Cretaceous) and purely aquatic relative, Xenopus, family Pipidae[4]. Exceptions to the ranid mode of lens formation[3] noted by us include small size (e.g. at Stage V, the lens rudiment of D. pictus is only $^1/_4$ the diameter of that of the corresponding R. pipiens lens stage); and late persistance (after Stage X) of a lens vesicle remnant. These did not alter, however, the consistent immunofluorescence profile for γ crystallins that we obtained; we submit that overwhelming evidence, both intra- and interphyletic, confirms this class of lens-specific structural proteins as one of the most valuable probes available to the developmental biologist today.

On the biomechanical function of the liver capsule[1]

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Summary. We have carried out histomechanical studies on the human and bovine liver capsule under tension loading. Results: Nonlinear force-time curve under extension with constant speed, initial upper and lower summit decrease of the dynamic relaxation curve, amplitude diminution phenomenon of the dynamic relaxation in the lower nonlinear part of the extension-time curve, dynamic (cyclic) force recovery.

The main components of the liver capsule are connective tissue cells, nerves, collagen, and elastic fibres. The physiological intrahepatic tissue pressure interacts with the tension of the capsule[3–6]. The liver capsule expands in consequence of an acute gain in volume with certain diseases of the liver. Impacts in a blunt abdominal trauma can result in high tensions of the liver capsule as well as in ruptures of the capsule and parenchyma. In this context, the question arises which mechanical properties the liver capsule embodies[7–10].

Materials and methods. We have studied the human and bovine liver capsule. The histomechanical examinations were performed 24–36 h after death. The specimens were stored about + 3 °C. A rectangle area was marked on the surface of the liver. The marginal lines of this rectangle were cut with a razor blade. After that the designated capsule area was carefully dissected. Then the specimen was once more measured and attached to the clamps of the testing machine. The histomechanical loading examinations were performed in physiological solutions of defined temperatures with the Dynatron after Meskat, Rosenberg and Hoffmann[11] for dynamical stress-strain curves and a statical universal testing machine[12, 13].

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3 G. Arnold, F. Gross, M. Zech and P. J. Macharey, Anat. Anz., in press (1977).

4 Y.-C. B. Fung, Biorheol. *10*, 139 (1973).

5 T. Nemetschek, in: Sitzungsberichte der Heidelberger Akademie der Wissenschaften. Math.-naturw. Klasse, Jahrg. 1974, 3. Abh. Springer-Verlag, Berlin/Heidelberg/New York 1974.

6 J. Schurz, Physikalische Chemie der Hochpolymeren. Springer-Verlag, Berlin/Heidelberg/New York 1974.

7 H. v. Mayersbach, Allgemeine Histologie. Gustav-Fischer-Verlag, Stuttgart 1973.

8 H. G. Vogel, Conn. Tiss. Res. *3*, 237 (1975).

9 A. Viidik, L. Sandquist and M. Maegi, Act. orthop. scand., Suppl. *79*, 5 (1965).

10 W. Worthmann, G. Arnold und H. Lippert, Int. Z. angew. Physiol. *31*, 77 (1973).

11 W. Hoffmann, Rheol. Acta *5*, 101 (1966).

12 J. Diamant, A. Keller, E. Baer, M. Litt and R. G. Arridge, Proc. r. Soc. Lond. B. *180*, 293 (1972).

13 F. Hartmann, in: Neue Gesichtspunkte zu den Folgen der chronischen Polyarthritis. Symposium am 25./26. Mai 1973 in Timmendorfer Strand, p. 40. Ed. F. Hartmann. Byk Gulden Pharmazeutika, Konstanz 1974.

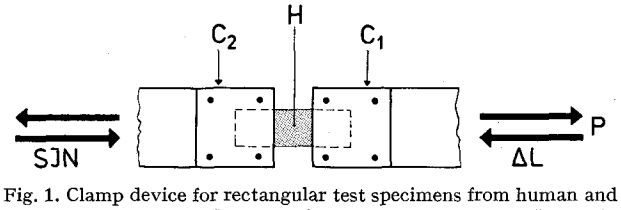

Fig. 1. Clamp device for rectangular test specimens from human and bovine liver capulse. C_1 right clamp which induces a change in length (ΔL) at the capulse specimen (H) with a certain preselected constant speed. P force (load) measurement device attached to the right clamp. C_2 left clamp which brings about sinus-shaped changes in length.

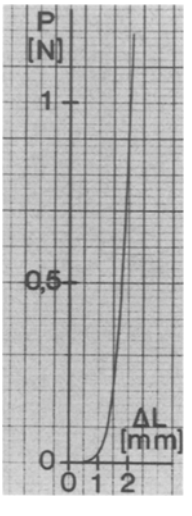

Fig. 2. Force-extension diagram from the bovine liver capsule. Size of the capsule test specimen before dissection 'in situ' 30 × 10 mm. After the piece of capsule had been dissected, a certain contraction could be observed. The mechanical test was performed in Ringer solution. The diagram was taken from a steady state series (after preconditioning). Abscissa: increase in length ΔL in mm. Ordinate: force (load) P in Newton. The initial length between clamps before preconditioning and in a perfect release from tension was 10 mm.

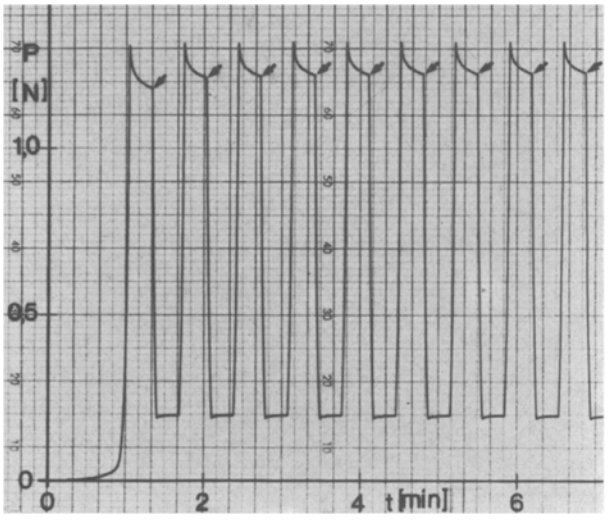

Fig. 3. Loadings and partial tension releases at constant time intervals with interposition of isometric phases (constant length) which give rise to relaxation and low recovery curve sections. Right angle test specimen, 30×10 mm before dissection ('in situ' size), 29.8×8.6 mm after dissection, examination in a physiological solution (PBS) at 36.5 °C, initial length 10 mm between clamps and total release from tension prior to the force-extension tests (without preconditioning); speed of change in length $v_{\Delta L}$ = 17 mm/min. Abscissa: time t in min. Ordinate: force (load) P in Newton.

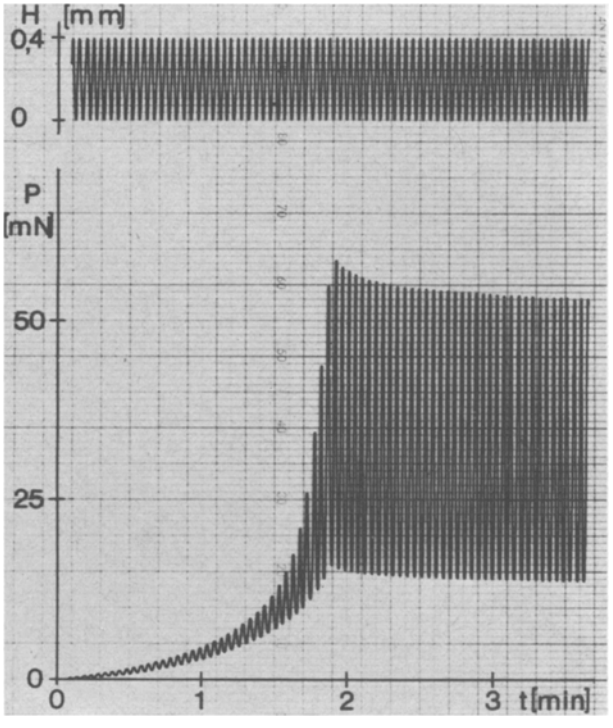

Fig. 4. Nonlinear dynamic (cyclic) force increase (first part of diagram) and dynamic (cyclic) relaxation (second part of diagram). A right angle test specimen was dissected from bovine liver capsule. 30×10 mm before dissection, 28×9.5 mm after dissection, initial length between clamps and total release from tension L_o = 10 mm, Ringer solution, temperature about 33 °C, speed of change in length $v_{\Delta L}$ = 1.6 mm. Upper curve: sinus shaped length-time input function; the linear ramp length-time input function is not represented in the figure; lower curve: force-time output function, H = heave (double amplitude) in mm, P = force in 10^{-3} Newton, time record synchron for both curves in min. Mechanical history: numerous similar tests within damage-free stress-strain ranges had been performed prior to the represented diagram under consideration of time and speed conditions.

Results. The test specimen from the liver capsule was attached to the clamps of the testing devices as shown in figure 1. The right clamp is denoted with C_1 in figure 1. With this clamp, increases or decreases in length (changes in length) of the test specimen can be carried out with preselected constant velocities. The force-extension diagram shows initially a low increase of force under a relatively large increase in length. After the curved part of the diagram the force increases sharply and approximately as a straight line.

When the strain process performed on the liver capsule is interrupted in such a manner that the length is kept constant, a time dependent stress or force decrease can be observed which is called relaxation (figure 3). The strain and inverse strain proceedings are recorded with the time as independent variable on the abscissa. This advantageous in most histomechanical tests. In the test of figure 3, the mechanical stress-strain history should be taken into account: After numerous loadings within the damage-free force range, a pause of 2 min duration was interpolated under total release of the load. The representation of figure 3 demonstrates for example a histomechanical (historheological) transient process of the relaxation curves. The relaxation angle points are labelled with arrows. The first relaxation angle points are lower than the following ones. The last relaxation angle points are situated on an equal force level. The tissue is now in a viscoelastic (rheological) steady state.

In figure 4 the liver capsule is stretched with a constant (linear) strain rate of the clamp C_1 (figure 1). After 1.9 min, the strain is kept constant (isometric condition). From the beginning, an additional sinus strain is superponed with clamp C_2 (figure 1). A dynamical (cyclic) force-

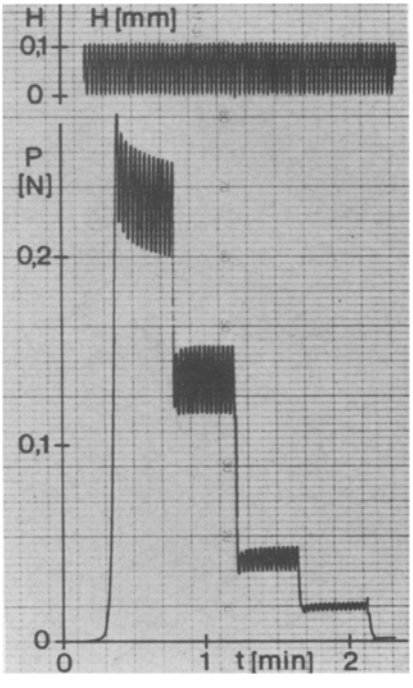

Fig. 5. Dynamic (cyclic) relaxation and cascade dynamic (cyclic) force recovery curves (mechanical recreations). Bovine liver capsule, 30×10 mm before dissection, 29×8 mm after dissection, Ringer solution, temperature about 34 °C, L_o = 10 mm, $v_{\Delta L}$ = 17.6 mm/min. Mechanical previous history: numerous similar loadings with similar equal time intervals had preceeded the diagram represented. H = heave (double amplitude) length-time input function in mm, P = force-tome output function in Newton, time t in min synchron for both curves. The C_1 input function (multiple ramps) is not represented in the figure.

time output function results from the 2 input functions. The force increases curvilinear with an overproportional heightening of the force amplitudes. In the dynamical relaxation phase, we observe in figure 4 a pronounced upper and a slight lower summit decrease. The force difference of the upper and lower summit decreases initially. This appearance is called amplitude (or heave) diminution phenomenon of the dynamic relaxation in the lower nonlinear part of the extension-time curve. Figure 5 shows after a fast stretch under constant length and superponed sinus wave extensions a short dynamic relaxation phase. After a repeated partial relieve of the tension on account of an induced decrease of the length and repeated interposition of isometric conditions, an initial increase of the force amplitudes can be observed (figure 5). This appearance is called inverse dynamic (cyclic) relaxation or dynamic (cyclic) force recovery. The amplitudes of the sinus wave strain input function can be seen in the upper part of figure 5. The amplitudes of the output function recovery curves are small in the lower force regions.

Discussion. The main component of the liver capsule are collagen fibres. They have biomechanical functions[14]. The considerable initial compliance with a high resistance force under further elongation is an important protective mechanism with blunt abdominal injuries. The substantial elongation properties of the liver capsule at the beginning of the force-extension or force-time diagram are essential in acute volume expansions of the liver in as much as the tissue pressure of the parenchyma increases protacted initially.

The histomechanical diagrams are reproducible provided that a series of identical curves in considerations of equal time distributions are generated, and on the condition that we take into consideration the curves of the steady state phase after the transient phase, for example the last cycles of figure 3. Figure 2 represents a curve out of a series in the steady state phase. After a partial force release, the liver capsule does not contract instantaneously like an ideal elastic body because the elastic reset forces are damped by viscous elements of the tissue[15]. Under isometric conditions (constant length) after a partial force or length release, a time dependent static or dynamic (cyclic) force recovery arises.

Our experiments have shown a complex mechanical behaviour of the liver capsule. The biomechanical properties of the liver are evidently versatile and adapted to physiological and pathological stress-strain conditions and within certain limits to traumatic impacts[16].

14 R. Fricke, in: Biopolymere und Biomechanik von Bindegewebssystemen, p. 149. Ed. F. Hartmann. Springer-Verlag, Berlin/Heidelberg/New York 1974.
15 H. Greiling, in: Neue Gesichtspunkte zu den Folgen der chronischen Polyarthritis. Symposium am 25./26. Mai 1973 in Timmendorfer Strand, p. 25. Ed. F. Hartmann, Byk Gulden Pharmazeutika, Konstanz 1974.
16 B. Kummer, in: Biopolymere und Biomechanik von Bindegewebssystemen, p. 19. Ed. F. Hartmann, Springer-Verlag, Berlin/Heidelberg/New York 1974.

Sister chromatid exchanges in human lymphocytes exposed to 8-methoxypsoralen and long wave UV radiation prior to incorporation of bromodeoxyuridine

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Summary. Human lymphocytes exposed to the effects of long wave UV radiation in the presence of 8-methoxypsoralen prior to stimulation by PHA show dose related sister chromatid exchanges after 2 replication cycles in vitro. This has implications for interpreting the repair processes involved and for monitoring DNA damaging agents in vivo.

8-Methoxypsoralen (8-MOP) is a furocoumarin compound which, on being excited by 360 nm radiation, forms covalently bound adducts with pyrimidines[1]. The molecule may form adducts at either or both ends so that monoadducts and cross links affecting both strands of the DNA molecule are formed[2]. Clinically it has been used in the treatment of psoriasis[3] and suggestions that the treatment may cause chromosome damage in man[4] and be potentially mutagenic or carcinogenic have given rise to concern.

The recently developed techniques[5,6] for staining chromosomes after cells have been grown for 2 rounds of replication in the presence of bromodeoxyuridine (BrdU), which substitutes for thymidine, make it possible to distinguish between chromatids in which both DNA strands have been substituted and those retaining the original 'old' DNA strand. This has permitted the clear demonstration of sister chromatid exchanges (SCEs) when these occur[7]. A number of known carcinogens and mutagens when present in the culture medium have been shown to have marked effects on the rate of SCE in cultured chinese hamster cells[8], or human leukocytes[9], presumably because these substances cause damage to the DNA.

We have used the BrdU technique, essentially as described by Perry and Wolff[6] with minor modifications, to examine the effect of 8-MOP and long wave UV radiation (UV-A 315–390 nm) on the production of SCEs in human lymphocytes in vitro when the treatment is administered prior to the addition of BrdU to the cultures. This has shown that the effect of certain DNA damaging agents in producing SCEs can be demonstrated without the necessity of labelling the chromosomes before the administration of the agent.

1 L. Musajo, G. Rodighiero and F. Dall'Aqua, Experientia *21*, 24 (1965).
2 R. S. Cole, Biochim. biophys. Acta *217*, 30 (1970).
3 J. A. Parrish, T. B. Fitzpatrick, L. Tannenbaum and M. A. Pathak, New Eng. J. Med. *291*, 1207 (1974).
4 G. Swanbeck, M. Thyresson-Hok, A. Bredberg and B. Lambert, Acta derm. (Stockholm) *55*, 367 (1975).
5 S. A. Latt, Proc. nat. Acad. Sci. USA *70*, 3395 (1973).
6 P. Perry and S. Wolff, Nature *251*, 156 (1974).
7 S. A. Latt, Proc. nat. Acad. Sci. USA *71*, 3162 (1974).
8 P. Perry and H. J. Evans, Nature *258*, 121 (1975).
9 B. Beek and G. Obe, Humangenetik *29*, 127 (1975).