Induktion geschlechtsgebundener Letalfaktoren durch Äthylurethan (ClB-Methode)

Nr. des Versuches	Injizierte Substanzen	Konzentration	Zahl der geprüften X-Chromosome	Zahl der gefundenen Letalfaktoren	% Letalfaktoren
1	Äthylurethan	m/3 + m/7,8	283	10	3,5
	+ KCl	m/4 + m/7,8	213	7	3,3
Kontrolle	KCl	m/7,8	310	1	0,3
2	Äthylurethan	m/3 + m/7,8	270	8	3,0
	+ KCl	m/4 + m/7,3	197	9	4,6
Kontrolle	KCl	m/7,8	370	3	0,8
3	Äthylurethan	m/4 + m/7,8	64	2	3,1
	+ KCl	m/8 + m/7,8	300	9	3,0
Kontrolle	KCl	m/7,8	408	3	0,7
Summe Versuch 1–3	Äthylurethan + KCl	m/3, m/4, m/8 + m/7,8	1 327	45	3,4
Summe Kontrollen	KCl	m/7,8	1 088	7	0,6
	$\chi^2 = 21\,430$ $N = 1$ $P < 0{,}00001$				

*than*serien eine *Erhöhung* der Mutationsrate auf Werte zwischen 3,0–4,6%. Der Unterschied zwischen den Urethan- und Kontrollserien ist, wie der in der Tabelle wiedergegebene P-Wert zeigt, statistisch gesichert.

Neben den geschlechtsgebundenen Letalmutationen wurden in den Urethanserien auch eine Reihe *sichtbarer* Mutationen beobachtet, die in den Kontrollserien fehlten. Es waren dieses vier *rezessive* geschlechtsgebundene Mutationen (*lozenge, echinus,* eine *aristopedia*ähnliche und eine die Borsten mosaikartig verändernde Mutante), ferner eine Reihe *dominanter* Mutanten (zwei *Minutes,* eine autosomale *brown*-ähnliche und eine autosomale *Deformed*-ähnliche Mutante, schließlich eine den *white*-Locus genetisch und zytologisch umfassende *Notch-Deficiency,* die als Halbseitenmosaik in der F_1 auftrat). Es scheint also das Chromosomen*brüche* bewirkende Äthylurethan bei *Drosophila alle* jene Mutationstypen auszulösen, die auch nach Einwirkung verschiedener Strahlenarten oder der Gruppe der Senfgase auftreten.

Die weitere Analyse der Induktion sichtbarer Mutationen durch Urethan ist Objekt einer zweiten Versuchsanordnung, über die an anderer Stelle berichtet werden soll.

Zum Schluß sei noch kurz die Frage der *Spezifität* der durch Urethan induzierten Mutationen angeschnitten. Wie schon aus der obigen Aufzählung der sichtbaren Mutationen hervorgeht, läßt sich bisher eine Auslösung spezifischer, sichtbarer Mutationen durch Urethan *nicht* erkennen. Ähnliches ergab sich aus der genetischen Analyse 20 induzierter Letalmutationen. Sie zeigte eine zufallsmäßige Verteilung über den gesamten euchromatischen Teil des X-Chromosoms (7 Letalfaktoren lagen im Intervall zwischen *yellow* und *crossveinless,* 5 zwischen *crossveinless* und *vermilion,* 5 zwischen *vermilion* und *forked* und 2 rechts von *forked.* Ein letzter Letalfaktor gab abnorme Spaltungszahlen in der linken Hälfte des X-Chromosoms und konnte daher nicht mitberücksichtigt werden). Von besonderem Interesse ist, daß in 3 Fällen, in denen zwei Letalfaktoren im gleichen Männchen entstanden waren, sich auch diese an ungleichen Stellen des X-Chromosoms befanden. Die zufallsmäßige Verteilung der Letalfaktoren über die gesamte Länge des euchromatischen Teiles des X-Chromosoms deckt sich mit bisher noch unveröffentlichten Feststellungen Dr. MARQUARDTS, die eine gleichmäßige Verteilung 190 analysierter Translokations- und Fragmentationsbrüche über die Chromosomen der *Meiosis* von *Paeonia tenuifolia* ergaben.

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Summary

Injection of sublethal doses of ethylurethane into *Drosophila melanogaster* males increases the mutation rate for sex-linked lethals up to about 3·4%, as compared with 0·6% in the controls. In addition, several recessive and dominant visible mutations were obtained in the urethane series, while none were found in the control series. Up to the present there is no evidence for an induction of specific mutations by ethylurethane.

Effects of Colchicine and other Plant Constituents on Blood Coagulation and of Thrombokinetic Agents and Anticoagulants on Plants

The following are the working hypothesis on which were based the preliminary experiments outlined below: (1) The analogies found by the author between the responses of plants and animals to colchicine and other plant constituents, whether polyploidogenic or not[1,2]. (2) The antagonistic actions in plants and animals of *Viscum album* preparations[3] and heteroauxin to colchicine[2,4]. (3) The influence of colchicine and heteroauxin on protoplasmic viscosity[5] and the rôle of this influence in cell division and in the polyploidizing action of these agents[6]. (4) The analogies between blood clotting and

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Table I

Treatment	Rate of germination %	Length of shoots mm (av.)	Weight of 1 shoot mg (av.)	Lenght of roots mm (av.)	Weight of roots per plant mg (av.)	No. of tumours on roots per plant (av.)	No. of roots per plant (av.)	Weight of tumours on shoots per plant (av.)
Thrombin II	30·0	193	87	146	58	·	4·	·
Thrombin II + Colchicine	45·0	93	57	30	35	3·14	4·2	14·8
Inactivated Thrombin .	15·0	147	62	145	47	·	3·6	·
Inactivated Thrombin + Colchicine	27·5	87	58	41	42	2·26	3·8	6·7
Heparin	22·5	178	88	121	40	·	3·8	·
Heparin + Colchicine	47·5	81	54	44	32	2·26	3·5	6·9
Heparin + Thrombin	17·5	172	82	170	61	·	4·2	·
Vitamin K	62·5	100	50	52	19	·63	5·8	·
Vitamin K + Colchicine	42·5	32	41	11	12	1·76	3·1	20·4
+ Vitamin K + Heparin	17·5	122	60	108	33	·	5.7	·
Colchicine	80·0	27	36	10	21	2·37	2.6	13·4
Control H_2O	27·5	146	68	173	67	·	3.8	·

the initiation of cell division[1], as well as between blood coagulation and certain mechanisms in the formation of precipitation membranes by plant protoplas²m. (5) The logical postulate, frequently confirmed by empirical facts, that the above reactions do not remain restricted to cryptic manifestations, but also find expression in growth phenomena or other macroscopically discernible effects[3].

The following were the chief questions for which experimental data had to be collected: (1) Do thrombokinetic agents and anticoagulants such as affect blood clotting time (B.C.T.) elicit responses in plants, whether administered alone or combined with colchicine? Do colchicine, or other plant constituents, have an effect on B.C.T. in activating thrombine *in vitro*? (3) Do colchicine, or other plant constituents, have synergetic effects with, or antagonistic effects to, thrombokinetic agents or anticoagulants in plants ("C-tumours"!) or in animals?

The experimental approach to the problem was attempted by the following methods:

(a) *Germination Experiments on Wheat and Radishes*

The seeds were sown on filter paper placed in Petri dishes and soaked with watery solutions or suspensions of the following substances: (a) Thrombin I (activity 1—2 U/mg), thrombin II (40—50 U/mg), both prepared by Dr. M. GERENDÁS, thrombine III (American make); (b) Dicoumarol; (c) Vitamin K ("Vitacolan", Richter, Budapest) 1:2,000, 1:5,000; (d) Hirudine 1:2000; (e) Heparin 400 U/mg 1:2,000; (f) Inactivated thrombin 1:3,000 (control); (g) Colchicine (Boyer Paris) 1:5,000. These substances were either administered alone or simultaneously with colchicine. The plants were also treated with mixtures of the above thrombokinetic agents and anticoagulants.

In this preliminary account only the most striking results can be given. Table I shows the responses of wheat in a representative series of experiments. It must be mentioned, however, that in some other series of experiments where thrombin III was used, stimulation of straight growth was not as distinct, the controls having caught up with the thrombin-treated seedlings at the end of the experiment.

Other experiments with a different thrombokinetic agent, vitamin K, gave more homogeneous results. Moreover, vitamin K produced some other interesting effects. For instance, fasciated roots and shoots were found in several of the treated seedlings and tumour growth was frequently observed on the root-tips. Tumours were also obtained on some of the stems of young bean plants smeared with lanolin containing a 1:2,000 solution of vitamin K. Enlarged stomata were observed in both wheat and beans, as well as fasciated leaves and flowers in the latter[1]. These different findings confirm the polyploidizing action of vitamin K[2]. (The chromosomal analysis of our material will be published in the final account.)

As to the anticoagulants which were tested, the effects of heparin on wheat can be seen in Table I. In similar experiments hirudin produced like results, whilst dicoumarol, at least in the concentrations used, did not seem to have any effect.

In radishes, 3 mg of thrombine I administered to 40 seedlings in the course of the experiments retarded germination. When colchicine 1:20,000 was added to thrombin the proportion of germinated seeds was that of one fourth of the seeds treated with thrombin alone, one fifth of those treated with colchicine alone and one sixth of the water controls. When heparin alone was applied to the seeds it had an effect on germination

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[2] N. NYBOM and B. KNUTSON, Hereditas *33*, 58 (1947).

Table II

Material	Concentration	B. C. T.	Remarks
Colchicine	1:150	15s	stimulation
	1:5,000	15	
	1:10,000	14	
	1:20,000	15	
Viscadyl (alcoholic *Viscum* extract)	1:10	20	inactive
	1:5	48	inhibition
	1:3	58	inhibition
	1:2	70	inhibition
	concentrated	∞	total inhibition
Viscum album (fresh watery extract)	concentrated	∞	total inhibition
Viscum (extract of "Berries")	concentrated	16	stimulation
Isolated from *Viscum*: Saponin	saturated	20	inactive
Isolated from *Viscum*: Cholesterin	saturated	20	inactive
Isolated from *Viscum*: Oleanolic Acid	saturated	20	inactive
Isolated from *Viscum*: Ammoniated Phosphate of Mg	saturated	20	inactive
Loranthus europaeus (watery extract)	concentrated	12	stimulation
	1:2	14	stimulation
	1:4	16	stimulation
	1:8	18	stimulation
	1:16	20	inactive
Sedum (pressed sap)	concentrated	20	inactive
Spinach (watery extract)	concentrated	45	inhibition
Coumarin	saturated	16	stimulation
Heteroauxin (indole-acetic acid)	1:100	∞	total inhibition
	1:250	14	stimulation
	1:500	14	stimulation
	1:1,000	16	stimulation
	1:5,000	18	?
Mucus of Leech	concentrated	20	inactive
Resactor (embryonal liver extract)	5:1,000	15	stimulation
Control (see text)		20	

similar to thrombin, but combined with colchicine the difference in the rate of germination, as compared with heparin alone, was insignificant. The surface area of the leaves of the thrombin plants was significantly larger than that of the water controls, but colchicine being added this growth stimulation was practically neutralized. The fact may be significant that morphological aberrations, including a crateria, and enlarged stomata were found in several of the thrombin-treated seedlings.

(b) *Effects of Colchicine and other Plant Constituents on Blood-Clotting Time*[1].

The effects of the following substances were investigated: Colchicine (Boyer); watery extracts of *Viscum album*; "Viscadyl" (Richter, Budapest), an alcoholic extract of *Viscum*; various constituents of *Viscum* (kindly isolated by Zyma Ltd., Nyon); watery extracts of *Loranthus europaeus*; watery extracts of spinach; heteroauxin (Schuchardt, Görlitz); the pressed sap of *Sedum maximum*; coumarin; the mucus of *Hirudo medicinalis*; "Resactor" (an embryonic liver extract of the Hungarian Pharmaceutical Products Co., Budapest).

The experiments were set up according to the following plan:

	Control	Experiments
Oxalated blood plasma	·1	·1 ml
0·9% solution of NaCl	·2	·1 ml
Substance tried	·	·1 ml
10 mg/ml thrombine	·1	·1 ml
Blood clotting time	20·	see Table II

To the following remarks may be added: *Loranthus europaeus*, although belonging to the family of *Viscum*, had an effect contrary to the latter on B.C.T. The various *Viscum* fractions tried did not affect B.C.T. at all. In the belief that the high amount of solids present in the Viscum fractions used might be responsible for the inhibition of blood clotting, extracts of spinach and *Loranthus* containing the same amount of dry material were prepared, but spinach was not nearly as active as *Viscum*, and, as already stated, *Loranthus* had opposed effects. The viscous sap of *Sedum* and the mucus of leeches, tried for similar reasons, did not alter the B.C.T. The influence of heteroauxin (indole-acetic acid), the plant hormone, was investigated because, as the author has shown[1, 2], it has effects in many respects similar to those of colchicine, but, on the other hand, effects antagonistic to those of the alkaloid in plants and animals[2, 3]. It was reported moreover that, like colchicine,

[1] These experiments were kindly carried out by Dr. M. GERENDÁS.

[1] L. J. HAVAS, Growth *2*, 257 (1938).

[2] L. J. HAVAS, Soc. Roy. Sci. méd. et nat. Bruxelles, Conf. 8 janv. 1 (1940).

[3] R. I. WALKER, J. Arnold Arb. *19*, 158 (1938).

it increases protoplasmic viscosity[1]. (Viscosity measurements of the above and other substances, as well as of blood treated by the same *in vitro* and *in vivo* are in progress.)

(c) *Effects of Colchicine on Blood Sedimentation*

The Westergreen method was used. To 4·5 ml cattle blood mixed with a 3·8% solution of Na-citrate (1:5) was added 0·5 ml colchicine (1:200 and 1:10,000). Table III shows the rate of sedimentation of the red blood corpuscles.

Table III

Treatment	Rate of sedimentation (mm)		
	1·h	2·h	3·h
Control (blood alone)			
Control (blood alone)	·	·5	2·5
Blood—colchicine 1:10,000 . .	·	·5	3·5
Blood—colchicine 1:200 . . .	1·	2·5	4·5

(d) *Effects of Colchicine on Leeches (Hirudo medicinalis L.) and Earthworms (Lumbricus terrestris L.).*

Some preliminary tests were undertaken to examine whether leeches which, as is known, produce the anticoagulant hirudine, would show increased resistance to colchicine as compared with other *Vermes* which do not excrete hirudin. This actually proved to be the case: For instance, a leech weighing 2·9 g injected with 1·5 ml colchicine (1:200) resisted during 11 days, while a *Lumbricus*, weighing 2·8 g, injected with 1 ml colchicine, died within 7 hours.

In other experiments I have tried to ascertain whether the action of colchicine could be neutralized by the simultaneous administration of the anticoagulants *Viscum* and heparine. The earthworms were placed in Petri dishes into which was poured 1 ml of each of the various substances. It was found that animals treated with *Viscum* extract (62:1,000) died within about one day, whilst the animals submitted to a treatment of a mixture of colchicine (1:200) and *Viscum* survived for two days. Heparin added to colchicine increased the period of survival in a similar way. The circumstance that colchicine caused arterial dilatation, while both heparin and *Viscum* induced the dilatation of the venous system, may contribute to the explanation of some of the compensatory actions of *Viscum* and colchicine.

Conclusions

In summing up the principle experimental data outlined above, the following preliminary conclusions can be made:

(1) The circumstance that the polyploidogenic and oncogenic agent, colchicine, has an influence on protoplasmic viscosity in plants, and also influences blood plasma viscosity in stimulating the action of thrombin points to the similarity of at least this mechanism among those responsible for the many analogous effects of the drug in plants and animals.

(2) The circumstance that, on the other hand, the two thrombokinetic agents tested, thrombin and vitamin K, act in many respects synergetically with colchicine as oncogenic agents (C-tumours) and that vitamin K is, moreover, a polyploidogenic agent, even when applied alone, enforces the assumption that these mechanisms are similar.

(3) The finding that *Viscum* acts, like heparin, as an anticoagulant, and that both have a tendency to counteract in plants the oncogenic influence of the thrombokinetic activators colchicine and vitamin K, restricts the circle still more.

(4) Finally, the discovery that, proportionally to its concentration, heteroauxin may influence B.C.T. both as a thrombin activator and as an anticoagulant, is doubly important in consideration of the circumstance mentioned above, that heteroauxin increases protoplasmic viscosity in plants and elicits, like colchicine, oncogenic and polyploidogenic effects[1]. The antagonistic actions of heteroauxin to colchicine, including tumour growth in plants and hormonal actions in plants and animals may thus find a new explanation.

L. J. HAVAS

Hungarian Institute for Biological Research, Tihany, September 10, 1947.

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[1] W. H. GREANLEAF, J. Heredity *29* (No. 12), 452 (1938).

Über die bakteriostatische Wirkung von Anthocyanen und verwandten Stoffen

G. F. GAUSE[1] isolierte aus *Proactinomyces cyaneus antibioticus* eine farbige Verbindung mit ausgeprägter antibiotischer Wirksamkeit. Diese isolierte Substanz besaß Indikatoreigenschaften. Sie wurde auf Grund einiger Farbreaktionen, die allerdings für ihre chemische Konstitution nicht beweisend sind, von GAUSE[1] und BRAZHNIKOVA[2] als Anthocyanidinderivat bzw. als nahe mit diesen natürlichen Farbstoffen verwandte Substanz bezeichnet. Für das Vorhandensein eines Anthocyanidinderivates wurde von GAUSE[1] auch auf eine Arbeit hingewiesen, in der KRISS[3] aus einem nahe verwandten Aktinomyzeten ein Anthocyan isoliert haben wollte. Bei der genaueren chemischen Nachprüfung dieser Angabe durch R. ROBINSON und Mitarbeiter[4] wurde jedoch festgestellt, daß der aus *Actinomyces violaceus-ruber* Waksman isolierte Farbstoff kein Anthocyan darstellte. Nach den bisher vorliegenden Literaturangaben[5] ist es auch wenig wahrscheinlich, daß Anthocyane in Mikroorganismen vorkommen. Sie wurden bis heute im Pflanzenreich nur von den Moosen aufwärts gefunden.

Da uns bisher keine Untersuchungen über die bakteriostatische Wirkung von Anthocyanen aus der Literatur bekanntgeworden sind, schien es uns von Interesse zu sein, einige Anthocyane und chemisch verwandte Stoffe auf ihre bakteriostatische Wirkung zu prüfen. Wir untersuchten außer Rubrobrassicin, einem acylierten Anthocyan aus Rotkohl, das uns aus früheren Versuchen[6] zur Verfügung stand, Cyanin, Pæonin und das Aglycon Pæonidin. Die drei letzten Präparate stellte uns Herr Prof. Dr. P. KARRER, Zürich, freundlicherweise zur Verfügung, wofür wir ihm auch an dieser Stelle bestens danken möchten. Ferner bezogen wir zwei Flavonolglykoside (Quercitrin und Rutin) und drei Fla-

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