after the adsorption period enhanced the HA production. There was linearity with the increase of DMSO concentration and virus yield, the optimum being 60 vol./100 administered during adsorption. The addition of more than optimum DMSO concentration did not have further activator effect, thus is in agreement with others who worked with the RNA of poliovirus[6].

Discussion and conclusions. The positive serologic reactions and infectivity tests prove that the progeny is indeed PyV originating from the isolated genome used as inoculum. The biosynthesis was enhanced considerably by DMSO. The mechanism of action of this chemical is not yet clarified[8]. Our results confirm, in a different system the findings of BAYREUTHER et al.[5] hitherto not reproduced by others, emphasizing the universality of the DNA code[10] and demonstrating the penetration of the free viral genome during the initiation of the infective process in Protista[2-5,7,8]. Details, including infectivity titrations and findings with EMC-RNA will be published separately.

Zusammenfassung. Die Infektion von Schimmelpilzen, *C. albicans*, durch Polyomavirus mit isolierter Polyoma-DNS und die aktivierende Wirkung von DMSO werden beschrieben.

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[10] The assistance of Mr. G. KOLOMPÁR and B. BUCZ during these assays is gratefully acknowledged.

Isoenzyme of Some Dehydrogenases and Nonspecific Esterase in Experimental Virus Hepatitis in Mice

Some biochemical characteristics and the morphology of the development of experimental virus hepatitis in mice have been described in our previous papers[1,2]. The results concerning alkaline phosphatase, LDH and GOT suggest that some of their multiple forms (isoenzymes) originating in liver cell cytoplasm and subcellular particles are delivered to the blood from liver cells damaged to different degrees.

The present report deals with some qualitative changes of isoenzymes of LDH (lactate dehydrogenase, i.e. L-lactate: NAD oxidoreductase, EC 1.1.1.27), MDH (malate dehydrogenase, i.e. L-malate: NAD oxidoreductase, EC 1.1.1.37), ICDH (isocitric dehydrogenase, i.e. threo-D_s-isocitrate: NADP oxidoreductase [decarboxylating], EC 1.1.1.42), GlDH (glutamic dehydrogenase, i.e. L-glutamate: NAD(P) oxidoreductase [deaminating], EC 1.4.1.3), G-6-PDH (glucose 6-phosphate dehydrogenase, i.e. D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49) and nonspecific esterase during a slightly mitigated virus hepatitis in mice. LDH isoenzymes are used as well-known indicators of development of the infection. A strain of hepatotropic ectromelia virus, derived from the so-called Motol virus[1-4] was used. The virus was propagated in mouse fibroblast cells and partially purified by the method of end-point dilution.

Adult Swiss albino mice (strain 'H') weighing 21–23 g, fed a standard diet and water ad libitum were given i.p. injections of 0.1 ml containing approximatinelly $10^2 LD_{50}$ virus in phosphate buffer or only with vehicle (control group). 8 mice were sacrified each day by decapitation up to 7 days after the inoculation. Enzyme changes in the liver and blood plasma were analyzed separately in each mouse. Blood was collected in Heparin (Spofa) in order to obtain non-hemolyzed plasma. The liver was removed, washed with cold saline, dried with filter paper, weighed and homogenized for 40 sec in $4 \times 10^{-2} M$ veronal buffer at pH 8.3 (w/v) at 0 °C using a glass Potter-Elvehjem homogenizer (600 *g*). Sodium deoxycholate (Koch-Light) was added to a final concentration of 0.2%. The mixture was rehomogenized, stored at + 4 °C for 10 min and centrifuged for 30 min at 20,000 *g* at 4 °C. The supernatant was used for spectrophotometric determination of the total activity of enzymes and for electrophoresis. A quantitative micromodification of electrophoresis in agar gel[5] was employed. Electrophoresis was carried out in 1.5% purified Bacto-Agar (Difco) in $4 \times 10^{-2} M$ veronal buffer at pH 8.3 and ionic strength of 0.3 for 60 min at + 3 °C, 30 V/cm and 4 mA/cm. After separation, the electrophoreograms were incubated at 37 °C for 60 min (or for 20 min when determining LDH and esterase). The incubation mixtures for LDH, MDH and GlDH contained $10^{-1} M$ sodium lactate (Spofa), $5 \times 10^{-2} M$ sodium malate (Lachema) and 5×10^{-2} sodium L-glutamate (Koch-Light) as substrates respectively, and $2 \times 10^{-4} M$ PMS (N-methyl-phenazonium methosulfate, Calbiochem), $2 \times 10^{-3} M$ NAD (Boehringer), $1.5 \times 10^{-3} M$ NBMT (2-/*p*-nitrophenyl/-5-phenyl-3-/3, 3'-dimethoxy-4-diphenylyl/-tetrazolium chloride)[6]. The ICDH assay mixture contained $2.7 \times 10^{-3} M$ DL-isocitric acid trisodium salt (Koch-Light), $2 \times 10^{-4} M$ PMS, $2.5 \times 10^{-3} M$ NBMT, $1.8 \times 10^{-3} M$ NADP (Boehringer), and $10^{-2} M$ $MnCl_2$. The G-6-PDH mixture contained: $1.3 \times 10^{-3} M$ D-glucose-6-phosphate disodium salt (Calbiochem), $8 \times 10^{-4} M$ PMS, $7.5 \times 10^{-4} M$ NBMT, $2.3 \times 10^{-3} M$ NADP, and $5.5 \times 10^{-2} M$ $MgCl_2$. The esterase mixture consisted of $5.3 \times 10^{-4} M$ 2-naphthyl acetate (Lachema), and 0.1% Echtblausalz BB (Hoechst). $4 \times 10^{-2} M$ veronal buffer at pH 8.3 was used in all reactions. After incubation the electrophoreograms were washed with 3% acetic acid. It should be pointed out here that a better separation was achieved at the higher voltage as compared with previous experiments[2] using a prolonged time of electrophoresis. In addition, a different agar batch was used.

The infection culminates 7 days after inoculation, i.e. 1–2 days later, as compared with results obtained if using larger doses of the virus[1,2]. The morphological characteristics of the liver were analogous with those already described[1]. Total activities of all blood plasma enzymes studied increases considerably, beginning on day 3–5 and culminating at 7 days. The changes in total activities of liver extracts were not significant. The changes in isoenzymes originating as a result of damaged liver are

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summarized in Figures 1–3. In the extracts of liver homogenates, only quantitative shifts in proportions of individual isoenzymes are mostly observed, whereas the changes in blood plasma are not only quantitative but also qualitative (mainly the increase in all LDH_5 subfractions, LDH_4, and LDH_3) beginning 4 days after inoculation. Changes in some enzymes were not significant (GlDH, ICDH); on the other hand, striking changes were observed in MDH and G-6-PDH. Very active fractions of MDH and G-6-PDH, absent in controls, were found in the blood plasma of infected animals. Their electrophoretic mobilities and the increase of the activity during infection correspond to those of the liver fractions. New fractions of esterases occurred in blood plasma already 4 days after the infection. The blood plasma was then flooded with liver nonspecific esterase isoenzymes at the peak of the liver damage.

It should be pointed out that it was not the aim of the present communication to explain the fact that more LDH isoenzymes were observed as compared with the previous paper[2]. It may be assumed that low molecular dissociation products of enzymes[7], NAD-dependent cytoplasmic dehydrogenase ('nothing dehydrogenase')[8] or perhaps even adsorption to proteins[9] are responsible for the finding. However, the fact that additional fractions, originally present only in liver at the advanced stage of the disease, were found even in blood plasma appears to be of importance.

This finding complements our previous observations[2] concerning liver damage development. The process begins with an injury of the liver cell membrane resulting in the passage of cytoplasmic enzymes (e.g. cytoplasmic MDH and G-6-PDH) into the blood and terminates in the occurrence of cell particle isoenzymes in the blood. The blood is thus flooded with cytoplasmic as well as liver cell particle isoenzymes of the peak of infection, and the composition of the spectrum of their isoenzymes resembles considerably that of isoenzymes of liver cells.

The micromethods used can be applied to studies concerning the activities of enzymes and their fractions during experimental hepatitis and may hence contribute to our knowledge of the development of pathological changes at the subcellular level.

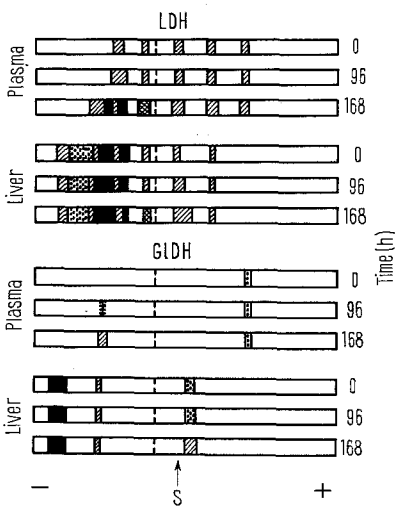

Fig. 1. Schematic outline of changes of LDH and GlDH isoenzymes in blood plasma and liver homogenate extracts during experimental hepatitis (mean values). (—), anode; (+), cathode; S, start; 96 and 168, h after inoculation. Full bands, very intensive staining; dashed bands, medium staining; dotted bands, (very) weak staining.

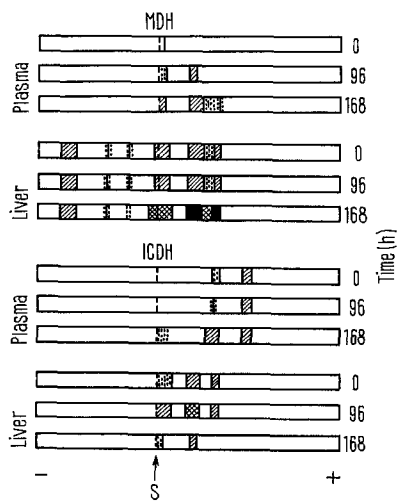

Fig. 2. Schematic outlines of changes of MDH and ICDH isoenzymes in blood plasma and liver homogenate extracts during experimental hepatitis (mean values). Explanation see Figure 1.

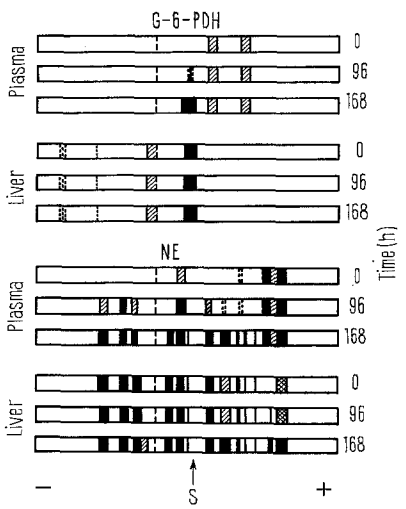

Fig. 3. Schematic outline of changes of G-6-PDH and nonspecific esterase isoenzymes in blood plasma and liver homogenate extracts during experimental hepatitis (mean values). Explanation see Figure 1.

Zusammenfassung. Verschiedene Dehydrogenasen, die physiologischerweise nur in der Leber vorkommen, wurden bei Virushepatitis der Mäuse in fortgeschrittenem Stadium auch im Blut gefunden.

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