On voit que la diastase purifiée ne donne lieu généralement qu'à une faible consommation d'oxygène et à une formation d'ammoniaque pratiquement nulle; au contraire, la D-AAD purifiée «reconstituée» par addition de flavine-adénine-dinucléotide donne des résultats nettement positifs, et si les chiffres sont plus faibles qu'avec la préparation brute, ils se correspondent mieux.

En conclusion, l'action de la D-AAD purifiée du rein de porc sur le plasma sanguin conduit à des résultats analogues à ceux que nous avions obtenus avec les extraits bruts, et nos déductions antérieures concernant la présence dans le sang d'un substrat de la D-AAD se trouvent ainsi confirmées.

P. BOULANGER, G. BISERTE et R. A. GRIFFIÉ

Services de biochimie et de physiologie de la Faculté de médecine et de l'Institut de recherches sur le cancer, Lille, le 25 juillet 1949.

Summary

When raw D-acidaminodehydrase (AAD) from pig kidney acts upon blood plasma, O_2 is consumed and NH_3 is formed; this suggests that an oxidative deamination is taking place, and the occurrence in the blood of a substrate for the D-AAD seems probable. These results are confirmed by the use of purified D-AAD and by the striking activation of the reaction when pure riboflavin-adenine-dinucleotide is added.

Über die Hemmung von Desoxyribonucleotide spaltenden Fermenten durch Colchicin

Die Wirkungsweise der Mitosegifte ist nur für einen kleinen Teil dieser chemisch außerordentlich verschiedenen Substanzen bekannt. Wir haben uns daher die Frage vorgelegt, ob Mitosegifte durch die Beeinflussung derjenigen Fermente wirken können, welche möglicherweise am Stoffwechsel von Zellkernsubstanzen beteiligt sind und eine Rolle bei der Vermehrung der Kernsubstanzen während der Zellteilung spielen. Diesbezügliche Untersuchungen sind in der Literatur bisher nicht mitgeteilt worden. Lediglich Urethan ist hinsichtlich der Pharmakologie der Narkose eingehender fermentchemisch untersucht worden, während Colchicin wegen der Beeinflussung der Gicht von KEESER[1] geprüft worden ist, ohne daß Zusammenhänge zwischen dem Einfluß auf diese Krankheit und der Mitosegiftwirkung vermutet oder untersucht worden sind.

In unseren Versuchen hat sich Colchicin als recht wirksam erwiesen; deshalb soll über diesen Teil unserer experimentellen Daten hier kurz berichtet werden. Die Prüfung der Desoxyribonucleotidase erfolgte nach dem Vorgehen von GREENSTEIN und Mitarbeitern[2], als Substrat dienten aus Rindermilz nach BREDERECK[3] dargestellte Desoxyribonucleotide. Dabei zeigte sich eine Hemmung der Phosphatabspaltung um über 50% (siehe Tab. I).

Diese Ergebnisse decken sich mit den uns erst während unserer Untersuchung bekanntgewordenen Befunden von AHLSTRÖM, v. EULER und v. HEVESY[4], wonach der Phosphatumsatz in Zellkernen des Jensen-Sarkoms, gemessen mittels P^{32}, durch Colchicininjektion bei Ratten um etwa 20% gesenkt werden kann.

Tabelle I

Zunahme an anorganischem Phosphor in γ je Ansatz nach			
	1 Stunde	2 Stunden	3 Stunden
Ohne Colchicin	+38,0	+62,0	+57,0
Mit Colch. $1{,}2 \cdot 10^{-2}$ mol	+28,0	+16,0	+21,0

Die gleichzeitig von uns untersuchte enzymatische Desaminierung von Desoxyribonucleotiden ist ebenfalls durch Colchicin um etwa 40% hemmbar, wie aus Tab. II hervorgeht (Ansatz nach GREENSTEIN[1]).

Tabelle II

Zunahme an N in γ je Ansatz nach		
	2 Stunden	4 Stunden
Ohne Colchicin	+8,1	+19,5
Mit Colchicin $1{,}2 \cdot 10^{-2}$ mol	+5,1	+ 7,8

Die Versuche werden fortgesetzt und sollen zusammen mit anderen hier nicht erwähnten Befunden in Kürze an anderer Stelle publiziert werden.

Der Firma Hoffmann-La Roche AG. Basel sind wir für die liebenswürdige Überlassung von Colchicin zu großem Dank verpflichtet.

K. LANG, G. SIEBERT und H. OSWALD

Physiologisch-chemisches Institut der Johannes-Gutenberg-Universität Mainz, den 12. Juli 1949.

Summary

The authors raise the question, if enzymatic processes possibly linked with the mitotic cell division may be influenced by mitotic poisons. The presented date show an inhibition of the dephosphorylation of desoxyribonucleotides at a rate of about 50% and of the deamination of about 40% by colchicine (final concentration $1 \cdot 2 \cdot 10^{-2}$ M).

[1] E. KEESER, Arch. exp. Pathol. Pharmakol. *197*, 187 (1941).
[2] J. P. GREENSTEIN, C. E. CARTER und H. W. CHALKLEY, Cold Spring Harb. Symp. Quant. Biol. *12*, 64 (1947).
[3] H. BREDERECK, Z. physiol. Chem. *253*, 170 (1938).
[4] L. AHLSTRÖM, H. v. EULER und G. v. HEVESY, Ark. Kemi etc. *24A*, 12, 1 (1947).

[1] J. P. GREENSTEIN, C. E. CARTER und H. W. CHALKLEY, loc. cit.

Inhibition with Merthiolate of the Mucooligosaccharase Fraction of Testis Hyaluronidase (Mesomucinase)

The observation that the aqueous extract of mammals' testicles, as well as the aqueous extract of leech head, the poison of some snakes, and the filtrates of agar cultures of some microorganisms could split hyaluronic acid, first by lowering the viscosity of a solution of that acid and, only after a certain period of time, by liberating its components (acetylglucosamine and glucuronic acid), led even the first investigators (FAVILLI[1], MEYER and coworkers[2]) to suppose that testis extract contained a mixture of enzymes.

[1] G. FAVILLI, Boll. Ist. Sieroter. Milanese *19*, 481 (1940).
[2] K. MEYER, E. CHAFFEE, G. HOBBY, and M. H. DAWSON, J. Exp. Med. *73*, 309 (1941).

Later on, EAST, MADINAVEITIA and TODD[1] demonstrated that in addition to the mucopolysaccharase also a β-phenyl-*N*-acetylglucosaminase was present in the testis extract of mammals.

Subsequently HAHN[2] succeeded by means of fractioned precipitation with ammonium sulfate, sodium chloride, and copper sulfate in splitting three fractions: a depolymerizing one called mucopolysaccharase or MP fraction, one which determines the liberation of acetylglucosamine and glucuronic acid called mucooligosaccharase, or MO fraction, and one which has the power of splitting β-phenyl-*N*-acetylglucosamine, called by HAHN GL fraction. The fractions thus obtained show, however, a remarkably lowered activity in respect to the hyaluronidase *in toto*.

Finally ROGERS[3] demonstrated that bacterial hyaluronidase is a complex of various fractions.

It was recently pointed out by MCCLEAN[4], ROGERS[5], GUERRA[6], and SWYER[7] that various substances such as heparin, sodium salicylate, etc. have an inhibiting action on hyaluronidase; but so far it has never been observed whether such inhibiting substances had a different action on the various enzyme fractions.

In the course of some investigations for which it was necessary to keep the enzyme-substrate system a long time in thermostat, for which reason bacterial development had to be prevented by means of an antiseptic, it was observed that when merthiolate was added (final dilution of approximately 1:7,600), the reaction of MORGAN and ELSON for acetylglucosamine[8] failed to take place even when it should have been clearly positive (see Table I).

Table I

Acetylglucosamine reaction after the action of testis hyaluronidase with addition of merthiolate.

Determination after hours	Hyaluronic acid solution + 10% hyaluronidase + H_2O	Hyaluronic acid solution + 10% hyaluronidase + merthiolate*
8	+ + —	— — —
16	+ + +	— — —
24	+ + —	— — —

* The merthiolate was used in an aqueous solution at a concentration of 1:1,000. As source of the enzyme an aqueous extract of bull testis powder in a concentration of 1:10; or an aqueous solution of purified hyaluronidase[9] in a concentration of 1:1,000 was used.

Explanation of signs:

Reaction
- — — — negative
- + — — evident traces of acetylglucosamine
- + + — clearly pinkish colour
- + + + red colour

On the other hand, the fall of viscosity took place in the normal way as demonstrated by the experiment reproduced in Table II, which shows that merthiolate, in the concentration used, has but a very slight action on the depolymerizing fraction (MP fraction of HAHN) of testis hyaluronidase.

Table II

Action of merthiolate on the depolymerizing fraction of testis hyaluronidase. Lowered percentage of the viscosity of a solution of hyaluronic acid following the effect of the aqueous extract of bull testicle with and without addition of merthiolate.

Determination after minutes	Hyaluronic acid solution + 10% hyaluronidase + H_2O	Hyaluronic acid solution + 10% hyaluronidase + merthiolate
Immediately	100	100
5	17	32
10	13	18
15	6	11

Examining the above-described results it is clearly seen that merthiolate, in the concentration used, completely inhibits the acetylglucosamine reaction.

It could, nevertheless, be thought that merthiolate inhibited the course of the reaction and not the enzyme activity. However, merthiolate (in the same concentration used in previous experiments) does not modify MORGAN and ELSON's reaction when it is added to a hyaluronic acid solution, previously kept in thermostat at 37°C with hyaluronidase for 16 hours, so as to have acetylglucosamine liberated in a fairly appreciable amount.

It must therefore be concluded that the lack of acetylglucosamine reaction in the enzyme-substrate-merthiolate mixture was due to an inhibition of the mucooligosaccharase fraction caused by the latter substance.

The previous observations which had led the various authors to suppose the existence of a number of differently active fractions in hyaluronidase, which would not be a single enzyme but a complex of enzymes successively acting upon different substrates, are quite confirmed by the present investigations.

In particular, the results described in the present paper agree with those of HAHN, who, as said before, could split from hyaluronidase a depolymerizing fraction and a fraction acting as an oligosaccharase.

It is clear that the two fractions are quite distinct entities, not only on account of their different action, but particularly on account of the different effect produced on each of them by merthiolate in the same mixture.

Nevertheless merthiolate is active not only on the MO fraction of hyaluronidase; in a higher concentration (final dilution of approximately 1:2,700) it can also inhibit the hyaluronidase *in toto*, but it is possible, by means of that substance, to obtain a selective inhibition of the different fractions.

The action has been tested of merthiolate on hyaluronidases coming from different sources (leech head, poison of *Bothrops jararaca*, etc.): there too, merthiolate developed a remarkably inhibiting action on the oligosaccharase fraction.

Furthermore, it was tested whether the inhibition of the mucooligosaccharase fraction (MO) of testicle extract had altered the diffusion phenomenon (which takes place when hyaluronidase is injected into the dermis of an animal together with an indicator, such as for instance Indian ink), although that was very improbable, since diffusion is a phenomenon to be connected with the depolymerizing action of the enzyme,

[1] M. EAST, J. MADINAVEITIA, and R. TODD, Biochem. J. *35*, 872 (1941).

[2] L. HAHN, Arkiv för Kemi, Mineral. och Geol. *21*, Ser. A. n. I (1945).

[3] H. J. ROGERS, Biochem. J. *42*, 633 (1948).

[4] D. MCCLEAN, J. path. Bact. *54*, 284 (1942).

[5] H. J. ROGERS, Biochem. J. *40*, 583 (1946).

[6] F. GUERRA, Science *103*, 686 (1946); J. Pharmacol. *87*, 193 (1946).

[7] G. I. M. SWYER, Biochem. J. *42*, 28 (1948); Biochem. J. *42*, 32 (1948).

[8] W. MORGAN and L. ELSON, Biochem. J. *28*, 988 (1934).

[9] I am indebted to the "Vismara Terapeutici" of Casatenovo (Como) for a generous gift of purified testis hyaluronidase.

and that is the reason why it takes place immediately. The inhibition of the MO fraction does not alter the diffusion phenomenon.

The intimate mechanism of action of merthiolate is now being investigated and also the action of other hyaluronidase inhibitors (heparin and sodium salicylate) on the various enzyme fractions. The results of such experiments will be reported *in extenso* in a further paper.

M. CAMPANI

Institute of General Pathology of the University of Modena, July 18, 1949.

Zusammenfassung

Merthiolat zeigt eine hemmende Wirkung auf die Hyaluronidase des Hodens. Dennoch ist es mittels verschiedener Konzentrationen jenes Stoffes möglich, eine selektive Hemmung der Mukooligosaccharase-Fraktion (MO) bei fast vollständiger Schonung der depolymerisierenden Fraktion (MP) zu erzielen.

Noradrenaline and Adrenaline in the Suprarenals of the Guinea-Pig

HOLTZ and SCHÜMANN[1] have given evidence to show that extracts of suprarenals of cattle in addition to adrenaline probably contain noradrenaline. This finding has been confirmed by EULER and HAMBERG[2] and by GOLDENBERG *et al.*[3]. L-noradrenaline has subsequently been isolated in pure form from suprarenals of cattle (BERGSTRÖM, EULER, and HAMBERG[4] and from standard samples of epinephrine (TULLAR[5]).

The present experiments have shown that guinea-pigs suprarenals also contain noradrenaline as estimated with the colorimetrical method of EULER and HAMBERG[6] which permits quantitative estimation of both noradrenaline and adrenaline in a mixture.

The animals were killed by a blow on the neck and the suprarenals immediately prepared and extracted with 10 p.c. trichloracetic acid which was removed from the filtrate with ether. The following figures were obtained:–

No.	*l*-Adrenaline µg per g	*l*-noradrenaline µg per g	per cent *l*-noradrenaline
(1) single pair	276	52	15·9 %
(2) single pair	272	122	31·0 %
(3) 5 pairs	223	70·5	24·0 %

The relative amount of noradrenaline compares well with the figures obtained by EULER and HAMBERG on 7 extracts of fresh suprarenals from cattle, 18·0–27·4 p.c., with an average of 23·6 p.c.

[1] P. HOLTZ and H. J. SCHÜMANN, Naturwiss. *35*, 159 (1948).
[2] U. S. v. EULER and U. HAMBERG, Nature *163*, 642 (1949).
[3] M. GOLDENBERG, M. FABER, E. J. ALSTON, and E. C. CHARGAFF, Science *109*, 534 (1934).
[4] S. BERGSTRÖM, U. S. v. EULER, and U. HAMBERG, Acta chem. Scand., *3*, 305 (1949).
[5] B. F. TULLAR, Science *109*, 536 (1949).
[6] U. S. v. EULER and U. HAMBERG, Acta physiol. Scand., in the Press (1949).

L-Noradrenaline, which has previously been recognized as a neuroergone in adrenergic nerves[1] thus also occupies the position as a true hormone.

U. S. v. EULER, U. HAMBERG, and A. PURKHOLD

Department of Physiology, Faculty of Medicine, Karolinska Institutet, Stockholm, August 3, 1949.

Zusammenfassung

In Nebennierenextrakten von Meerschweinchen wurde folgendes gefunden: sie enthalten *l*-Noradrenalin, das 15,9–31 % der gesamten Katecholverbindungen ausmacht.

[1] U. S. v. EULER, Acta physiol. Scand. *16*, 63 (1948).

Measurement of the Pressure in Schlemm's Canal and in the Anterior Chamber of the Human Eye

It has been discussed if the pressure in Schlemm's canal is higher or lower than the intraocular pressure[1]. The mechanism of the outflow depends on that question, but it has never before been possible to measure this pressure.

The discovery of the aqueous veins by ASCHER[2] and independently by GOLDMANN[3] has opened the possibility of bloodless measurement of the pressure in Schlemm's canal. If an aqueous vein is blocked by compression so near to the limbus corneæ, that it has not yet joined any veins, the pressure above the block will be the same as in Schlemm's canal. Since there are some twenty connections between the canal and the episcleral veins this compression of a single one will not significantly increase the physiological pressure in Schlemm's canal. Determinations according to this principle have been made by means of a small pressure chamber connected to a manometer[4] and fitted with a transparent rubber membrane and built-in lighting. The aqueous vein was blocked by a small pelotte, the pressure chamber applied above the block and the pressure adjusted until the collapse of the blocked vessel. The whole measurement was made under a magnification of 30.

By compression of the jugular veins by means of a pneumatic cuff the pressure in the episcleral veins can be raised. When it is high enough the flow of aqueous humour first stops and then turns in retrograde direction. This can best be seen where an ordinary vein empties into the aqueous vein. Just at the moment of balance when the flow has stopped the boundary pulsates. At this moment the pressure is the same in the aqueous and episcleral veins, in Schlemm's canal and in the anterior chamber of the eye and can be measured with the pressure chamber.

Thus, this is a new bloodless method of measuring the intraocular pressure (I.O.P.). However, the stasis does not leave the I.O.P. unaffected and the method does not directly yield values of the physiological I.O.P. In order to obtain such values the following procedure was adopted. First of all a tonometry was performed with a Sklar-Schiötz tonometer yielding a value of P_1 mm Hg. Then the cuff is inflated and the venous pressure at which aqueous flow ceases is determined (P_2). Immediately after this and without change in the degree of stasis a new tonometry is performed (P_3). The physio-

[1] S. DUKE-ELDER, Brit. J. Ophthal. *25*, 287 (1926).
[2] K. W. ASCHER, Amer. J. Ophthal. *25*, 31, 1174, 1301 (1942).
[3] H. GOLDMANN, Ophthalmologica *111*, 146 (1946); 112, 344 (1946).
[4] E. SEIDEL, GRAEFE'S Arch. 112, 252 (1923).