Table II. Effects of a 24-h pulse of ecdysone analogue on wing disks cultured in vitro without fat body

Compound	Cuticle (%)	Evagination (%)
α-Ecdysone	0	0
β-Ecdysone	50 (2.0 μg/ml)	100 (2.0 μg/ml)
Ponasterone A	35 (0.1 μg/ml)	65 (0.1 μg/ml)
Inokosterone	0	15 (0.5 μg/ml)
Podecdysone A	0	0
2-Deoxycrustecdysone	25 (10.0 μg/ml)	55 (10.0 μg/ml)
22,25-di-Deoxyecdysone	0	0

body. Both evagination of the disk and deposition of cuticle were scored in each test. Each test was run at least twice with a minimum of 20 disks. The ecdysone compounds were tested at concentrations of 0.1, 1.0, and 10.0 μg/ml of medium in each of the continuous exposure assays, and also at the most effective concentrations as determined in these tests and in earlier pulse experiments.

The effects of continuous exposure at the minimum effective concentrations are reported in Table I. The 24-h pulse data are shown in Table II. Exposure to solvent controls (10% or 50% ethanol) and a synthetic isomer, 22-iso ecdysone, had no effect in any of the assays.

The data show clearly that the mode of exposure to the hormone and the type of response examined influenced the relative activity of the test compound (Tables I and II). This difference was also observed when *Drosophila* disks were cultured in vitro. Here, opposite conclusions about the relative activity of α- and β-ecdysone were reached by workers who used either evagination[13] or cuticle[14] deposition as their bioassay. In our experiments we found that cuticle deposition was a better criterion of activity than evagination, but it was best to examine both responses. On the basis of both evagination and cuticle deposition in the three in vitro bioassay systems described, we suggest the following order of activity for the ecdysone analogues tested: β-ecdysone > ponasterone A > 2 deoxycrustecdysone > inokosterone > α-ecdysone > 22,25-dideoxyecdysone > podecdysone A.

Probably, because β-ecdysone, 2-deoxycrustecdysone and ponasterone A (all with 20-hydroxy groups) stimulate both cuticle deposition and evagination after a pulse treatment without fat body, they are acting as hormones per se. However, two other analogues (α-ecdysone and inokosterone) that required continuous exposure or the presence of fat body to become effective in our in vitro bioassays may be converted to more active forms. It is interesting that the addition of the ethyl group to the side chain of β-ecdysone produced a compound (podecdysone A) that was even less active that one (22,25 di-deoxyecdysone) without the 20-hydroxy group. On the basis of our study, the activity of ecdysone analogues may be easily affected by altering the side chain structure[15,16].

Résumé. Le développement des disques imaginaux isolés en culture in vitro en présence de β-ecdysone a été comparé au développement en présence des substances analogues.

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DNA Degradation During Organ Storage

One of the critical areas in organ transplantation is the viability of the transplanted organ prior to re-establishing organ blood flow. Preservation of cadaver kidneys is needed to allow for preparation of the recipient for transplant surgery if the donor and recipient are in the same hospital, or to permit transport of the kidney to a suitable recipient in another hospital. For kidney transplantation, the organ is exposed to warm ischemia during removal from the donor and to cold ischemia during storage. Events occurring during these ischemic periods that prevent long term viability are not fully understood. Knowledge of the cellular defects that occur during storage will present areas to be modified for improving preservation. Long term organ survival may require an intact DNA memory bank. This study was designed to examine the effects of organ storage on the integrity of the nuclear DNA.

Materials and methods. The size of DNA molecules was examined by low shear viscometry[1]. Viscosity measurements allow evaluation of the size of high molecular weight DNA. Charles River strain male mice, 6–10 weeks old, were sacrificed; the kidneys flushed with 0.15 *M* NaCl to remove the blood; and the kidneys excized. One kidney was either stored at 37 degreees (warm ischemia) or at 0 degrees (cold ischemia) in 2 ml of 0.15 *M* NaCl. The contralateral kidney was taken as the control, and nuclei isolated immediately[2]. The nuclei were lysed overnight at 50 °C by 0.5% SDS and 5 m*M* EDTA (pH 6.2). After storage, nuclei were isolated from the kidney[2] and lysed overnight. The viscosity was then determined at 50 °C using a low shear viscometer[1] (Beckman Instruments, Palo Alto, California). The viscosity was stable over an 8-h period (16–24 h after lysis was initiated). The intrinsic viscosity determined for the lysed nuclei is, for practical purposes, dependent on the DNA, while the

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Storage conditions	No. of experiments	Control n (dl/g) [a]	Test n (dl/g) [a]	Control MW × 10^{-8}	Test MW × 10^{-8}	% Change in n (in MW)	P [b]
37 °C for 0.5 h	8	593	476	2.37	1.71	−20 (−28)	< 0.01
37 °C for 1.0 h	8	695	433	3.01	1.49	−38 (−50)	< 0.01
0 °C for 72 h	7	593	426	2.37	1.45	−28 (−39)	< 0.001

The nuclei were lysed overnight at 50 °C in 0.5% SDS, 5 mM EDTA (pH 6.2); 0.125 mM $MgCl_2$; 0.30 M sucrose. The n_{rel} was determined at a shear stress of 0.0044 dynes/cm² at 50 °C in the low shear viscometer (Beckman Instruments). The n_{rel} was determined at 3 DNA concentrations of 2–10 μg/ml. [a] n was determined by the average of the three ln n_{rel}/C. [b] The p value for the change with storage was determined by the t-test for paired samples.

protein and RNA do not significantly contribute to the viscosity[3]. The presence of pronase 100 μg/ml in the lysis solution did not affect the viscosity. The viscosity determinations were done at 3 DNA concentrations. The concentration of DNA, C, was determined by the diphenylamine reaction[4]. The specific viscosity expressed in ln n_{rel}/C is equal to the intrinsic viscosity within experimental error[3]. The average of ln n_{rel}/C at 3 different DNA concentrations from 2–10 μg/ml was used to determine the specific viscosity n in dl/g.

Results. The results presented in the Table show that warm ischemia for 30 min resulted in a significant decline in the viscosity, and indicate that this decline was greater with 1 h of warm ischemia. When a kidney was stored in the cold for 72 h, there was also a significant decline in the viscosity of the DNA. Significance was determined by utilizing the student t-test for paired samples. The test kidney was compared to the contralateral control kidney. The viscosity of DNA between normal mice varied as shown by the 3 control values. A similar variation was seen for rat liver DNA[3].

Viscosity determinations were done at lower salt concentrations than used by others[3,5] (30 mM compared to 195 mM). When the experiment was repeated with 195 mM NaCl, it was more difficult to get reproducible readings. The values of n were 16% lower at 195 mM than at 30 mM salt. Accordingly, the molecular weight of the DNA was determined by reducing n 16% before using the equation of CROTHERS and ZIMM[5].

Discussion. The decrease in viscosity is due to double strand and not single strand DNA breaks; it has been demonstrated that single strand nicks do not decrease the viscosity[6]. The decrease in the size of the DNA that follows a period of storage – both warm and cold – may contribute to potential organ survival. Radiation injury of cells offers a possible analogy. A correlation between survival and primary lesions in the DNA exists for radiation induced breaks in the DNA[7,8]. With double stranded breaks, information important for long term viability may be lost after organ storage. Also, the break in the DNA and the reduced molecular weight could explain previous findings of decreased ability of nuclei to synthesize RNA[2] and the lower molecular weight of the synthesized RNA after storage (LAZARUS and HOPFENBECK)[9].

Prior studies have shown that the molecular weight of the DNA decreased during the process of aging[3]. In the aging process, the survival of the entire organism decreases along with the decrease in DNA size. The chance for survival of the stored organ may also decrease as the molecular weight of the DNA decreases. Storage decreases the size and therefore the integrity of the DNA. The storage periods studied without perfusion (1 h of warm ischemia and 72 h cold ischemia) cause organ death[10,11].

The degradation of the DNA during storage may reach an irreversible level not correctable by the repair mechanisms. To insure that an organ remains viable, protection of the DNA may be necessary.

Zusammenfassung. Es wurden Kerne aus isolierten Mäusenieren, die unter warmen und kalten Bedingungen aufbewahrt worden waren, isoliert. Nach der Bestimmung der Viskosität wurde das Molekulargewicht der kernhaltigen DNS während der Konservierung reduziert. Die Abnahme der DNS-Molekulargrösse bewirkt möglicherweise eine Kürzung der Überlebensdauer des isolierten Organs.

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