The amount of $^{14}CO_2$ produced from 1-^{14}C-galactose is unaffected in normal liver slices by the administration of actinomycin D; whereas, in regenerating rat liver there is a marked decrease approaching the control values (Table II). Furthermore, in regenerating rat liver slices ethanol inhibition is attenuated approaching the normal values if the antibiotic is injected at the beginning of partial hepatectomy (Table II).

The observations correlate with the reports that the activities of mitochondrial and microsomal enzymes are altered during the early phase of regeneration and return to normal levels upon the restoration of liver mass[5]. However, this is not true with all biological processes; for instance, polyploidy remains high after complete restoration of tissue mass by the liver[10].

The return of normal metabolic activity in the regenerated liver may result from either regulation of enzyme activity or newly synthesized proteins. The abolition of the increase in galactose metabolism and decrease in ethanol inhibition by actinomycin D would indicate that the synthesis of nucleic acids and thus, the elaboration of a new protein might be needed for metabolic adaptation of the regenerating rat liver.

This experimental approach of inhibiting nucleic acid synthesis has been successfully used to demonstrate de novo formation of several enzymes. For instance, the injection of actinomycin D to animals suppressed the adaptive synthesis of glucokinase[11], glucosyl transferase[12] and tryptophan pyrrolase[13]. It should be emphasized that the evidence obtained by these experiments, however valuable, can only be deemed tentative until the actual turnover of enzyme-proteins is demonstrated. With this limitation in mind, it is concluded that the activities of enzymes of galactose metabolism are adapted to the process of regeneration, being regulated by the synthesis of newly formed proteins.

Zusammenfassung. Es zeigt sich während des frühen Regenerationsstadiums bei der teilweise hepatektomierten Rattenleber, dass ein Anstieg im Galaktose-Metabolismus mit gleichzeitiger Abnahme seiner Hemmung durch Ethanol einhergeht. Nach vollständiger Geweberegeneration kehrt der normale Metabolismus zurück.

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Fibrinogen-Fibrin Conversion as Determined by Polymer Bead Sedimentation Technique

A newly-developed, sensitive method for determining the clotting time of fibrinogen involves addition of suitably-sized bead particles to a fibrinogen solution followed by exposure of the mixture to coagulating enzyme. The time required for gel formation to occur is related to the extent of particle sedimentation observed and can be readily assessed at any time after clot formation by measuring the absorbance of the coagulated system. The influence of several parameters on the results obtained with this technique suggests that it may be useful in the determination of low fibrinogen concentrations and low levels of thrombin clotting activity.

Methods. Bovine fibrinogen (65% clottable, Pentex, Kankakee, Illinois, USA) was dissolved in 0.05 *M* Tris/HCl buffer, pH 7.4, containing 0.15 *M* NaCl (hereafter referred to as 'buffered saline') to give a final concentration of 0.125% (w/v) (in terms of 100% clottable fibrinogen) and briefly centrifuged. Bio-Gel P-2 (200–400 mesh, Bio-Rad

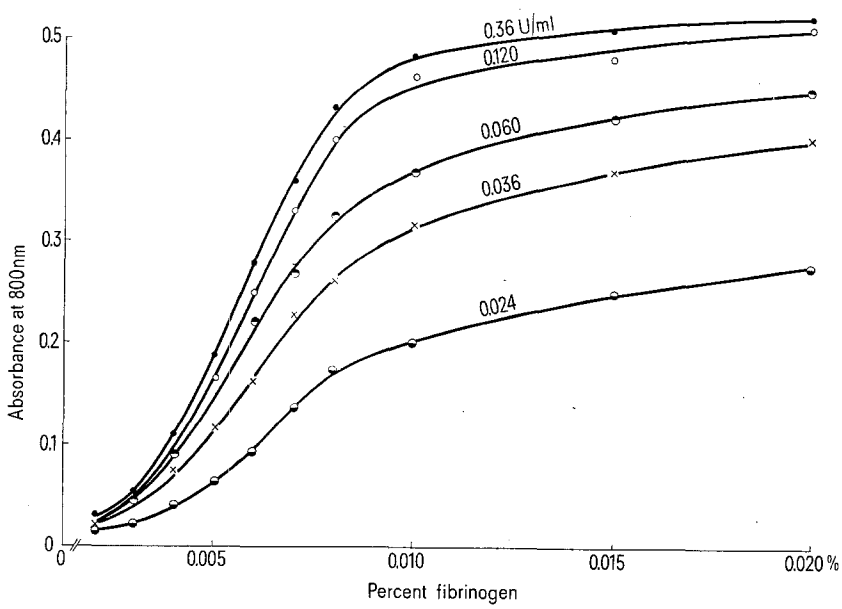

Fig. 3. Effect of fibrinogen concentration on clot absorbance at several thrombin levels, with fixed concentration of Bio-Gel P-2 (1%) in 'buffered saline' at 22 °C. *Legend:* Final thrombin concentrations. ●, 0.36 U/ml; ○, 0.12 U/ml; ◓, 0.060 U/ml; ×, 0.036 U/ml; ◒, 0.024 U/ml.

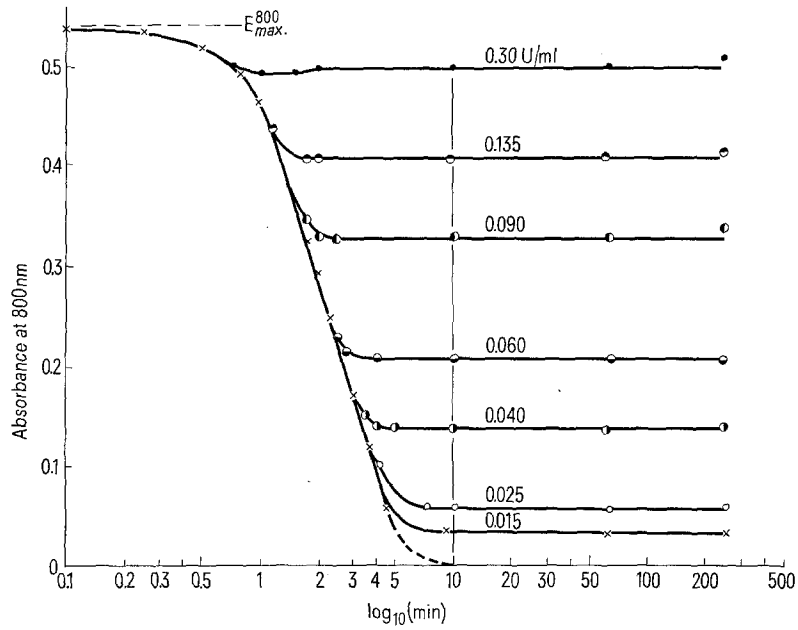

Fig. 1. Clot absorbance plotted against time (after combination of reagents), with fixed concentrations of fibrinogen (0.1%) and Bio-Gel P-2 (1%) in 'buffered saline' at 22 °C., for several concentrations of thrombin. *Legend:* ●, 0.30 units per ml; ◓, 0.135; ◐, 0.090; ◒, 0.060; ◑, 0.040; ○, 0.025; ×, 0.015.

Laboratories, Richmond, California, USA) was added to the supernatant to a final concentration of 1.25% (w/v). The resulting suspension was maintained at the desired temperature by slow stirring in a double-jacketted vessel. 2.0 ml aliquots were rapidly dispensed by syringe into 12 × 75 mm cuvettes containing various concentrations of purified bovine thrombin[1] in 0.5 ml buffered saline. After brief mixing, the cuvettes were set vertically in racks placed in water baths at appropriate temperatures. Final incubated mixtures contained 0.10% fibrinogen, 1.0% Bio-Gel, and various concentrations of thrombin in 2.5 ml. Absorbances were determined in a Coleman Junior II spectrophotometer. To minimize turbidity of fibrin itself, a wavelength of 800 nm was used[2].

Results. Firstly, to establish an incubation time, absorbance of samples at room temperature (22 °C) was measured as a function of time. From Figure 1 it is apparent that absorbance did not vary appreciably beyond each clotting time, or in any case beyond 10 min.

In the 2nd experiment, the final absorbance with a range of thrombin concentrations was studied at different temperatures, with fibrinogen concentration kept constant. In the 3rd experiment, fibrinogen concentration was varied at several thrombin levels, with temperature maintained constant. In the latter case, the thrombin solution and Bio-Gel suspension were pre-mixed and added by syringe to varying fibrinogen concentrations in buffered saline.

The effect of varying thrombin concentration at several temperatures is shown in Figure 2. It is apparent that the effect of thrombin concentration on absorbance varies with temperature, although it is not clearly seen at which particular temperature maximum sentitivity is achieved. For this reason, thrombin concentrations producing equal

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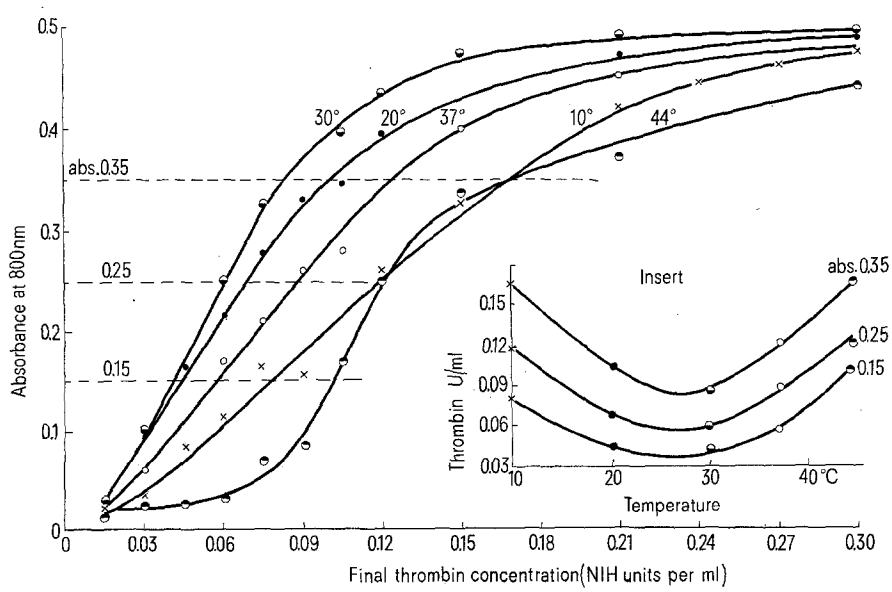

Fig. 2. Effect of thrombin concentration on clot absorbance at several temperatures, with fixed concentrations of fibrinogen (0.10%) and Bio-Gel P-2 (1%) in 'buffered saline'. *Insert*: Variation of thrombin concentration at fixed clot absorbance levels with temperature. *Legend:* ×, 10 °C; ●, 20 °C; ◒, 30 °C; ○, 37 °C; ◓, 44 °C.

absorbance were plotted against temperature, and the curves obtained for 3 different absorbance levels are shown in the insert of Figure 2. The results obtained indicate that the required thrombin concentration is minimal and, therefore, that sensitivity is maximal, somewhere in the vicinity of 25–26 °C. It is probable that increased viscosity of the gelforming mixture at lower temperatures is responsible for making the particle sedimentation method more sensitive just above room temperature than it is at 37 °C. Thus, under suitable conditions of temperature and fibrinogen ccncentration, thrombin levels of less than 1 U/ml can be readily determined by means of this procedure. Even lower concentrations should be similarly measurable using polymer beads which settle more slowly, eg. Bio-Gel P-2 (minus 400 mesh).

In the 3rd experiment, a range of fibrinogen concentrations was studied at different thrombin levels, with temperature being maintained at 22 °C (room temperature). From the data shown in Figure 3 it is apparent that fibrinogen concentrations as low as 0.003% can still be determined quantitatively by means of this method. One reason for this marked sensitivity probably is the lack of agitation in the incubated samples. The possibility of adapting this procedure to the quantitative determination of extreme hypofibrinogenemia would appear to be indicated[3].

Zusammenfassung. Die photometrische Absorptionsmessung für Fibrin-Gerinsel mit zum Teil sedimentierten Polyacrylamid-Partikeln ermöglicht eine quantitative Bestimmung sehr niedriger Thrombin- und Fibrinogen-Konzentrationen.

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[3] Acknowledgments: The authors are grateful to Mr. J. A. Reid for purification of the bovine thrombin. This work was carried out under a grant from the Medical Research Council No. MA 3618.

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The Antibiotic Edeine IX: The Isolation and the Composition of Edeine D

Previously we reported that the antibiotic edeine, produced by *Bacillus brevis* Vm4, appeared to be a complex of 4 biologically active principles named edeines A, B, C and D which could be separated on paper or thin layer chromatography[1]. We have also reported the preparative isolation of edeines A and B on cation exchange resins[2], carboxymethyl cellulose[3] and Sephadex[4]. Both compounds can also be isolated by counter current distribution[5]. Preparations of edeines A and B thus obtained are usually contaminated by their very closely related inactive isomers which could be removed in high voltage electrophoresis[6].

The composition[5,7] and the structure[6] of edeines A and B have been established. Both compounds are oligopeptides and conjugates of polyamines. Amino acid composition of both antibotics is identical. They contain one residue of each: glycine, 2-hydroxy-3-amino-propionic acid (isoserine), β-tyrosine, 2, 3-diaminopropionic acid and 2, 6-diamino-7-hydroxy-azelaic acid. Edeines A and B differ in the nature of polyamine moiety which is spermidine or guanylspermidine, respectively. In the present communication the preparative isolation and the determination of composition of edeine D is reported.

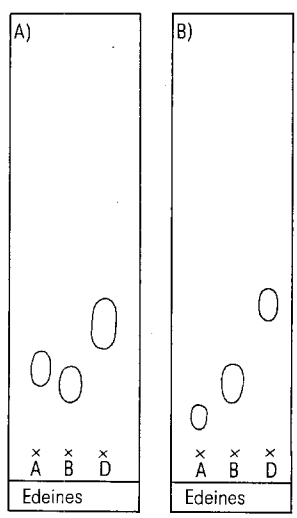

Fig. 1. Paper chromatography of edeine on Whatman No 1. A) Descending chromatography for 17 h in the solvent system: n-propanol: methanol:ammonia:water = 8:2:3:0,54. B) Ascending chromatography for 26 h on filter paper buffered with phsophate-citrate buffer (pH = 5) in the solvent system: phenol:buffer (phosphate-citrate pH = 5) = 1:1. Visualization with ninhydrin.

The crude edeine complex was obtained according to the previously described procedure[2] and further purified by counter current distribution[7] in solvent system prepared with one part of 80% aqueous phenol and one part of ammonium acetate buffer (0,15 M ammonium acetate and 0,3 M acetic acid), appliyng 500 transfers of upper phase.

The purified edeine complex obtained contained varying amounts of edeine D depending on the fermentation conditions and composition of fermentation medium. In some preparations substantial amounts of edeine D were present, although usually this component is produced as minor constituent of the complex.

The preparative isolation of edeine D from the complex could be effectively done by means of column chromatography in solvent system: isopropanol: ammonia: water = 60:35:5 and silicagel (particles below 0.08 mm) as absorbent or n-propanol: methanol: water: ammonia = 8:2:3:0,54 and cellulose. Using the former system, which is preferable, 70 mg of pure edeine D were obtained. Paper chromatography of edeine D as compared with edeines A and B is presented on Figure 1.

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