Sulfanilanilamido-2,6-dimethoxypyrimidin, Sulfadimethoxin, Madribon						0,19±0,09
3-Sulfanilamido-6-methoxypyridazin, Sulfamethoxypyridazin, Kynex						0,18
3-Sulfanilamido-1-phenylpyrazol, Sulfaphenazol, Orisul						0,23

intraperitoneal infizierten weissen Mäusen geprüft. Die Substanzen wurden den Tieren in Dosen von 10 mg/kg einmal täglich 5 Tage nacheinander gegeben und nach weiteren 5 Tagen die Ergebnisse gewertet. Aus der Überlebenszeit der einzelnen Versuchstiere und der Kontrollgruppe, das heisst der infizierten, aber nicht behandelten

[1] Diese Mitteilung wird ungekürzt in der Zeitschrift Československ. farm. erscheinen.

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