begin to show GGTP synthesis. This enzyme fluctuation in the postnatal period, and after deviation of portal blood supply, can be understood if one considers the fine circulatory situation in the hepatic lobulus. There is a zonal relationship between cells constituting the lobulus and their blood supply. The hepatocytes situated close to the axial terminal branches (vena portae and arteria hepatica propria) are the first to be supplied with fresh blood, rich in oxygen and nutrients. They form the most active and resistant core of the lobulus: they are the last to die and the first to regenerate[7]. In our case, they are the last to stop and the first to start to re-synthesize GGTP. The more distant the cells are from the site where the terminal portal and arterial branches empty into sinusoids, the poorer is the quality of blood that bathes them.

After the portacaval shunt, all blood originating from the intestines, spleen and pancreas (insulin, glucagon) stopped flowing through the liver. The blood is then provided by the arteria hepatica propria coming from the aorta.

Whether it is the change in substrate or oxygen supply, or both ,which is responsible for the high enzyme activity arizing in shunted rats, requires further studies.

Zusammenfassung. Mit Hilfe der histochemischen Methode konnte nachgewiesen werden, dass die Leberzellen nach der portocavalen Shunt-Operation die Fähigkeit der GGTP-Synthese wiedererlangen. Die Enzymabnahme in der postnatalen Periode und ihre Zunahme nach der portocavalen Anastomose stimmt sowohl zeitlich als auch mengenmässig im histochemischen Präparat mit den biochemischen Ergebnissen überein.

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[7] A. M. RAPPAPORT, Z. J. BOROWY, W. M. LONGHEED and W. N. LOTTO, Anat. Rec. *119*, 11 (1954).

[8] Chemisches Zentrallabor, Inselspital und Institut für klinische Pharmakologie der Universität, CH–3010 Bern (Switzerland).

Has Ricin an Enzyme Inductive Effect?

Ricin is a component of the common castor oil seed (*Ricinus communis*, Euphorbiaceae) and is one of the representatives of the socalled phytotoxins. The phytotoxins are very toxic proteinaceous compounds of plant origin with a molecular weight of some 10,000. They play important physiological roles in plants, and they resemble the true bacterial toxins in many respects, especially the diphteria and tetanus toxin.

According to HAUSCHILD[1], ricin is one of the 5 most toxic materials known: tetanus toxin, botulinus toxin, diphteria toxin, gramicidin, ricin. FUHRMAN[2] considers that ricin is the most toxic substance of plant origin.

The signs and symptoms of ricin intoxication vary very much, according to the size of the dose. More than 6% of the cases are fatal (SOLLMANN[3]). Recently BALINT[4] has published an exhaustive review on ricin. It was reported that in late (subacute) ricin intoxication the smooth endoplasmic reticulum of liver cells hypertrophies and the mitochondria are shrunk (BALINT[5,6]).

REMMER[7] showed that in the case of an enzyme induction, caused by different drugs, the hexobarbital sleeping-time is reduced in rats, due to the induction of the drug metabolizing enzyme system in the liver.

In this communication our preliminary results are reported which raise the possibility that ricin also could have an enzyme inductive effect in the liver.

[1] F. HAUSCHILD, *Pharmakologie und Grundlagen der Toxikologie*, 1st edn. (VEB G. Thieme Verlag, Leipzig 1956), p. 1106.

[2] F. A. FUHRMAN, Scient. Am. *217*, 60 (1967).

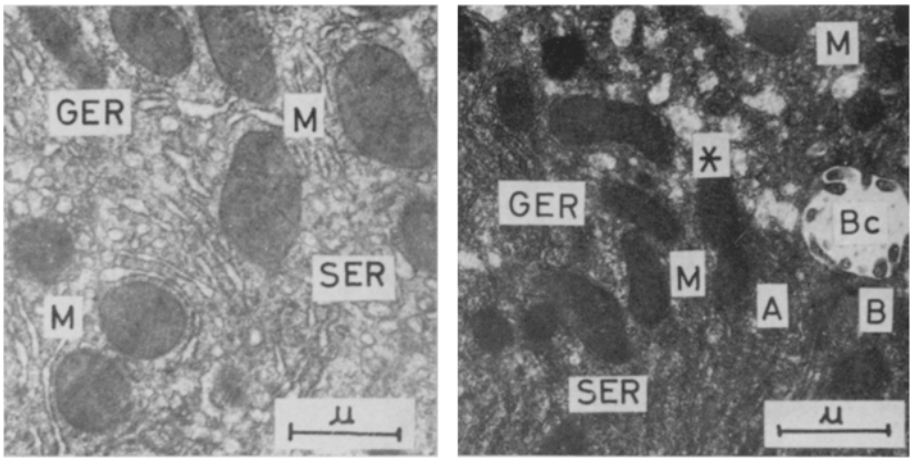
[3] T. SOLLMANN, *A Manual of Pharmacology and its Applications to Therapeutics and Toxicology* (W.B. Saunders & Co., Philadelphia 1957), p. 1535.

[4] G. A. BALINT, Toxicology *2*, 77 (1974).

[5] G. A. BALINT, Ph. Doctoral Thesis, Makerere University, Kampala, Uganda.

[6] G. A. BALINT, Biochem., exp. Biol., in press (1974).

[7] H. REMMER, Eur. J. clin. Pharmac. *5*, 116 (1972).



Comparative electron micrograph of a section of rat liver treated with 2×10 μg/kg ricin, taken 1 week after first ricin administration. Glutaraldehyde fixation, araldite embedding. Uranyl-acetate-lead-citrate contrasting. Left side: Control animal. M, mitochondria; SER, smooth endoplasmic reticulum; GER, granulated endoplasmic reticulum. Right side: treated animal. M, mitochondria; SER, smooth endoplasmic reticulum; GER, granulated endoplasmic reticulum; A and B, 2 neighbouring cells; Bc, bile canaliculus; * unidentified material, due to ricin (BALINT[5,6]).

Materials and Methods. Female Wistar rats, weighing 200–250 g, were pretreated with 2×10 µg/kg ricin, i.p. Ricin was prepared by us (BALINT[5]) with the help of KOBERT's method and it had a true resemblance to a Merck's preparation, which was stated also to have been produced according to KOBERT's method (E. MERCK Ltd., Darmstadt, W. Germany, Charge No.; Br-23-1776-W. 42721-722721). The preparation was an unfractionated ricin with high enough purity. Its LD_{50} value on rats was 38,15 ± 0,412 µg/kg, i.p. (BALINT[5]).

Between the 2 ricin doses, 4 days elapsed, and the experiment was carried out on the 3rd day after the second ricin dose. The animals belonging to the 2 control groups received treatment as follows: Control group No. 1 was treated with 2×0.25 ml sterile, pyrogen-free normal saline solution, i.p., while the control group No. 2 received 2×80 mg/kg phenobarbital (sodium-salt) i.p. Both control groups received the treatment at the same time when ricin was administered. Each of the groups contained 15 animals.

After the above-mentioned pretreatment, the animals were narcotized i.p. with 100 mg/kg hexobarbital ('Evipan-Natrium', Bayer, W. Germany) and the sleeping-time was measured. As 'sleeping-time' was considered the time interval which elapsed from the injection of hexobarbital till the animals tolerated lying on their back.

The effect of ricin on the hexobarbital sleeping-time on rats

Treatment	Sleeping-time (min) Mean values ± S.D.	*P*
Control (normal saline)	102 ± 12	—
Control (phenobarbital)	57 ± 15	< 0.05
Ricin	68 ± 16	0.05

During the experiments, electron microscopic investigations were carried out also, on another group of animals which received the same treatment. The results of these experiments are reported elsewhere (BALINT[6]) but to show the hypertrophy of the smooth endoplasmic reticulum (Figure 1). The increase in the density of the whole treated cell is one of the most noticeable features.

Results. The experimental results were analyzed statistically with the help of Student's *t*-test and are listed in the Table.

Discussion. The results, listed above, give further suggestions that ricin might have an inductive effect on the drug metabolizing enzyme system in the liver. This experimental result appears unique in the literature, as no material of plant origin is known to have this effect, and no material with so high molecular weight (about 66.000–70.000) as ricin is known to have a similar effect. Further investigations are necessary on this question[8].

Zusammenfassung. Vorversuche mit Rizin, dem toxischen Protein von Rizinussamen (*Ricinus communis*) ergaben einen Verkürzungseffekt der Hexobarbital-Schlafzeit bei Ratten, was für einen induktiven Effekt auf das drogenabhängige metabolische Enzymsystem der Leber spricht.

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Isolation of Thrombin-E and the Evolution of Enzyme Activity from Prothrombin[1]

As a prerequisite for the clotting of blood, thrombin must first be derived from prothrombin. The proteolytic and esterolytic functions of this enzyme are dissociable properties, and during the activation of prothrombin, esterase activity appears first. This is followed by the emergence of proteolytic activity or the capacity to induce the coagulation of fibrinogen. This latter property then disappears leaving only esterase activity. These facts were presented in several papers from this laboratory beginning in 1957[2–4]. At that time, the technology of protein chemistry was not adequately developed to enable an interpretation of the activation sequence in terms of protein structure.

What structure does the protein have when the enzyme can hydrolyze *p*-toluenesulfonyl-L-arginine methyl ester (TAMe), and how is it changed when this property is retained while the capacity to clot fibrinogen evolves and subsequently disappears? This report, based on the use of technology previously described[5–9], provides information on the question formulated above. Thrombin has the capacity to hydrolyze TAMe and to 'clot fibrinogen'. Itis a two chain structure in which the A chain is connected to the B chain by a disulfide bond[10]. Our new experiments serve to demonstrate that the B1 portion[8] of the B chain is removed by autolysis and the remaining structure, called thrombin-E, possesses only the quality to hydrolyze TAMe. We present a simple procedure for the isolation of thrombin-E and the B1 chain. We have found that the

[1] This investigation was supported by research grant No. HL 14142-04 from the National Heart and Lung Institute, National Institutes of Health, U.S. Public Health Service.

[2] W. H. SEEGERS and R. H. LANDABURU, Am. J. Physiol. *191*, 167 (1957).

[3] R. H. LANDABURU and W. H. SEEGERS, Am. J. Physiol. *197*, 1178 (1959).

[4] W. H. SEEGERS, R. H. LANDABURU and J. F. JOHNSON, Science *131*, 726 (1960).

[5] W. H. SEEGERS, *Prothrombin* (Harvard University Press, Cambridge 1962).

[6] W. H. SEEGERS, E. R. COLE, C. R. HARMISON and E. MARCINIAK, Can. J. Biochem. Physiol. *41*, 1047 (1963).

[7] W. H. SEEGERS, E. MARCINIAK, R. K. KIPFER and K. YASUNAGA, Arch. Biochem. Biophys. *121*, 372 (1967).

[8] W. H. SEEGERS, J. REUTERBY, G. MURANO, L. E. MCCOY and B. B. L. AGRAWAL, Thromb. Diath. haemorrh. Suppl. *47*, 325 (1971).

[9] W. H. SEEGERS, L. E. MCCOY, J. REUTERBY, N. SAKURAGAWA, G. MURANO and B. B. L. AGRAWAL, Thrombosis Res. *1*, 209 (1972).

[10] S. MAGNUSSON, Thromb. Diath. haemorrh. Suppl. *38*, 97 (1970).