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## I. Surgical Technique, Ureter and Urinary Diversion

### Splintless Microsurgical Uretero-Ureteric-Anastomosis in the Dog

W. Kramer, D. Jonas and W. Weber

The use of splintless microsurgical uretero-ureteric anastomosis was investigated in the dog: 1. Do microsurgical techniques improve the results in end-to-end ureteric anastomosis? 2. Is absorbable 8-O Vicryl® an apt microsurgical suture? 3. Does exact microsurgical end-to-end anastomosis of the ureter save postoperative splinting?

Under Halothane® anesthesia the right ureter (av. 5,8 Charr.) in 24 female mongrel dogs (av. 17 kg body weight) was divided obliquely at its abdomino-pelvic transition. For anastomosis under the surgical dissecting microscope suture techniques were applied as follows (one in 6 dogs): Submucous interrupted resp. running suture and all layer interrupted resp. running suture, with stitches at 1 mm intervals in all 4 techniques and 8-9 knots in the interrupted suture; a watertight anastomosis was aimed for. Results were assessed 3 months postoperatively by IV. urograms, light and scanning electron microscopic investigation of the anastomotic site, pre- and postoperative microscopic and bacteriological examination of bladder urine in all animals.

Differences of the results of all suture techniques were minimal. In 22 of 24 ureteric anastomoses (2 poor results due to faulty technique) good anatomical and functional results were obtained. The use of Vicryl® and without ureteric splinting proved a success. The method of microsurgical ureteric anastomosis is recommended for the human patient in small anatomical proportions. Exact approximation of the ureter was of greater importance than the choice of suture technique.

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### Morphometric and Karyometric Studies on Hydroureters During Complete Distal Obstruction and after Restoration of Urinary Continuity by Uretero-Cyst-Neostomy

U. Stöber and B. Aeikens

We wanted to determine the influence of the ureter on the regeneration of tubule-cell-function after removal of a complete obstruction.

The restoration of urinary continuity after a complete ligature of the distal ureter was performed by uretero-cyst-neostomy or by splints, placed into the pelvis of the kidney. The regeneration of the tubule cells and the alterations of the single layers of the ureter were determined histologically by morphometry and karyometry.

The recovery of renal function was more complete after continuity was restored by splints. After advanced obstruction and restotration of urinary continuity by uretero-cyst-neostomy a relative increase of pressure of the ureter persists. This is the result of a functional obstruction, caused by replacement of the muscle cells by connective tissue. The proliferation of connective tissue is probably induced and maintained by extravasation of urine into the layers of the ureter and that after abolition of the special function of the tunica mucosa. The defects of the mucosa are probably caused by metabolic disturbance after a pressure induced decrease of blood supply.

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### Carbon-Polymer Stomata in Experimental Cutaneous Ureterostomy

R. Harzmann, St.H. Flüchter, L.I. Kobashi and K.-H. Bichler

Next to the nephrostomy, cutaneous ureterostomy is the simplest form of urinary diversion. It does have, however, one drawback: the problem of stoma stenosis. In addition, there are the usual problems with urological stomata — skin irritation, unpleasant smell and intolerance of the appliance. On the basis of encouraging results with experimental and clinical im-

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plantation of stoma prosthesis of carbon-polymers for vesicostomies, implants of this material were studied in the ureter.

Dilatation of the ureter was produced in 16 mongrel dogs by knotting a chrome catgut thread over a 4 F splint which had been introduced into the ureter. After 7 days the ureter was removed prevesically and a carbon-polymer stoma with Dacron sleeve and 5 F lumen was implanted in the ureter and fixed pararectally. X-rays were taken every two weeks. At 3, 6 or 9 postoperative months the stoma was explanted and examined histologically. 24 of the 32 stoma implants were tolerated well and provided continent urinary drainage, slight incrustation and, radiologically, a good flow of contrast medium. 10 of these 24 cases had had transient leakage of urine. 8 of the 32 stoma implants were unsuccessful because of insufficient healing, urinary extravasation, stoma hernia or incrustations. One major problem proved to be the tendency of the ureter to form folds at the level of the stoma.

The results of these experiments on animals would seem to justify a clinical study. It is conceivable that in this way stoma stenosis of the cutaneous ureterostomy can be avoided.

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## Protective Ureteric Displacement into a Silicone-Sheath for Induced Retroperitoneal Fibrosis: Microsurgical Experiments on Rats

L.V. Wagenknecht, H.M. Langendorff and H. Schäfer

At present, the "therapy of choice" for patients with retroperitoneal fibrosis is ureterolysis with intraperitoneal transposition. This procedure, however, leaves the upper and lower part of the ureter vulnerable to recurrent fibrotic stricture since these portions still remain within the retroperitoneal space. In order to protect the ureter in its entire length an alloplastic cover might offer a better alternative.

Following experimental induction of retroperitoneal fibrosis (phenol mandelic oil) in rats the entire ureter was displaced into a silicone envelope. Under the operation microscope the silicone sheath was closed around the renal pedicle by separate sutures 6/0 prolene. The upper and lateral sealing of this pouch was done by continuous sutures and silicone adhesive type A and the lower opening of this silicone envelope was fixed to the bladder wall.

Progressive retroperitoneal fibrosis caused anterior displacement of the silicone pouch but neither a fibrotic infiltration into this cover nor ureteric stenosis was noted.

Histologic investigations of these animals in comparison to the control group showed effective protection by the silicone cover. Experiments with 5 dogs confirmed these positive results with rats. Longterm results will show whether this procedure might be applied clinically.

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## Replacement of the Ureter: Animal Experiments with a Modified Loop of the Small Intestine

K. Weigner, H. Kiel and S. Lymberopoulos

The problem of a replacement of the ureter in the case of kidneys that are worth being preserved has not been solved yet. Experiments with homo- or heterological material has not achieved satisfactory results, up to now. The right ureter of 15 Beagle bitches was exposed by transperitoneal median approach and the middle third was extirpated. Then, analogous to the ileum conduit, the resection of an adequately long loop of the small intestine was performed. The intestine loop was transected longitudinally at the level of the radix, the mucosa was removed by means of the scalpel and the intestinal wall was tilted over and stitched up. This way the serosa formed the lining of this segment, that was to be interpolated, splinted and anastomosed with the distal and proximal end of the ureter.

Four weeks after the removal of the splint nine of the Beagle bitches showed a good output of the contrast medium of their kidney without any significant congestion. Two animals died immediately after the operation, two of them after 7 days, showing signs of urinary peritonitis. Two lost their ureter splints during their first postoperative days and developed a urinary fistula. These early results of ureter replacement by a modified loop of the small intestine are encouraging. A partly active urinary conduit caused by the peristalsis of the loop of the small intestine is to be discussed in those cases where no congestion occurred. However, safe interior splinting and adequate fixation in order to avoid a dehiscence of the suture seems to be a problem with dogs.

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## The Ileal-Conduit-Syndrome

E. Wilhelm and P. Thierauf

Ileal conduit urinary diversion was carried out in 15 mongrel dogs. 12 animals survived longer than 3 weeks with an average follow-up of 8.2 weeks. All dogs surviving that long were assessed with loopogram and infusion urography after the third week. When they died or were sacrificed the ileal segment including mesenterial stalk was histologically examined. The findings were compared with control sections from the entero-enterostomotic segment.

All of the conduits examined exhibited the following constant features, seen also in coeliac disease:

1) Radiographic features: straightening, thickening, separation and finally disappearance of the valvulae conniventes.

2) Histological features: atrophy of the ileal mucosa, inflammatory infiltration of mucosa and submucosa and enlargement of the regional lymph nodes due to reactive hyperplasia.

In addition to the susceptibility of the ileum to motor functional disturbance with obstruction, this Ileal-Conduit-Syndrome could, theoretically, be a màjor factor in explaining the poor late results of ileal conduit urinary diversions.

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## Can Urinary Continence be Established by the Use of Free Autologous Transplants of Smooth Musculature?

D. Hauri, R. Ackermann, H.-R. Osterhage and H.-J. Leisinger

We aimed to develop a stoma for urinary continence according to the method described by Schmidt et al. (Chirurg

50, 96 (1979)).

Segments of ileum and colon were isolated in anaesthetised dogs. Both stomata of these segments were anastomosed to the skin. Proximally to the stomata, free autologous transplants of ileum and colon were implanted according to Schmidt's method. These transplants differed in length and tension.

Two and six months after operation we studied the dogs clinically and by manometry. We filled the bowel-segments through one stoma via infusion of saline solution and were able to study the other stoma. Then we recorded simultaneously pressure in the segments and in the newly formed zone of continence. Our results showed that —

1. No stoma was continent for saline solution.
2. The pressure curve in the "zone of continence" was depressed at the moment when pressure in the segment increased. This phenomenon is contrary to normal sphincter action.

Our conclusion is that this procedure does not produce an active sphincteric mechanism that can guarantee continence for liquid. It is, however, a passive continence mechanism sufficient for faecal continence.

Zürich/Würzburg/Schaffhausen

## Augmentation Cystoplasty with Autologous Skin in the Rat

G. Carmignani, E. Belgrano, P. Puppo, F. Gaboardi and P. Ceppa

We describe a new experimental model, the augmentation cystoplasty in the rat, which allowed us to study the behaviour and the characteristics of the cutaneous graft, free or pedicled. 50 Wistar rats were divided into groups as follows: group A: partial cystectomy and simple closure — group B: partial cystectomy and augmentation cystoplasty with free cutaneous graft sutured with the epidermal face outside — group C: the free cutaneous patch is sutured with the epidermal face inside — group D: augmentation cystoplasty with pedicled epigastric skin flap.

The free cutaneous grafts were reabsorbed almost completely within 30 days. On the contrary the pedicled grafts underwent only a moderate degree of fibrosis. In group B the urothelium covered all the inner surface of the graft within 10 days from operation. In group C the squamous epithelium covered the internal face of the patch until its complete reabsorption. In group D the squamous epithelium remained unmodified. The skin is a suitable material for cystoplasty. The pedicled graft offers no advantage when compared to the free graft. It is better to suture the skin with the epidermal face outside.

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## II. Surgical Technique, Endoscopic and Laser

## Experimental Studies on the Use of a Tissue Adhesive (Chirurcoll/Polfa) in Operations on the Kidney Parenchyma

A. Musierowicz and M. Chilimoniuk

Healing of renal parenchyma wounds closed with the tissue adhesive and catgut sutures was studied in 40 white Wistar rats. The operated animals were divided into three groups. In two groups, 15 animals each, longitudinal nephrotomy was performed; in one of them — with additional opening of the renal pelvis. In both the groups the wounds were closed with Chirurcoll. The same operations were performed in 10 animals of the third group but the wounds were closed with sutures. 5 non-operated animals made the control group for histochemical studies. Material for microscopic examination was collected after 24 hours, 3 and 7 days, after 10 weeks and 12 months. The sections were fixed in the fluid CORNAU, dyed with haematoxylin, coasine and azan; P.A.S. reaction was performed. The frozen sections were examined for amber dehydrogenase as well as for acid and alkaline phosphatase. The renal parenchyma wounds, closed with the adhesive, and catgut, were healing through the formation of the connective tissue. The use of the adhesive did not check necrosis of the renal parenchyma following injury of the blood vessels. Results of morphological and histochemical examinations have demonstrated that Chirurcoll, applied in the closure of the kidney parenchyma wounds, causes less damage to the active kidney parenchyma, as compared with the catgut sutures, and is non-toxic for the remaining kidney parenchyma.

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## Urethral Temperature and Low Frequency Currents During TUR-P

F. Hamann, H. Melchior and R. Niederhagen

The leak of high frequency alternating current (HF AC) in TUR-P is supposed to cause strictures of the male urethra (1, 2). Pathophysiologically, electric current can damage human tissue via Joule-heat (6), neuromuscular irritation (3), and electrolysis (4). Since there is no muscle of any importance in the urethra distal to the external sphincter, this leaves heat and electrolysis.

In a clinical study, we recorded the submucous heat development by means of microthermocouples, and in addition the AC-amount of frequencies below 30 cycles/s. During 153 minutes of permanent transurethral resection of the prostate, we found no submucous temperature above 36°C, neither under normal working conditions, nor under construction of a short circuit by adenoma tissue wedged between resection loop and shaft. The voltage of the low frequency currents lay in the area of a few millivolts, the amperage in the area of µA.

Thus we consider the leak currents during TUR-P not to be responsible for the development of post-TUR urethral strictures, as long as there is ≤150 W of electric power applied (6), and a solution of ≥50 kΩ·cm specific resistance is used for irrigation (5).

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## Electromagnetic Interference (EMI) of Demand Pacemakers (DPM) During TUR-Resections

R. Zink, G. Staehler, J. Beyer, A. Laubenthal and E. Roos

Patients who depend on DPM may be seriously jeopardized by EMI during TUR-resections.

In 15 DPM patients multichannel recordings of ECG, resectoscope on-off impulses and arterial pressure or capillary pulse were performed. In each of 3 dogs with chronically implanted leads up to 20 DPM-lead-combinations under the same conditions were tested. The HF-impulses were also recorded with a groundfree oscillograph. An in vitro model was used to quantify the EMI sensitivity of 19 DPM models by measuring the minimal distance of a TUR-sling from a lead tip without EMI.

Resectoscope on-off impulses overlap widely in their frequency those of DPM, and interactions are highly probable.

The DPM function may be blocked completely, or partially, or not at all or even be triggered above 130 beats per minute, according to the DPM model.

EMI is a DPM model specific reaction, which seems to depend mainly on the characteristics of the respective input filters.

EMI could be avoided in dogs by "grounding" the heart by an additional intracardial electrode.

It is recommended that during TUR-resections on patients with DPM, the DPM should be switched to a fixed rate by the use of a magnet. This is a safe measure and avoids the hazards of EMI.

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## Pathological and Anatomical Results of Coil Embolisation of the Internal Iliac Vein of the Dog

D. Molitor, N. Jaeger, B. Sewing, E. Doppelfeld, M. Schmidt and B. Raque

The Gianturco coil was placed in the internal iliac vein of 17 dogs in order to demonstrate the practicability of occlusion of the venous system.

Catheterisation was via the external jugular vein. The control of the coil position and occlusion of the vein was done by X-ray. Pulmonary complications were excluded by szintigraphy. Over the period of 4 months postoperatively we made a histological investigation every 4 weeks of the embolized internal iliac vein. We found no perforation, no reaction of the perivascular sheet or dislocation of the coil.

There was an incomplete occlusion of the vein in 3 dogs. The reason may be sought in the difference between the size of coil's helix and the vein diameter

With further development of this technique, coil embolisation of the human testicular vein may be the beginning of a new approach to the treatment of varicoceles.

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## Temperature Measurements in Animal Tissues Subjected to Thermal Loads

W. Weinberg, A. Kronester, E. Keiditsch, G. Staehler and R.C. McCord

The use of lasers in medicine is based on their thermal effects, the decisive factors for which are the temperature increases within the irradiated tissue and the duration of the exposure. We have examined methods that would allow us to manipulate these factors with measurable accuracy.

In the first set of experiments, laser-based temperature changes (measured with specially designed contact thermosensors) were recorded at distinct intervals and the resulting curves were analyzed. In another set of experiments, constant temperatures were allowed to act on tissues for set time periods. The thermal load was achieved by a heat-generating cell. The heat-exposed tissue was analyzed histologically after 3 to 5 days.

Temperature-time curves recorded during laser irradiation continue to show irregularities as effected temperatures do not remain constant. For that reason, it is not yet possible to correlate discrete temperature values over set time periods with definite histological damage. However, similar histological findings allow a positive correlation between the variable temperature-time profiles obtained during laser irradiation to the constant temperature-time curves obtained from our second set of experiments. The factor of self-absorption is measured and eliminated from the calculated data through mathematical means. This factor allows conclusions as to the penetration depth of the laser beam as well as to any possible changes occurring in the conductivity of the tissue.

Temperature measurements are a valid means towards an improved understanding of thermally-based mechanisms leading to tissue damage. In order to arrive at safety and effectiveness in laser therapy through temperature measurements, numerous distorting factors must be eliminated.

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## Limits of Thermal Loading on the Intestine During Irradiation of the Urinary Bladder with the Nd:YAG Laser

A. Kronester, G. Staehler, W. Weinberg, E. Keiditsch and A. Hofstetter

In the clinical application of lasers for thermal destruction of urinary bladder tumours, the adjacent intestine must be excluded from harmful effects which could result in perforation. We have therefore examined the major effects of thermal loading in two comparative sets of experiments on rabbit intestine: 1) the intestine was exposed directly to a heat-generating cell; 2) the intestine was subjected to indirect heat penetrating across the inner bladder wall during laser irradiation. In both cases the temperature loads were adjusted to varying degrees. Using a microthermo-element and a measurement analyzing system, each set of temperatures could be measured with an accuracy of $\pm$ 0.5°C and immediately recorded (temperature range of 35°C-80°C; exposure times of 2 s-3 min).

The exposed intestinal sections were removed after a survival time of 3-5 days and analyzed histologically.

In the development of thermally-caused damage to the intestine, temperature is of considerably greater impact than exposure time. The critical limits of thermal loading are over 64°C for durations of up to 6 s, and over 61°C for durations of up to 12 s of exposure.

Penetration into the intestine must be prevented through proper selection of the beam intensity and the duration of exposure. In a first estimate according to Lambert-Beer's law, the following limits may be considered safe in endoscopic laser irradiation in air: A power of ≤40 W with a pulse duration of ≤2 s, a focal radius of ≥2 mm and a bladder wall thickness of ≥3 mm.

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## Experiments to Optimise the Dose for Endovesical Irradiation with the Neodymium-YAG Laser — Investigations in Bladders of Rats, Rabbits and Corpses

K. Rothenberger, J. Pensel, F. Frank, E. Keiditsch and A. Hofstetter

The endovesical coagulation of urinary bladder tumours by means of the Neodymium-YAG laser is now an alternative to electro coagulation. It may be combined with TUR. With this therapy, deep homogenous necrosis of tissue may be obtained by careful selection.

This volume of the tissue correlates with the laser properties to an extent that it permits sufficient irradiation of neoplasms provided the extension of the tumour is calculable. The tissue can be measured intravesically by the Endo-Ultrasonic Transducer, which was developed by us. In an animal experiment we determined the distribution of temperature in space and time at the external wall of the bladder during intravesical irradiation. Comparing measurements on human bladders of corpses and in vivo bladders confirmed the transferability of the results obtained by animal experiments to human medicine. The temperature measurements agree with the histological appearance of the laser necrosis. With a laser power of 45 Watts we obtained 5-6 mm deep homogenous necrosis. In endangered areas, where the bowel is directly adjacent to the bladder wall, transmural necrosis can be obtained without damage to neighbouring tissue by both, laparascopic control and removal of the bowel from the bladder by way of abdominal gas-filling. In spite of deep tissue lesion there is no perforation risk of the wall. These results have shown that, the intravesical measurement of tissue by ultrasound and the transurethral irradiation by the Neodymium-YAG laser can be used for optimal and sufficient therapy of bladder tumours. The operation can be carried out without tissue contact and without anaesthesia.

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## Laser Investigations of the Normal and Strictured Dog Urethra

H. Bülow, U. Bülow and H.K. Wullstein

The effects of the Neodymium-YAG laser beam on normal and on granulation tissue of the dog urethra were investigated in vitro. For performing a transurethral urethrotomy using the Neodymium-YAG laser of 40 W a laser focal spot diameter of 1.5 mm seemed to be advantageous because a smaller one would barely be seen through the endoscope; with a larger focus, the power density would not be sufficient to remove tissue. The lesions in granulation tissue were less marked than in normal tissue. In order to remove granulation tissue using a focal spot diameter of 1.5 mm, it was necessary to apply an impulse of approximately 3 sec, whereas for normal tissue an exposure time of 2 sec was sufficient. In a focal spot of 1.5 mm a perforation of the urethral wall, which is approximately 4 to 6 mm thick, was to be expected after application of more than four individual impulses, each of 2 sec duration. After determination of the most favorable diameter of the focus and the pulse duration of the laser beam, endoscopic laser examinations were performed on normal and experimentally strictured urethrae in dogs. A suitable laser endoscope was designed and a surgical technique for laser-beam treatment of the strictured urethra was developed. The postoperative follow-up period was up to 6 weeks. There was no need for postoperative urinary diversion. Neither bleeding nor perforations were observed.

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## Experimental Investigations of the Effect of Nd—YAG Laser Irradiation on Prostatic Tissue

R. Böwering, W. Weinberg, E. Keiditsch, A. Kronester and A. Hofstetter

Endoscopic local irradiation after TUR by Nd—YAG laser may be an effective new method of treatment of prostatic carcinoma.

Human cadaver prostate tumours and canine prostate glands in vivo were irradiated by Nd—YAG laser with different power levels and pulse durations.

Temperature measurements were carried out by an arrangement of micro thermo couple, digital volt meter and desk top computer in different layers of the prostate and the rectum.

Histological investigations showed circumscript thermal lesions, e.g. density changes by coagulation and alterations of the blood vessels, despite post mortem changes. The temporal and spatial temperature measurements showed a good correlation with the histological findings. There was a large penetration depth resulting in necrosis, but no perforation and no changes in the rectum were to be found also after high power laser irradiation.

Neodymium-YAG laser irradiation proved:

1. An efficient photocoagulation of prostatic tumour. Penetration depth as total necrosis of tissue from 2-4 mm was observed.
2. In situ thermal necrosis without remarkable tissue ablation.

These results are encouraging and suggest that endoscopic Nd: YAG laser irradiation of prostatic carcinoma in man might be effective.

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## III. Urological Nephrology, Urinary Tract Infection, Prostatitis

### The Effect on Renal Function and Morphology of the Commonly Used Methods of Intrarenal Access

J.M. Fitzpatrick, M.W. Sleight, M. Marberger and J.E.A. Wickham

The aim of this study was to investigate the effect on renal function of the four commonly used methods of intrarenal access. Experiments were performed on 16 dogs, with 4 dogs in each group. Each dog had an operation performed on the left kidney, with the right kidney serving as a control. After 48 hours, clearance studies (creatinine, inulin and PAH) were performed on both sides. The four methods of renal access were: extended sinus approach, radial nephrotomies, anatrophic nephrotomy and bivalve nephrotomy. The pelvis was opened in all cases; the renal artery was clamped for 30 minutes in the latter three approaches.

There was no significant difference in creatinine, inulin or PAH clearances between the extended sinus approach and its controls; there was no significant difference in creatinine or inulin clearance between the radial approach and its controls, although there was a difference in PAH clearance. There was a significant difference in the 3 clearances between the anatrophic and bivalve nephrotomies and their controls. There was no significant difference in creatinine and inulin clearance between the anatrophic and bivalve nephrotomies.

Morphological studies were performed by injecting resin into the renal arteries and veins of all the kidneys and then corroding the kidney. The extended sinus and radial approaches were associated with minimal parenchymal loss, whereas parenchyma was lost with the anatrophic and bivalve procedures. It is suggested on the basis of these studies that the optimal method for removing staghorn calculi is the extended sinus approach with multiple radial nephrotomies for the intracaliceal fragments not removed by the former approach.

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### The Influence of Angiotensin II Antagonist on Post Ischaemic Acute Renal Failure

H. Huland, H.J. Augustin and T. Engels

In 34 dogs the effect of 90 min unilateral renal warm ischaemia on renal perfusion and function was studied with (group B) and without (group A) treatment of ($Sar^1$, $Ile^8$)-Angiotensin II. 15 min before ischaemia of one kidney 10 µg $kg^{-1}$ x $min^{-1}$ ($Sar^1$, $Ile^8$)-Angiotensin II was given 15 dogs i.v. (group B) and also for 15 min before and for 30 min after the renal ischaemia was stopped. Renal and intrarenal perfusion using the Xenon-133-washout method, split renal inulin clearance, diuresis, sodium-rat-transport, urinary osmolarity and split renal venous renin study and angiographic studies were done up to three hours after a 90 min period of unilateral warm ischaemia and compared with the data of 15 dogs having the same period of ischaemia without ($Sar^1$, $Ile^8$)-Angiotensin II treatment (group A) and a further group of 4 dogs being sham operated.

In the first three hours after release of unilateral renal artery occlusion a significant influence of the Angiotensin II antagonist on changes in post ischaemic vascular resistance, intrarenal hemodynamics and renal function could be demonstrated. All of these measurements were well preserved in the treated (group B) dogs.

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### The Role of Inosine in the Prevention of Warm Ischaemic Damage in Renal Transplantation

J.M. Fitzpatrick, M. Marberger and J.E.A. Wickham

Although Collins' solution is widely used in renal transplantation, the problem of warm ischaemia before cold preservation has not been solved, with universally poor results when it is 15 minutes or longer. Inosine is known to protect against in situ warm ischaemia in dogs and humans, so this study was aimed at investigating a possible protective role by inosine against warm ischaemic damage when followed by cold preservation with Collins' solution and then autotransplantation.

Experiments were performed on 10 dogs. Five dogs (Group 1) were submitted to 15 minutes warm ischaemia followed by 24 hours cold preservation in Collins' solution; in 2 of these, 30 mg/Kg inosine was injected 10 minutes prior to renal artery ligation, and in 1, 60 mg/Kg inosine. A further 5 dogs (Group 2) had 15 minutes warm ischaemia follwed by 12 hours cold preservation; in 3 of these, 30 mg/Kg inosine was injected. Following preservation, contralateral nephrectomy and autotransplantation of the preserved kidney were performed.

In Group 1 there was no difference in serum creatinine between controls (0.12 mmol/l preoperatively and 1.04 mmol/l on 3rd day) and either dose of inosine (0.12 to 1.09 mmol/l with 30 mg/Kg, and 0.11 to 0.85 mmol/l with 60 mg/Kg). In Group 2 there was no difference between controls (0.12 to 0.91 mmol/l) and inosine (0.13 to 0.87 mmol/l), but 2 of the 3 dogs who were given inosine survived, with gradually decreasing creatinine. However, it seems that inosine fails to protect against warm ischaemia when followed by cold preservation with Collins' solution.

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### Lessening of Post Ischaemic Damage of the Microcirculation and Protection of Nephron Structures by Blockade of the Renin-Angiotensin System (RAS)

A. Schilling, R. Bassermann, E. Pratschke, G.E. Rindfleisch and U. Zirkelbach

Post ischaemic impairment of the microcirculation is partly due to an increase in renin activity. Blockade of the RAS was obtained by 1) inhibition of angiotensin II by pre-ischaemic and post ischaemic intrarenal injection of 5 mg Saralasin and 2) suppression of renin production by chronic loading with 12 mval sodium/kg/day.

Dog kidneys were made ischaemic for 120 minutes by transfemoral balloon occlusion of the renal artery.

Measurements taken before as well as at 10 and 60 min. after a 120 minutes ischaemic period were PAH- and inulin-clearance. The kidneys were examined histologically.

Results:

| | | untreated controls n = 8 | Sodium loading n = 8 | Saralasin injection n = 8 |
|---|---|---|---|---|
| Inulin post ischaemia | 10 min | 1,0 | 3,5 | 2,8 |
| | 60 min | 1,3 | 8,3 | 10,5 |
| PAH post ischaemia | 10 min | 0,6 | 9,1 | 6,9 |
| | 60 min | 1,7 | 12,4 | 21,8 |
| Glomerular collapse | | +++ | (+) | (+) |
| Necrosis of tubules | | +++ | + | ++ |
| Tubule ostruction | | +++ | + | ++ |

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## Evaluation of Kidney Function in Unilateral Hydronephrosis by 99m-TC-DMSA-Scanning (DMSA)

D. Jocham, E. Moser, Ch. Chaussy, U. Büll and M. Beer

It has been stated that in unilateral hydronephrosis there is an over-estimation of the function of the hydronephrotic kidney by the determination of the 131-I-Hippuran clearance. We investigated Hippuran clearance in comparison to DMSA. The results of both methods of 19 healthy individuals were compared to those of 16 patients, who had unilateral hydronephrosis diagnosed by IVP. In 10 Beagle dogs the determination of the kidney function by DMSA was carried out first in the healthy kidney, secondly after complete unilateral ligation of the ureter and thirdly after draining the pelvis of the hydronephrotic kidney. The amount of radioactivity drained from the hydronephrotic pelvis was determined. In healthy patients both methods showed equal results. In a unilateral hydronephrotic kidney the results of the Hippuran clearance were on average 4,5% higher than the results of the DMSA. This over-estimation was calculated to be significant. In the dogs the function of the hydronephrotic kidney decreased from 54,2 ± 2% to 18,5 ± 5% 7 days after occlusion of the ureter and to 16,2 ± 7% after 10 days. After draining the hydronephrotic kidney the function was calculated 1% lower by DMSA than before. This was shown to be significant. It was shown, that DMSA is the most precise method to determine the function of a hydronephrotic kidney. Hippuran clearance may still be preferred since a small overestimate of renal function has little clinical importance.

Urologische Klinik und Radiologische Klinik der Universität München, Klinikum Großhadern, Marchioninistrasse 15, D-8000 München 70

## Experimental Investigations of the Intravascular and Extravascular Antibiotic Concentrations of the Renal Parenchyma

J. Djulepa, J. Potempa and W. Wundt

We have determined and compared intravascular and extravascular antibiotic concentrations in the renal parenchyma.

The main tests were carried out on healthy kidneys and experimentally induced inflamed kidneys in 29 dogs and 30 Wistar rats using gentamicin and cephalosporins and subsequently with trimethoprim + sulphadiazine in various diseases of human kidneys. First we produced an experimental infection of the animals kidneys with E. coli, enterococci, Proteus, Pseudomonas aeruginosa and Klebsiella. These bacteria were isolated from patients with a chronic infection of the urogenital system and inoculated into the kidneys. We continued these investigations on 21 human kidneys (renal tumours and pyelonephritically contracted kidneys). In the tumour kidneys, the antibiotic concentrations were determined intravasally and extravasally in the healthy portion, and in the entire cortex in the pyelonephritically contracted kidneys. Renal papillae with a large proportion of urine were removed. The perfusion provided almost blood-free kidneys. A photometric method enabled the total blood in the homogenized renal parenchyma to be determined quantitatively. At the same time the possible proportion of blood as well as the antibiotic concentration determined microbiologically after perfusion could be calculated in the entire renal parenchyma and separately in the intravascular and extravascular renal tissue in healthy and infected kidneys in µg. All results of our experiments with gentamicin, cephalosporins and trimethoprim + sulphadiazine have been summarized graphically.

Result: From our animal experiments and the investigations performed on human kidneys, it is to be concluded that the concentrations of the test antibiotic in the renal parenchyma are very much higher intravascularly than extravascularly. This is manifested especially in various inflammatory processes in the kidneys. When these are advanced, such as infected hydronephrosis and pyelonephritic contracted kidney, the extravascular portion is lowest (ca. 20%). This is significant for the therapy of chronic inflammatory processes in the kidneys.

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## The Effect of Histamine on the Canine Kidney

R. Tauber, H.J. Reimann, A. Gebauer, W. Permanetter and Ch. Chaussy

Histamine is an important factor in many pathophysiological processes. We have found a very high level of tissue-histamine in the area surrounding a human renal carcinoma but tissue histamine level of histologically normal parenchyma are not increased. We were interested in the significance of these high histamine levels in the surrounding of kidney tumours.

Tissue and plasma histamine were determined as described by Lorenz et al. (1970). Mast cells were stained by the free-dried section-technique according to Reiman et al. (1977). In animal experiments intraarterial injections of 5, 10, 25 µg histamine via the renal artery showed:

a) Occurrence of macro-hematuria more so with higher histamine level.

b) The plasma-histamine level measured in the renal vein increased in proportion to the histamine injections.

c) The histological investigation of the kidney revealed features similar to a shock reaction. The circulation of that time was nearly stable.

d) The heart rate showed a maximal increase of 15 p.m.

It is commonly accepted, that haematuria in renal carcinoma is caused by tumour infiltration of blood vessels. On the other hand this haematuria may also be an effect of histamine.

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## Morphometric Investigations of Glomerulus, Proximal and Distal Tubule in the Solitary Kidney of the Rabbit

E. Schindler, B. Aeikens, P. Kolle and H.-G. Drimalla

60 mongrel rabbits were unilaterally nephrectomized; morphometric evaluation of anatomical structures in the remaining kidney by means of the Leitz semi-automatic image analysis system was done after 1, 2, 3, 4, 5, 7, 14, 28, 56 and 84 days.

Morphological changes in the residual kidney of the rabbit start immediately after contralateral nephrectomy: the main alterations take place in the first days; no significant changes occur after 8 weeks. The epithelial area of the proximal tubule increases by 55 per cent from 650 $\mu^2$, to 1000 $\mu^2$, its circumference from 129 $\mu$ to 160 $\mu$ (or by 25 per cent). Similar growth rates are encountered with the lumen of the proximal tubule: the epithelial area grows by 68 per cent, its circumference by 29 per cent. Changes in the distal tubule are less impressive and occur rather late. The relative portion of the interstitium within the residual kidney (appr. 10 per cent) does not change at any time.

Diameters of capillary convolutions and volumes of cortical glomeruli increase in the beginning, only to shrink again after several weeks. A similar "reactive hyperaemia" was found in previous studies of the ERPF of the residual kidney of the dog. These facts together with a hypertrophied macula densa underline the importance of vascular changes in renal compensatory hypertrophy.

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## Enzymatic Concentrations of Various Morphological Structures Quantitatively Analysed in Normal and Pathological Human and Rat Kidneys

G. Heinert, J.E. Scherberich, W. Mondorf and W. Weber

Ischaemia and hydronephrosis cause a decrease of kidney function. Enzymehistochemically stained tissue sections were analysed for structural morphological alterations and decreased enzyme concentrations. Indicator enzymes of the proximal tubules from kidney cortex such as alkaline phosphatase (AP, EC 3.1.3.1), alanine-aminopeptidase (AAP, EC 3.4.11-) and β-glucuronidase (β-Gl, EC 3.2.1.31) were measured in human (n=202) and rat (n=88) kidneys. As a substrate naphthyl-1-phosphate sodiumsalt, DL-alanine-β-naphthylamide-HCl, sodium-6-brome-2-naphthyl-β-D-glucuronid were used. The quantitative evaluation of enzymatically stained kidney tissues was performed using an automatic, electronic image analysis device "Micro-Videomat" 2, Zeiss. Histograms showed regular high enzymatic patterns in normal kidneys of rats (AP 321,7 ± 94,9 U, n=64), (APP 708,8 ± 122,3 U, n=24) and human kidneys (AP 222,0 ± 59,0 U, n=10; AAP 790,9 ± 109,1 U, n=17; β-Gl 609,0 ± 74,6 U, n=11). There were significant ($p<0,05$, Wilcoxon-test) decreases of enzyme concentrations densitometrically analysed in human kidney cortex after alterations following hydronephrosis, pyelonephritis, ischaemia etc. (AP 35,9 ± 21,2 U, n=39; AAP 410,3 ± 153,6 U, n=21 and β-Gl 339,1 ± 92,2 U, n=14). The enzymehistochemical investigations of renal cortex sections may be helpful in prognosis, diagnosis and therapeutic aspects of clinical and experimental evaluation of kidneys.

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## Quantitative Enzymatic Image Analysis in Normal and Kidneys Altered by Hydronephrosis and Ischaemia Based on Animal Experiments

W. Rauh, G. Heinert, J. Scherberich, W. Mondorf and W. Weber

Patients suffering from acute tubular cell alterations caused by renal disease displayed increased amounts of enzyme concentrations in the urine. Marker enzymes of the proximal tubules from the kidney cortex such as alkaline phosphatase (AP, EC 3.1.3.1 n=188) and alanine-amino-peptidase (AAP, EC 3.4.11-) were measured. As a substrate naphthyl-1-phosphate sodium salt and DL-alanine-β-naphthylamide-HCl were used. Sections of kidneys from 166 Wistar rats were analysed densitometrically by an automatic device "Micro-Videomat" 2, Zeiss. The following groups of kidneys were measured: 1) unilateral ureteric obstruction for 10 and 2) for 21 days, 3) ligature of the renal vein for 2 days, 4) ischaemia for 2 hours, 5) 24 hours and 6) 2 hours with 14 days post ischaemic recirculation, and 7) control group. Significant ($2p<0,01$, Wilcox-on-test) decreases of enzymatic units (U) were found in kidneys of group 1) and 2); 10 days of ureteric obstruction: AP 39,7 ± 34,3 U, n=13; AAP 443,2 ± 282,3 U, n=13. In addition, kidneys after 2 days ligature of the renal vein exhibited significantly ($2p<0,05$) decreased enzyme concentrations (AP 137,2 ± 93,9 U, n=10; AAP 584,3 ± 148,9 U, n=8). Ischaemic kidneys displayed a significant decrease of enzyme concentrations in the cortex of group 6) ($2p<0,01$), (AP 68,2 ± 56,1 U, n=11; AAP 532,5 ± 193,3 U, n=11), 5) (AP 68,6 ± 71,2 U, n=10) and in group 4) (AP 271,5 ± 63,2 U, n=12).

The quantitative analysis of enzymatically measured kidney tissues may be advantageous as criteria of morphologic alterations.

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## Cimetidine as a Possible Stimulator of the Immune Response in Patients with Prostatitis

H.-W. Bauer and J. Schüller

The first investigations dealing with the immunological situation in prostatitis were carried out by Gray et al. (1974).

In this study the immunoglobulins were determined in the expressed prostatic fluid by means of the radial immundiffusion and diagnostic as well as prognostic conclusions were drawn. Further development of this investigation was achieved by replacing the radial immundiffusion by the nephelometric measurement of the immunglobulins and the "acute phase" proteins.

Since cimetidine treatment was associated with an augmentation of delayed hypersensitivity we tried in a pilot study to give cimetidine as an adjuvant in

male adnexitis. Ten patients received only antibiotic therapy after taking a culture of the EPF, ten patients received additionally 1 g cimetidine per day. Before and after four-week treatment immunoglobulins (IgG, IgA, IgM) were studied in the prostatic expressates by the above mentioned nephelometry. Before and after two-week therapy the "acute phase" proteins haptoglobin, $\alpha_2$-macroglobin and $\alpha_1$-glycoprotein were determined in prostatic expressates.

We found that the "acute phase" proteins normalised earlier in the group treated with cimetidine and that four weeks later the immunglobulins IgG and IgM were lower when cimetidine was given as an adjuvans. In contrast IgA as a possible local protective factor was higher than in the control group.

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## Prostatitis in Female Mastomys Natalensis by Klebsiella Pneumoniae — A Model for Experimental Prostatitis

W. Weidner, H. Brunner, J. Lerner and C.F. Rothauge

An experimental animal model is needed, to study several poorly understood aspects of prostatitis. As in man, the infection should be confined to the prostate without urinary tract infection. Female Mastomys natalensis seem to be suitable for these experiments.

About 150 animals were inoculated into the bladder with a suspension of K. pneumoniae, containing $10^8$ colony forming units (CFU). Control animals received sterile medium. Immediately after inoculation, one day later and at three days intervals until 30 day post-inoculation, urines, prostatic glands and kidneys of groups of animals were examined quantitatively for the presence of bacteria. Prostatic tissue and kidneys were also studied for histopathological alterations.

Nine to 12 days post-inoculation, K. pneumoniae ($10^4$ to $10^5$ CFU/g) could be isolated from the prostate of all infected animals, whereas urinary tract infection could no longer be detected.

Histologically, various patterns of prostatitis could be demonstrated. In control animals, morphological alterations were not seen. 25% of the infected animals developed chronic prostatitis, which was still seen 30 days after inoculation.

The results are encouraging for further studies on prostatitis caused by various microorganisms in this animal model.

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## Dynamics of Initial Antibacterial Effects in the Treatment of Bacteriuria

K. Naber and T. Ahrens

New antibiotics are often expected to show enhanced bactericidal activity. However, favourable experimental results in vitro and in animal infections need validation as to their clinical relevance, by controlled clinical studies.

Forty patients of both sexes with urinary tract infection (UTI) and showing bacteriuria with colony counts of at least $10^6$/ml in one of two consecutive specimens were treated orally with CGP 9000 or cephalexin at a dosage of 1 g t.i.d. for 5 days, in a double-blind, comparative study. Urine was taken (midstream in males and by catheter in females) twice before, and 1, 3, and 8 hours after the first dose, and then before the first morning dose on Days 2, 3, and 5 of the treatment. The antibiotic present in the samples under treatment was inactivated using a cephalosporinase from Enterobacter cloacae P 99 at a concentration of 5 mg to 5 ml of urine.

A colony count of less than $10^4$/ml, i.e. a reduction of at least 99%, was reached 8 hours after the first dose in 13 out of 18 patients receiving CGP 9000 and in 9 out of 18 receiving cephalexin. Twenty-four hours after the start of the treatment no difference could be demonstrated between the two groups.— CGP 9000 and cephalexin show different rates of bacterial killing in vitro and in animal experiments. Under clinical conditions in UTI, however, this difference could only be demonstrated as a transient trend, without statistical significance.

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# IV. Experimental Lymphography and Various Subjects

## Improvements of the Electrophoretic Mobility (EM) Test to Measure Lymphocyte Sensitization

Th. Zwergel, U. Zwergel and K.-H. Bichler

The electrophoretic mobility (EM) test is supposed to measure an unknown immunological parameter in human cancer patients. The electrophoretic mobility test depends upon the interaction of an antigen with sensitized lymphocytes, resulting in a reduced mobility of special indicator cells in the cytopherometer. However, universal acceptance of the test as a clinical test for cancer has been hampered by technical difficulties. The original method of timing with a stopwatch was replaced by two electric watches combined with the cytopherometer. The next improvement of timing at the cytopherometer consisted in the construction of an electronic controller and an electronic stopwatch connected to a minicomputer, data print out, and tape recorder. Automatic recording of data is time-saving and useful for correct evaluation of the EM-test. That can be now done directly on-line in a calculator with an EM-test evaluation program developed in our laboratory, and permits immediately a statistical evaluation. - Thus individual faults are reduced and a greater number of cells of one sample can be measured in order to increase the reliability of the results. We have also used and examined a television monitor for the observation of the indicator cells in the cytopherometer. Taking into account these technical modifications, besides all other improvements such as the use of sheep erythrocytes instead of guinea pig macrophages as indicator cells, the electrophoretic mobility test is no longer a capricious method, although some experience with the cytopherometer is still necessary to obtain reproducible results. In this case the EM-test is an additional help to check the immunological status of cancer patients.

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## Effect of Perchloric Acid and Heat Extraction and the Influence of Urinary Parameters on Reproducibility, Correlation and Clinical Applicability of CEA Measurement in Urine

P. Oehr, H.D. Adolphs, E. Rudel and C. Skirl

The results of CEA measurement in urine of bladder carcinoma patients differ widely. Some authors found a correlation between CEA level, tumour stage/grade and follow-up, respectively, others were unable to confirm such a relationship. Since CEA tests are originally designed for serum level determinations, their applicability to urine measurements remains to be proved. In order to extract CEA, we alternatively used heat- and perchloric acid (PCA)-extraction. The following test kits were employed: RIA Abbott-Dainabot-Japan, RIA and EIA (enzymatic) Abbott-Chicago. Samples of urine from bladder carcinoma patients were used. A concentration dependent loss of CEA was observed after PCA-extraction; heat-extraction and direct measurement gave similar results without loss of CEA. If heat-extraction followed PCA-extraction the lost CEA did not reappear. On the other hand, no loss of CEA occurred if heat-extraction was performed prior to PCA treatment. We did not find any influence of urinary and biochemical factors on the tests such as different pH-values and salt concentrations.

Conclusion: the PCA-extraction method which is used by most investigators, is not recommendable for urinary CEA determination. Heat-extraction does give high reproducibility. The tests being employed by us can be applied for both serum and urine CEA determination.

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## Indirect Lymphography of Urinary Bladder and Prostate: An Experimental Study with New X-ray Contrast Media

B. Kopper, B.I. Wenzel-Hora, H.M. Siefert and G. Konrad

Using conventional pedal lymphography regional lymph nodes of urogenital organs cannot be visualized radiologically. The results of indirect lymphography with oily X-ray contrast media were generally unsatisfactory. It was our aim to improve the radiological demonstration of the regional lymphographic system of the urinary bladder and prostate by using new contrast media.

Two contrast media, developed by Schering-Berlin, were injected into the submucosal layer of the bladder wall and into the prostate gland of beagle dogs and monkeys. One of the contrast media consists of a low-molecular emulsion of an iodinated oil; the second medium is water-soluble with low osmotic pressure and rather high molecular weight.

In general both media give a good contrast and allow a differentiated assessment on the morphology of the regional lymphatic nodes. The water-soluble medium is eliminated via the kidneys and 24 hours after application no contrast medium was detected by X-rays. Local and general tolerability of the water-soluble medium are excellent. However, not in all cases could we obtain a complete radiological demonstration of the total pelvic lymphatic system. The reasons are discussed. The experiments have shown that by interstitial injection of a new ater-soluble contrast medium the radiological demonstration of the lymphatic system is possible. The results so far obtained in animals are encouraging in view of the possible applications in clinical studies.

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## Experimental Chromolymphography

R. Harzmann, G. Haefelinger, H.V. Gärtner and U. Zwergel

With the intention of improving the completeness of lymphadenectomies, various chromolymphographic procedures using chlorophyll, xanthophyll and carbohydrates are described. Disadvantages proved to be a lessening of diagnostic certainty with the lymphogram. For this reason, an attempt to facilitate selective lymphadenectomy using high-contrast lymph node vital dying was carried out. For this procedure related dyes must meet the following requirements: they must be fat soluble, provide intense coloration of the lymph nodes, allow normal assessment of the lymphogram, be sterilisable, cause no skin discoloration and be harmless.

No data as to mutagenicity being at hand, each dye compound was studied for mutagenic effects. Of the fat soluble dyes sudan black, bright black, bright cresyl blue, oil blue, oracete blue, nile blue sulfate and Guajazulen, only sudan black and Guajazulen proved to be non-mutagenic. Simultaneous intralymphatic injection of either sudan black or Guajazulen was carried out on 10 mongrel dogs. Ten days later a paraaortal lymphadenectomy with histological evaluation was done. To ascertain the time required for resorption of the dye, the popliteal and paraaortal lymph nodes were removed at intervals of three days in another then dogs. Seven days subsequent to lymphography skin biopsies were performed to check for any skin discoloration from the treatment. Lymph node contrast was achieved quite successfully with sudan black and Guajazulen. Histology showed the same findings as after conventional lymphography. Sudan black did lead to lipiodol hindrance and thus to difficulties in interpreting the lymphogram. Guajazulen proved to be a potent contrast medium with no side effects.

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## Endourology: Development of Ultrasound-Guided Manipulation of the Urinary Tract

H.-U. Eickenberg, H. Dettmar and R. Heckemann

Percutaneous nephropyelostomy for urinary diversion is mainly done under X-ray control.

A technique is described for ultrasound-guided access to the renal collecting system. After dilation of the fistula tract manipulation within the pelvicaliceal system guided by ultrasound is possible. Experimental hydronephrosis was induced in 6 rabbits. The Günther Set (Cook) was placed into the kidney by guidance of a Multison 400 (Siemens). All sets could be localized by ultrasound within the collecting system. The various parts of the set were identified without X-ray. The movement of the intrarenal pigtail catheter was controlled by ultrasound. This technique — demonstrated for the fist time — will also be shown in a film. This concept of percutaenous ultrasound guided manipulation of the urinary tract should be called endourology.

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## Three-Dimensional Intraoperative Stone Localization

H. Melchior, G. Lang and F. Hamann

Progress in surgical treatment of nephrolithiasis depends on complete removal of the calculi as well as minimum traumatization of the renal parenchyma. Therefore, the exact localization of the calculi within the three-dimensional space of the pyelo-calical system is a basic requirement.

Our method of three-dimensional stone localization is based on the technique of stereoscopic X-ray control. By double X-ray exposure of the film from different directions, the stones and a marker on the surface of the kidney are shown twice on the film. The situation of the stones in relation to the marker shows the horizontal location of the calculi, their vertical position is calculated from the distances between the double images of both, the stones and the marker, based on the geometrical laws of triangles. In triangles with identical angles, the ratio of the heights (h) is equal to that of the baselines (c): $h_1/h_2 = c_1/c_2$. The distance between the marker on the kidney surface and the film ($h_1$) is identical with the thickness of the kidney, the distances between the double images of the marker ($c_1$) and those of the stones ($c_2$) are measured. Therefore, the vertical distance from stone to film is: $h_2 = h_1 \cdot c_2/c_1$.

There is an even less difficult, but only slightly orientating way: double X-ray exposure of the film after piercing the kidney with needles. If the distance between the double images of a stone is smaller than that between the needles, the stone is situated between the level of the needles and the film. If the distance between the images of a stone is larger than that between the needles, the stone is in the space in front of the needles.

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## Experience with a Technique for Microangiography of the Pig Kidney

A. Futterlieb, Ch. Rosenthal and H. Probst

We report our experience using microangiography to examine the fresh pig kidney. After nephrectomy the organ was perfused with Bretschneider solution until the perfusate in the venous effluent was clear. Filling with contrast medium was done at a constant pressure of 100-120 mm Hg, using a hydrostatic system and a Boyle-Mariotte bottle. The medium was stirred continuously to maintain its viscosity. The kidney was kept in a receptacle floating in saline solution at a constant temperature of 37-40°C. Optimal results were obtained from a commercial solution of barium sulphate in heparinized saline and containing 5% gelatine. The critical details of the technique were a constant pressure and correct viscosity of the medium which depended mainly on the temperature. Of lesser importance were the barium concentration and the period of filling provided it was longer than 30 minutes. Good results could then be obtained by this cheap simple method.

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# V. Renal and Testicular Carcinoma

## Transplantation of a Human Renal Cell Carcinoma (RCC) on NMRI nu/nu Mice

M.W. Köllermann, U. Otto, W. Klöppel, J. Dimigin, W. Linden, G. Klötzel and H. Rüdiger

Tissue from an RCC and lymph node metastasis of a 39 year old female patient were transplanted subcutaneously to NMRI nu/nu mice. The primary tumour showed two histological patterns, firstly a granular cell solid carcinoma and secondly sarcomatoid elements with polymorph and fusiform cells intermingled with abundant collagen fibers. The lymph node metastasis of the primary tumour showed only the sarcomatoid tissue of the former. Tumour in 100% of 80 animals transplanted in the first four passages. Chromosome analysis demonstrated the human origin of the transplants. The transplants of the lymph node metastasis grew more rapidly than the transplants of the primary. All transplants grew more rapidly in male than in female animals. Hormone receptors (androgen, oestrogen, and progesterone) were not found in any of the transplanted tumours. Histology of the transplant tumours showed only the sarcomatoid tissue of the original tumour. The solid carcinoma-type tissue was not found and probably not transplanted. Electron-microscopy showed no difference between the sarcomatoid part of the primary and the transplant tumours. Flowcytometric analysis of the transplanted tumours demonstrated two cell populations (DNA-content 6 and 10,8 pg). Transplantation of human RCC to nu/nu mice may be a valuable experimental model of the disease. Its usefulness must be demonstrated in further investigations.

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## The Biochemical Profile of the Tissue of a Human Renal Cell Xenograft on the Nude Mouse (NC65) Compared to the Profile of Human Renal Cortex (RCX) and Renal Cell Carcinoma (RCC)

G. Popelier, R. Geers, G. Verdonck, F. Schröder and W. Höhn

The level of protein in NC65 of 51 mg/g wet weight is twice as high as in RCC (x = 25 mg/g) and even higher than in RCX (x = 43 mg/g). The difference between RCX and RCC in the profile of 15 enzymes is mainly quantitative by a decrease of the activity of all but one enzyme (PK) in RCC. The profile in NC65 is completely different. Compared to RCX the activity of 5N is 2000%, of PK 900%, of Ald 800%, of LDH 220% and of MDH 150% higher in NC65 tissue. In NC65 half of the LDH isozyme is found in fraction IV and 30% in fraction III whereas in RCC 30% in LDH IV and 20% in LDH V, in RCX LDH I, II and III each represent 30%.

The content of the total fatty acids in NC65 is much higher than in RCX but still lower than in RCC (NC65 = 1.8 mg/g, RCX = 12 mg/g, RCC = 40 mg/g). Compared to RCX there is an increase in triacylglycerols and a decrease in polar lipids in NC65 as is found in RCC, but there is no increase in sterolesters. In NC65 the profile of the individual fatty acids shows, compared to RCX, a higher incidence of C18:1 and a lower one of C20:4 than in RCC. Compared to both RCC and RCX there is only a low incidence of C18:2. As the biochemical profile of NC65 is quite different from both RCX and

RCC, it cannot be compared as a renal cell carcinoma model.

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## The Sterol Content of Human Renal Cortex (RCX) and Renal Cell Carcinoma (RCC)

G. Popelier and P. Geuens

In renal cortex (RCX) 2.5% of the fatty acids occurs in the lipid subclass of the esterified sterols, in the renal granular cell carcinoma's (RGCC) 23% and in the renal clear cell carcinoma's even 38%. The lipid content of the RCCC is 4 to 6 x higher than in renal cortex but in RGCC it is only slightly higher. The tissue of 26 RCC and corresponding RCX have been analysed on their sterol profile analysed by thinlayer chromatography. The total sterol content of RCX is 4 ± 0.1 mg/g wet weight and among them 13% are esterified. Only cholesterol for 67% and 7-Dehydrocholesterol for 30% are present in detectable amounts. In RCCC a mean value of 20 ± 4.0 mg/g and in RGCC 14 ± 2.5 mg/g wet weight is measured. Depending on the type of RCC resp. 75 and 60% are esterified. 7-Dehydrocholesterol for 31% and cholesterol for 49% are likely found but moreover 18% Desmosterol. In half of the RCC part of the cholesterol occurs as dehydrocholesterol and accounts then for 18% of the sterols. No statistical difference is found between RCCC and RGCC concerning the incidence of the different sterols.

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## Analysis of Proliferative Compartments in Human Seminoma

U. Rattenhuber, H.M. Rabes, P. Carl, U. Löhrs, R. Lamerz, K. Mann, G. Rindfleisch and G. Staehler

Postoperative tumour perfusion was applied to 7 human seminomas to determine the proliferation characteristics. $^{3}H$- and $^{14}C$-thymidine were added to the perfusate. Measurements of metabolic parameters showed that physiological conditions remained constant throughout the perfusion. Whole-tumour autoradiography revealed a regular pattern of proliferation with a high labeling index in the peripheral areas and in perivascular zones. According to the distribution of the spermatogones, the parenchyma is evenly labeled (LI 15-20%). To get a quantitative evaluation of proliferative parameters in relation to the morphology, the labeling index was measured continuously from tumour periphery to the centre. The mean values for the labeling index were 0-1,1% in the central and 24,5% in the peripheral areas. In the perivascular tissue the labeling index increases up to 30,5%, with a rapid exponential decline further away. The theory that lymphocytes are inhibitors of tumour growth is supported by the fact that the labeling index of tumour cells immediately adjacent to lymphocytic infiltrations is low. Double-labeling experiments using $^{3}H$ and $^{14}C$-thymidine permit a determination of $t_S$. The mean value of $t_S$ was about 20 hours with a wide range of variation. The potential doubling time ($t_{pot}$) was 2-12 days for peripheral and 9-43 days for perivascular and intermediary zones. These data show that, in contrast to the teratocarcinoma, the proliferation of human seminoma is less dependent on cytological grading but mainly on the geometry of the tumour and its vascularization.

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## Cell Kinetic Analysis on Human Kidney Carcinoma After Embolization and Irradiation

U. Rattenhuber, H.M. Rabes, P. Carl and F.J. Marx

Vascular perfusion of renal carcinoma permits a characterization of proliferative compartments. By the addition of $^{3}H$- and $^{14}C$-thymidine cells in DNA synthesis are labeled and can be evaluated in autoradiograms. This method has been used in more than 20 renal carcinomas, and the inhibitory effect of preoperative irradiation on DNA synthesis has been proved. We report on a renal carcinoma which was embolized by autologous muscle homogenate and subsequently irradiated with 7.500 rad. Arteriograms 7 months later showed a partial recanalization and a significant reduction of the tumour size. After nephrectomy, histological examination revealed a low-differentiated carcinoma of the clear-cell type. Among large necrotic areas multiple tumour nodules were found. Whole-tumour autoradiography showed several proliferating subpopulations. Compared with the labeling index of the tumour centre the number of DNA-synthesizing cells in the peripheral areas of these nodules was significantly higher. The labeling index increased up to 12,5% at the borderline of the tumour nodules. The mean labeling index of proliferative compartments was 2,9%. Obviously as a result of embolization and irradiation, the overall labeling index of the whole tumour was only 0,07%, though the proliferative pattern of subpopulations was similar to carcinomas, which had not been treated before resection. Embolization and irradiation may suppress tumour growth only for a short time. Tumour progression in proliferative subpopulations is probably due to clonogenic cells.

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## Determination of Cell Kinetic Parameters of Human Transitional Cell Carcinoma of Renal Pelvis

H.M. Rabes, U. Rattenhuber and P. Carl

Cell kinetic parameters of transitional cell carcinomas of the renal pelvis can be determined by the newly developed method of extracorporeal perfusion of the tumour-bearing organ (Rabes et al., Cancer 44, 799, 1979). Immediately after nephrectomy, the organ is perfused via the vascular system with dextran-diluted, heparinized, oxygenated blood under normothermic conditions to simulate a physiological situation. Addition of $^{3}H$- and $^{14}C$-thymidine at 2 hr intervals permits, by autoradiographic evaluation, the estimation of the fraction of cells in DNA synthesis, determination of the intratumoural distribution of growing compartments, the calculation of DNA synthesis time, potential tumour doubling time and cell loss.

Whole-tumour autoradiography of two transitional cell carcinomas grade I revealed a regular distribution of growing compartments in a papillary or trabecular pattern. The mean labeling index was 4.5%. Its maximum (9.0%) was found in the immediate vicinity of the vascular stroma, with a linear decrease further

away. At a distance of 200 µm from the vascular pole, labeled cells were completely missing. With a $t_S$ of 25.8 hr, an average potential tumour doubling time of 17.7 days was calculated. Increase of $t_{pot}$ up to half a year or more in tumour subpopulations further away from the vascular pole as well as high cell loss by necrosis and cell shedding might be responsible for the much prolonged clinical tumour volume doubling time.

Supported by grants from Wilhelm-Sander-Stiftung.

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## Production of Xenogen Specific Antibodies Against Kidney Cell Carcinomas with Intra-Uterine Tolerance-Induction

W. Wieland, J. Birkmayer, G. Schmid, H.-E. Mellin, Ch. Chaussy, C. Hammer and W. Land

Immunological diagnosis and therapy of malignancies require specific antibodies against the tumour concerned. It has not yet been possible to isolate a pure membrane antigen from carcinoma of the kidney. By inducing tolerance against membrane proteins in normal kidney tissue an attempt was made to produce tumour-specific antibodies against membrane proteins in kidney carcinoma.

Via the jugular vein fetal lambs were each given at 2, 3 and 4 weeks prepartum intra-uterine injections of fractions of membranes of normal kidney cells from the kidney with the tumour. Starting in the eight postnatal week the animal was sensitised at fortnightly intervals through the fraction of cell membranes of the malignant part of the kidney. The sera were investigated for fractions of antibodies against kidney cell carcinoma with the aid of a double diffusion test with Agar gel and immune electrophoresis using alternating current.

Six of the nine fetal sheep survived. Two of them showed a significant antibody titre against antigen of the malignant kidney membrane. From our findings to date it seems that the timing of the intra-uterine antigen application (as far as possible from the partum) is an essential factor for inducing tolerance.

If it could be proved that the acquired antibodies in a sheep against a definite kidney carcinoma also show a specific reaction to kidney carcinoma (allogen cross reaction), it would be feasible to use this reaction in follow-through studies with patients having kidney tumours, particularly with reference to metastases. Finally these antibodies could also be used as carrier-systems for radioactively marked substances or for cytostatic therapy.

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## Longterm Observation of a Human Teratocarcinoma Xenotransplanted in Nude Mice

M. Wirth, R. Ackermann, S. Schmidt and A. Zimmermann

Model systems of human testicular tumours are needed to achieve further progress on the biology of this type of tumour. We have investigated whether xenografts of human non-seminomatous tumours on thymusaplastic nude mice would fulfil the requirements for such a model.

Specimens of a human teratocarcinoma were inoculated in nude mice on June 21st, 1978. These xenografts could be maintained by further transplantation in a total of 469 nude mice, yielding growing tumours in 196 animals. The tumour is now in the 10th animal passage. The percentage of positive takes increased from 25% until the 4th passage to 41,7% takes until the 10th passage. This could be due to in vivo cloning, but may also reflect a more skilful transplantation technique. DNA histograms carried out with tumour cells from different animal passages showed constantly a high hyperdiploid DNA peak. The architectural pattern of the anaplastic teratocarcinoma as determined by light microscopy, containing trophoplastic giant cells, did not change its appearance. The xenografts produced human chorionic gonadotropin (β-subunit) as the patients tumour did. The hormone could be identified on tumours of different animal passages by direct immunofluorescence. In context with earlier results, the observation suggests that the xenotransplanted teratocarcinoma retained its human characteristics even after longterm maintenance in nude mice.

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## Demonstration of Onkorna Viruses in Testicular Teratocarcinomas

R. Harzmann, R. Kurth and K.-H. Bichler

Onkorna viruses cause malignant tumours in a number of animal species. For this reason, the question of the existence of human onkorna viruses has been studied with great expenditure but little success. In 1971 particles having the morphology of onkorna viruses were demonstrated in the human placenta. Until just recently this remained the only indication of the existence of onkorna viruses in humans. The search for human onkorna viruses was first directed at whether a specific antiviral immunity existed in healthy subjects or in patients with testicular carcinoma. Antigens form ape onkorna viruses were employed, of which it could be assumed that they would have a close immunological affinity with human onkorna viruses. It was shown that only patients with teratocarcinomas of the testis gave evidence regularly of a significant antiviral immunity, not, however, those with seminomas. The teratocarcinomas which were subsequently taken in culture showed only a slight spontaneous production on onkorna viruses. This production could, however, be considerably increased with use of certain specific provocative measures. Thus, with teratocarcinoma of the testis, a malignant human tumour has been found in which reproducible onkorna viruses can be demonstrated. The anti-viral antibodies, which can only be demonstrated on teratocarcinoma patients might clear the way for development of a tumour marker specific for teratocarcinoma, so that close control of this tumour is conceivable.

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## Analysis of Proliferation of Human Testicular Teratocarcinomas

H.M. Rabes, U. Rattenhuber, P. Carl, U. Löhrs, G. Staehler, R. Lamerz, K. Mann and G. Rindfleisch

Data on kinetic parameters of the human testicular teratocarcinoma are unknown. The method of whole-tumour perfusion (Rabes et al., Cancer 44, 799, 1979) provides the possibility for a quantitative approach

to an analysis of cell proliferation kinetics.

The tumour-bearing testis was perfused under simulated physiological conditions in vitro. $^{3}H$-thymidine was infused for a short period, followed by $^{14}C$-thymidine after an isotope-free interval. The fraction of DNA synthesizing cells, duration of DNA synthesis, and secondary growth parameters were determined in autoradiograms. Combined cytological, histological, autoradiographic and immunohistochemical evaluation of whole-tumour sections render a quantitative description of proliferative activity in distinctive parts of the tumour possible. Besides adult, highly differentiated areas with low labeling indices, various degrees of dedifferentiation were related to a broad spectrum of proliferative activity. Foci of extremely high labeling indices, short DNA synthesis time and rapid potential tumour doubling time appeared most important for tumour progression though often localized in close vicinity of areas of necrosis, indicating an intrinsic coupling of high cell turnover and cell loss.

The results of this quantitative evaluation of the proliferative heterogeneity bear important implications for biology and therapy of the human testicular teratocarcinoma.

Supported by grants from Wilhelm Sander-Stiftung.

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## VI. Urodynamics

### Telemetric Urodynamic Investigations

J.W. Thüroff, U. Jonas, D. Frohneberg and E. Petri

The application of telemetry in urodynamics was evaluated by both standard and telemetric urodynamic techniques in 10 healty males. Reliability, practicability and indications for use were investigated. Two portable 2-channel transmitters were used for telemetric data transmission; data registration was performed by adaption of a receiver to the regular urodynamic unit. Telemetric data transmission included bladder pressure, rectal pressure and pelvic-floor-EMG; TV-observation made correlation of the data with the actual situation possible.

During bladder-filling, intravesical pressure could be registered in a lying, sitting and standing position. Stress conditions such as walking, running, climbing steps, knee-bends, push-ups and even handstands were tested. Intravesical pressure showed a gradual rise corresponding to augmented intraabdominal pressure due to increased stress. Desire to void occurred at a lower bladder volume than in regular cystometry. Micturition was performed in a standing position; additional information was gained by recording uroflow and volume voided. A normal voiding phase was recorded in all telemetric investigations, while 3/10 men displayed some abnormal micturition pattern in regular (sitting) cystometry.

Telemetric data transmission gives reliable and reproduceable data. The advantages are: 1) simulation of several stress conditions because of independence from stationary laboratory equipment 2) lowered psychogenic stress as the patient is left undisturbed and can urinate in a normal position 3) 24-hour investigations. This technique is recommended in: 1) incontinence, if no urine loss can be detected by regular cystometry 2) micturition disorders, if psychogenic inhibition of micturition is suspected 3) enuresis, if 24-hour investigations are desired.

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### The Effect of Luminal Ions on the Transurothelial Electrical Potential Difference of Normal and Diseased Human Bladder: A New Approach to Study Urothelial Function

G. Hohlbrugger, G. Jakse and M.Ch. Michailov

A high paracellular resistance against passive water- and ion fluxes as well as an active Na-transport are the basis of a transurothelial electrical potential difference (PD) in mammals. For measurement in humans, a millivoltmeter and Ag-$AgCl_2$-electrodes were applied. The reference electrode was placed at the site of an intradermal saline injection which was necessary to abolish skin potential differences. The bladder was filled up to urge with test-solution and then the measuring electrode connected with the opening of the catheter. This method provided adequate results since the test-solution represented a saline-bridge. With instilled 0,9% NaCl, striking circadian variations were found in normal bladders (n=5). In contrast, whenever a 0.9% NaCl value was immediately compared with that of another test-solution the resulting divergence showed constant degrees (n=3). Because of these results the effect of various Na-concentrations, of isotonic Cholin Cl-, $MgCl_2$-, Mannit-, $Na_2SO_4$- and KCl-solutions and Amiloride were studied. The highest divergences occurred with isotonic Mannit (+25) and isotonic $Na_2SO_4$ (-10). Therefore these solutions together with 0.9% NaCl were used for the clinical investigation. The corresponding PD-values in patients with different kinds of cystitis, with large dedifferentiated bladder tumours or with neurogenic bladder due to spinal cord injury were significantly different from normal. This indicates that the permeability of the urothelium was altered in these patients and may lead to changes in the electrolyte environment of sensory nerve endings and also of the detrusor muscle. This could account for an enhanced sensivity of these structures.

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### Changes in the Mechanical and Electrical Activity of Detrusor Muscle (Human and Guinea-Pig) After Cyclic Nucleotides, (cAMP, cGMP), Urol® and Other Drugs

M.Ch. Michailov, G. Hohlbrugger and W. Schneider

The influence of some drugs was examined on the tonic and phasic activity, on the contractions to electrical nerve stimulation (CENS: 10 Hz, 0.3 ms, 3s) and on the electrical activity of guinea-pig detrusor single cells via intracellular recording (method: Proc. Int. Un. Physiol. Sci. 13, 1497, (1977), Paris; Creed: Pflügers Arch. Phys. 326, 115, (1971)) cAMP (0.1-1 mM, dibutyryl salt), fenoterol (1-100 nM) and isotope (1-10 µM) decreased the detrusor muscle tone, the phasic activity and CENS in all preparations; these drugs induced hyperpolarisation accompanied by inhibition of spike and/or burst activity. cGMP (0.1-1 mM, dibutyryl salt) and buphenin stimulated the mechanical activity of the detrusor; depolarisation and stimulation of spike activity was observed after cGMP,

while buphenin inhibited the electrical activity similar to cAMP. Urol® decreased the mechanical activity of the detrusor in concentrations of 1-10 µg/ml and abolished it in concentrations of 100 µg/ml. Experiments with isolated human ureter and pelvis showed that the spontaneous mechanical activity and CENS were also decreased or abolished by Urol®. The drug acted in some preparations (also after an increase of potassium concentration) in very low concentrations (0.1-10 ng/ml). It is concluded that the effects of these drugs on the mechanical activity of the detrusor, correlate to their effects on the electrical activity. But in some cases, for example, with buphenin the contractile effects of the drug do not correlate with the electrical phenomena. Further, it is suggested that the inhibitory effects of Urol® on the mechanical activity of human detrusor, ureter and pelvis in vitro are the therapeutic basis of treatment in cases of nephrolithiasis.

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## The Determination of Contractile Properties of the Urinary Bladder from Isometric Contractions; A Pilot Study

R. van Mastrigt

A clinical method was developed for estimating the contractile properties of the urinary bladder. At the end of ordinary filling cystometry, patients were asked to start voiding. This yielded a pressure rise in which the first part (i.e. the part before flow started) was nearly isometric. This part of the pressure rise was analysed by computer in terms of a model for contracting muscle, resulting in a plot of the force exerted by the muscle as a function of its velocity of contraction. These plots could fit a hyperbolic function which is a very common finding in muscle physiology. By extrapolation of this function, an estimate was obtained of Vmax, the speed of contraction the muscle could reach if no force was exerted. This contraction speed was not actually reached during the contraction of the bladder muscle. In our pilot study this parameter was estimated for 21 patients. For 17 of these 21 patients the average value of Vmax was 36 mm/s with a relatively small standard deviation of 30%. The other four patients showed a much higher value of Vmax, and were all female incontinence patients. In the original 21 patients there were 10 female incontinence patients.

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## Precise Urodynamic Assessment of Urethral Obstruction

D.J. Griffiths

Urethral obstruction may be either anatomical or functional. Urodynamic assessment may be confused by artefacts: a urethral catheter may cause artefactual anatomical obstruction; the stress of the examination may lead to artefactual functional obstruction by the periurethral sphincter. It is important to distinguish these different types of obstruction and to recognize artefacts. We have performed standard pressure/flow studies of micturition in the presence of urethral catheters. Detrusor pressure is plotted against flow rate on a x-y recorder throughout micturition. The study is usually repeated at least once to check reproducibility. The material consists mostly of children, with some adults for comparison. By means of examples it is shown that the pressure/flow plot has different, reproducible forms for unobstructed and anatomically obstructed urethras. provided there is no variable sphincter activity during voiding. A thick urethral catheter (Ch.8) enhances the sensitivity to anatomical obstruction to a precisely measurable extent. In functional obstruction, sphincter activity usually varies during voiding, leading to characteristic (but not necessarily reproducible) features on the pressure/flow plot. Functional obstruction which becomes less and less pronounced in repeat studies is presumably an artefact. Pressure/flow plots, repeated and with a thick urethral catheter, enable a confident assessment of urethral obstruction and whether it is anatomical or functional in nature.

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## Urethral Smooth Muscle Activity Expressed in Urine Flow Rate Curve

A.E.J.L. Kramer, R.C.G. Gallandat Huet and P.J. Donker

Urine flow measurement is a routine technique in urological practice. Maximum flow rate and average flow rate, both related to voided volume, are the most used dianostic variables. How these data reflect the conditions in the lower urinary tract is known only partially. This paper tries to give a physiological explanation for the individual dependence of the flow rate on voided volume for voidings from normal men.

This dependence was studied for the average flow rate in 438 voidings (47-90 each) of 7 normal volunteers (ages 24-44). After a mathematical approximation of the normal trend flow rate, curves relating the cross-section of the urethral "compressive zone" and the flow rate can be derived.

For all seven volunteers the average flow rate grows linear with voided volume ($r \simeq .75$) up to a certain individual level. For higher volumes the flow rate remains constant. The flow rate curve can be fitted for the ascending limbs as: $Q = Qmax \cdot (1-e^{-10t/T})$ up to one half of the flow time T. The descending limb follows a cosine function. From these formulae it follows that $Qave = .65 \cdot Qmax$.

The flow past the compressive zone is given by the product of area and fluid velocity. This velocity also depends on the area. From the observations it thus follows that the area grows with initial bladder volume until at the critical volume a maximum area is reached. Combination of Laplace's law and the length-tension curve for the smooth urethral muscle leads to the conclusion that the activation degree of this muscle decreases as the initial bladder volume increases. After full relaxation no further increase in flow rate with volume can be expected.

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## Preliminary Results of a Method Designed for Quantitation of the EMG of the External Urethrals Sphincter and the Anal Sphincter by Computer Analysis

N.P. Tjon Pian Gi, A.E.J.L. Kramer, A. Oomen and P.J. Donker

During previous investigation of electrocystometrograms

it was observed that the appearance of the EMG of the urethral sphincter was different from that of the anal sphincter. With computer assisted analysis of the EMG of urethral and anal sphincters we investigated whether a reliable quantitation of the EMG was possible, and if significant differences could be found between the signals from the urethral and anal sphincters.

Electrocystometrograms were made in supine position. Filling rate is 0.5 ml/s. All signals were recorded by an Elema 8 channel ink jet recorder. Pressure lines in the bladder and rectum were connected to Statham transducers. Flowrate was measured by a Disa flow meter. Electric signals from the anal and urethral sphincters were registered through bipolar wire electrodes, introduced percutaneously with gauge 20 needles. Electric activity was then amplified by the Elema EMG-amplifier, recorded and monitored on an oscilloscope. The electric signals were recorded on magnetic tape, in a HP3960A FM tape recorder, after they had passed two special designed filters in order to minimize noise and movement artefacts.

The first step in quantitation of the EMG-signals has been the counting of the number of turns (changes in potential around the zero) at rest with the bladder empty, at maximal bladder filling, during physical exercise (coughing, etc.) and after evoked reflexes. Preliminary results in the first 3 patients showed that a reliable quantitation of EMG can be made of both the external urethral sphincter and anal sphincter. In these patients there was a difference in the absolute number of turns as well as different reactions during reflex stimulation. Further investigation will be done.

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## VII. Andrology

### Experimental Investigation on Vasography

L.V. Wagenknecht, H. Becker, H.M. Langendorff and H. Schäfer

Based upon an experience with more than 400 patients operated upon for excretory azoospermia in Hamburg's Refertilisation-Center we are strongly opposed to separate vasography as diagnostic procedure. The latter might result in a secondary obstruction caused by manipulations with the needle on the vas, by the contrast medium or by additional infection. In 90 rats the effects of six contrast mediums (see table) were tested in comparison to 3 control groups (Vas-preparation only; vas-ligation only; saline injection). Histologic examination showed considerable vas-damage (table). Vasography should be done during operation with hydrophilic contrast medium of low concentration.

| Groups of animals (10 rats per group) | Number of obliterations | Fibrosis of the wall | Inflammation infiltration | Fenestration septum formation | Disquamation of the epithelium |
|---|---|---|---|---|---|
| 1. Surgery only | - | - | - | - | - |
| 2. Vas ligation | - | - | - | - | - |
| 3. Saline injection | - | - | - | - | - |
| 4. Conray | 5 | ++ | ++ | ++ | ++ |
| 5. Urovison 58% | 2 | ++ | (+) | ++ | ++ |
| 6. Urovison 30% | 2 | + | (+) | ++ | + |
| 7. Telebrix | - | + | + | + | + |
| 8. Uromiro 80% | 2 | ++ | + | ++ | ++ |
| 9. Amipaque | - | - | (+) | - | - |

### On the Neuro-Muscular Transmission in Human Vas Deferens

M.Ch. Michailov, E. Elsaesser, F. Zettler, M. Boldt, R. Rachl

The neuro-muscular transmission in human vas deferens preparations (surgical material) was studied by the action of some drugs on the contraction to electrical nerve-stimulation (CENS) (method: Biophysik 11, 289, (1975)). Tetrodotoxin (0.1-1 µg/ml) and procain (100 µg/ml) abolished or strongly inhibited the CENS at electrical parameters of 10 and 100 Hz, 0.3 ms pulses and 3 s duration, i.e. predominantly the motor nerve elements of vas deferens were stimulated. The alpha-adrenergic blocking agents — phentolamine and phenoxybenzamine (1-10 µM), bretylium and guanethidine (100 µM) — drugs with blocking action on the post-ganglionic sympathetic neurones, the monoamine oxidase inhibitor iproniazid (1 mM) nearly abolished the CENS. Experiments using prostaglandins showed the following results : $PGE_1$, $A_1$ and $B_1$ in low concentrations, (0.1- 1 ng/ml) had an augmentory effect on CENS of human vas deferens; in higher concentrations (0.1-1 µg/ml) they had an inhibitory effect. The CENS of guinea-pig was diminished after $PGE_1$ (0.1-1 ng/ml), $PGA_1$, $B_1$ (10 ng/ml). After $PGF_2$-alpha (1 ng - 1 µg/ml) the CENS of human and guinea-pig vas deferens preparations was decreased. In higher concentrations all PGs (1 µg/ml) induced an elevation of basal tone and phasic contractions. It is suggested that the motor innervation of human vas deferens is adrenergic. Further it is probable that prostaglandins participate as cotransmitters or modulators on the neuromuscular transmission of this organ.

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### The Double-Layer Microsurgical Vasovasostomy in the Rat

G. Carmignani, E. Belgrano, P. Puppo and U. Repetto

The microsurgical technique of vasovasostomy introduced by Silber and Owen seems to give the best results. It is based on the assumption that a perfect muco-mucosal sperm-tight suture is the fundamental goal in vasovasostomy. We performed bilateral microsurgical two-layer vasovasostomy in 10 male Wistar rats. In 20 rats we performed only a unilateral vasovasostomy with a contralateral vasectomy. Fertility was tested in the first ten rats by caging the male with 2 female rats of proved fertility. In all the rats the anastomeses were controlled by dye injection and by histological examination. 35 out of 40 anastomoses were patent. Pregnancy was obtained in 7 out of 10 rats. Our results confirm that the microsurgical two-layer technique is the best method for vasovasostomy, especially when there is a difference in caliber between the two stumps of the vas. Specific training in microsurgical techniques is needed to perform this vasovasostomy. A new device, the gradual rotation clamp, makes the passage of the posterior muco-mucosal stitches easier, without the aid of an assistant and without any traction on the muco-mucosal sutures. A perfect microsurgical no-touch technique is then achieved.

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## Microscopical Anastomoses of Vas Deferens with Tubulus Epididymidis Using Absorbable Splints

E. Özdiler and A. Kelâmi

The fate of an epididymovasostomy is correlated directly with the patency of the lumen of the epididymidis. Recent laboratory and clinical experiments show the feasibility of a two layer microscopic anastomosis between vas deferens and the epididymidis. Our aim was to simplify this anastomosis using absorbable splints and absorbable suture material (A. Kelâmi, Alloplastische Spermatocele - Eine kritische Betrachtung und Verbesserungsvorschläge. Extracta Urol. 1: 245-251 (1978).

24 rats had bilateral procedures. Under 16 x magnification 32 anastomoses were done between the vas deferens and the tunica vaginalis of epididymis using 7/0 synthetic absorbable suture material. Before tying the sutures a 4/0 Plain-Catgut splint was inserted into the lumen on one side and vas deferens on the other side bridging both.

After 3-6 months an ejaculate by electroejaculation was obtained and examined microscopically. These early results show that the splints are absorbed and the anastomoses patent.

Further experiments are being made now to examine the patency in long term cases. This would be a step forward in the surgery of male infertility.

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## Vaso-Vasostomy Following Vasectomy with Preserved Innervation

P.-C. Esk and R. Pabst

With an increase in the number of vasectomies, one must expect an increase in the wish for vas reanastomoses. There exists a discrepancy between the number of patients with sperm in the ejaculate after reanastomosis and patients who actually cause conception. A possible explanation for this difference might be the denervation of the part of the vas deferens lying near the testes from which about 65% of the sperm are discharged by an effusion.

To elucidate this problem, ten dogs were subjected to vasectomy. In five dogs the nerve was carefully prepared and preserved and in the other five the usual vasectomy technique was employed. After six months, a vaso-vasostomy was done with an unilateral muscular and epithelial technique without splinting. The preserved innervation was proven by intact neural conductance in all five dogs.

In comparing the spermatological results before and after vasectomy, it was demonstrated that not only a reduced number of sperm per cmm but also a somewhat higher percentage of pathological sperm forms had resulted equally in both experimental groups. In contrast, the survival rate of the sperm was significantly longer in the group with the preserved innervation than in the other group.

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## Fertility After Experimental Torsion of the Spermatic Cord

G. Ludwig and J. Haselberger

In earlier studies the testicle tissue after torsion of the spermatic cord (TSC) in 255 Wistar rats was investigated by histological and morphometrical evaluation. We found the latency periods before the onset of irreversible tissue damage considerably shorter than generally assumed and also variable. The germ cell epithelium showed deterioration significantly earlier than the testosterone producing Leydig cells.

These morphological results were studied functionally through copulation experiments following TSC in 100 Wistar rats. In groups of 10 specimens the testicles of 100 rats were half unilaterally half bilaterally rotated 720° and retorted 1, 2, 4, 6 and 8 hours after torsion. Three weeks later each specimen and two female rats were put in the same cage. Fertility was demonstrated by pregnancy. 5 sham operated and 5 untreated animals of the same strain were used as control groups.

Independently of the torison period the fertility rate of the unilateral contorted specimens was 60% — as in both control groups. The bilaterally contorted animals were fertile in 40% from the 1h-group and in 30% from the 2h-group. If the torsion period was longer than 2 hrs the bilaterally contorted animals were infertile in all cases. The morphological results of our previous experiments were therefore verified functionally. There is no influence of a unilateral contorted testicle on the contralateral uncontorted testicle. Fertility rate is not different form the control group in these cases. In bilateral TSC the fertility rate is significantly reduced after a time period of 1 and 2 hours following the torsion. After 2 hours sterility results in bilateral contorted animals.

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## Experimental Studies on the Influence of Testicular Torsion on the Contralateral Testis and Blood Flow

G. Janetschek, F. Schreckenberg, S. Frank, G. Mikuz, W. Grimm and M. Marberger

Unilateral testicular diseases evoke subfertility in a high percentage of cases. Assuming that one healthy testis can maintain normal fertility, the contralateral testis must either be primarily defective or secondarily damaged. Testicular torsion mostly results in a haemorrhagic infarction. To simulate this condition in an animal experiment, blood flow studies were done using radioisotope-labelled microspheres (99Tc and 113In). These flow studies clearly show that only in those cases with a torsion of 540° with the tunica vaginalis was the arterial flow reduced to a minimum without provoking ischaemia. Using erythrocytes labelled with 99Tc we could further demonstrate that haemorrhagic infarction occurred at a torsion of 540°.

In 110 Wistar albino rats, ejaculates were collected by electrostimulation; testosterone, LH and FSH were determined by radioimmunoassay. The right testicle was then twisted 540°. A sham operation was done in the control group. 8 weeks after torsion, sperm output had decreased to half. The controls were unchanged. LH increase was statistically significant aftet torsion; the other hormones remained unchanged. The morphometry of the contralateral testis revealed no changes except a significant increase of the interstitial cells.

It has been shown that semi-castration in the rat does not reduce sperm output. There are therefore 2 possible explanations for the decrease in sperm

count observed in our study: 1) the destroyed but retained testis may disturb those mechanisms which otherwise would compensate the loss of one testis, 2) there may be direct damage to the contralateral testis.

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## Effect of Experimental Cryptorchidism and Winkelmann's Operation upon Spermatogenesis in Adult and Newborn Rats

Y. Mori, G. Konrad and W. Widjaja

Unilateral abdominal testes were produced in adult and newborn rats. Unilateral Winkelmann's operation and opening of tunica vaginalis only were additionally performed. Testes were removed after 6, 7, 9, 12, 42 days and 3, 5, 8 months, histologically examined and compared with the normal testes. In the cross-sectioned seminiferous tubules, Sertoli cells, spermatogonia, spermatocytes and spermatids were counted. Mean tubular diameter of 50 tubules and testicular weight were recorded.

In experimental cryptorchid testis on adult and newborn rats a reduction in number of spermatogenic cells was noted depending upon the time of operation. In the contralateral scrotal testes compensatory hypertrophy was prominent, but in 2 of 17 testes (12%) among the newborn rats atrophic changes in the scrotal contralateral testes were observed. In the testes after Winkelmann's operation as well as after opening of the tunica vaginalis only a temporary disturbance in the stage of spermatid was observed.

These results on rats, in which a compensatory hypertrophy in contralateral testis was shown, are somewhat different from those found in dogs where contralateral lesions are fairly frequent. The latter correlates more likely with the clinical situation.

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## Determination of Antibodies Against Spermatozoa in Experimental Cryptorchism

A. Rost, W. Ehrenberg, U. Fiedler and M. Butz

After we were able to demonstrate autoantibodies against spermatozoa in vasectomized and epididymectomized rats, this animal model was used for evaluation of the question as to whether the subfertility known in cases of cryptorchism might be of immunological genesis.

In 66 Sprague Dawley male rats, the testicles were unilaterally or bilaterally displaced intraabdominally. The indirect immunofluorescence technique was used for the detection of antibodies against spermatozoa. Examinations were done 3, 6 and 9 months after testicle displacement. Controls were performed with homologous normal testicles and homologous normal serum.

The cryptorchid testicles developed an average volume reduction of 55%, a decrease in the spermatogonia and an average reduction in the tubule diameter of 30 µ. 6 months after the operation; antibodies against spermatozoa were clearly found in both testicles after unilateral cryptorchism and to a lesser degree after bilateral cryptorchism. The homologous normal testicles behaved analogously, but the controls with homologous normal serum always remained negative.

On the basis of these results, there is the possibility that the impairment of spermiogenesis in cases of cryptorchism might have an immunological cause.

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## The Occurrence of a Carcino-Embryonic-like Antigen in Human Seminal Fluid

W. Krause, S. Peters and W. Weidner

Antigens similar to the carcino-embryonic antigen (CEA) were found in some tissue extracts and biological fluids. We used a CEA-specific radioimmunoassay to detect such antigens in seminal fluid.

The CEA-like substance was found in a mean concentration of 118 ng/ml as compared to the standard antigen, which is derived from colon carcinoma. Comparison of mean CEA concentrations from semen samples with different sperm counts showed no significant differences. The antigen is present also in azoospermia. Measurement of CEA in total seminal fluid and separated seminal plasma showed nearly the total amount being present in seminal plasma. No correlation with some other seminal parameters (fructose, immunoglobulins) could be demonstrated.

In column chromatography on Sephadex G-100 and G-200 the CEA-like substance was eluted within the void volume, i.e. the first fractions. The elution profile was the same as for standard CEA.

Our results suggest an immunological similarity between seminal CEA and the standard substance. No evidence for biological significance can be given.

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# VIII. Bladder Carcinoma

## Early Detection of Premalignant and Malignant Cells in Urine by Television Image Analysis System

H.J. de Voogt, J.S. Ploem, J.A.M. Brussee and C. Knepflé

AFS-staining of urinary sediment smears permits DNA-measurement by cytofluorometry, but also scanning of the absorption image. When the absorption image is brought on a television monitor screen, atypical and malignant cells can be selected by measuring chromatin contrast (using different grey levels of the image) and size of the nucleus. The product of these two is directly proportional to the relative DNA-content, which can be measured by fluorometry of the extinction image.

However artefacts, caused by cell clusters or by dirt particles have to be rejected. In the LEYTAS this is done by image transformation procedures. After selection of suspect cells and rejection of artefacts (which in the case of false alarms can also be detected visually), total absorption, mean absorption, surface and DNA-content (compared to standard diploid lymphocytes) are measured and stored in the computer. Finally the amount of malignant and suspect cells and the total cell-density are computed. This then provides an atypia-index, based on DNA-histogram and the composition of the total cell population.

The results of a pilot study of urine of 20 patients

have encouraged us to continue the project with larger numbers. It is expected that this could lead to a more refined and standardised cytological diagnosis.

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## A Microscopcytophotometric Study of Urothelial Cells

B. Aeikens and C.-E. Liedtke

The cytologic examination of the urine has been established as a screening test for tumours of the bladder. With the help of the urine cytology it is possible to detect cancer of the bladder frequently at a time, when an operative treatment is still successful. Tumours of low malignancy (grade I) reveal minor abnormalities and therefore these tumours are often overlooked using cytological methods. For these reasons it is desirable to develop methods for further characterisation of the tumours. Therefore, it appeared useful to examine the applicability of microscopic scanning cytophotometry to discriminate urothelial cells. A special combination of a Leitz MPV 2 microscope and a digitizer has been built to allow the interactive segmentation of the cell into nucleus, plasma and background.

Computer discrimination between benign, atypical and malignant cells by means of cytophotometry has been accomplished with a small error of classification, not greater than five percent.

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## Mapping Studies of Surgically Removed Bladders: A Comparative Histological and Histophotometrical Examination in Patients with Carcinoma of Bladder

K.H. Kurth and F.J.W. ten Kate

Several investigators have drawn attention to the existence of cystoscopically occult precancerous epithelial abnormalities such as flat carcinoma in situ and occult invasive carcinoma (Koss, L.G. et al., Urology 9: 442, 1977). Mapping of epithelial changes in the cystectomy specimens has the purpose of identifying unsuspected areas of invasive carcinoma.

Histophotometry examines in a rapid and automated way thick (1 mm) unprepared tissue slices. The method has been described in an earlier study (Kurth, K.H. et al., Urol. Res. 7: 113, 1979). The correlation between nuclear volume and nucleic acid content is determined in formalin fixed and fresh tissue samples. This ratio is characteristic for benign and malignant tissue. In this study six specimens were examined. All patients underwent cystectomy for invasive bladder carcinoma. After the gross photographs were taken the specimens were examined histophotometrically. The entire epithelial surface of the bladder was then cut into sequential tissue blocks, approximately 1 by 4 mm each and about 1 mm thick. Each block was examined separately. One hundred and thirty to one hundred and seventy blocks were required to process the entire bladder. For the histophotometrical examination six measurements were performed for each block. Histology was then performed on the same sample. As in other studies we found occult non-visible carcinoma. Histophotometrically occult carcinoma was detected in fresh tissue samples from the cystectomy specimens, but not always in formalin fixed samples. We believe that because the homogenous fixation demanded for the quantitative histophotometrical study was not always achieved, the measurements were not performed in a standard way and the results obtained were therefore not comparable.

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## Ultrastructural Analysis After Intravesical Chemotherapy of Superficial Bladder Tumours in the Rat

H. Rübben, H.H. Dahm and W. Lutzeyer

The aim of the experiment was to study the effect of cytostatic drugs, given intravesically to reduce the frequency of superficial bladder cancer induced by 0.05% BBN.

240 female Wistar rats were divided in groups of thirty. 210 received 0.05% BBN over a period of 12 weeks. In the seventh week instillation therapy was started. Group 1 NaCl solution, group 2 Adriamycin (ADM) 1.5 mg/ml, group 3 ADM 0.5 mg/ml, group 4 Mitomycin C (MMC) 1.5 mg/ml, group 5 MMC 0.5 mg/ml, group 6 MMC/ADM alternatively 1.5 mg/ml, group 7 MMC/ADM alternatively 0.5 mg/ml. Instillation time: 30 min., volume: 0.25 ml. interval: 3 days, frequency 10 times (1.5 mg/ml), 15 times (0.5 mg/ml). BBN feeding was stopped after the last instillation. 30 rats get instillation therapy only without BBN-tumour induction. 2 and 8 weeks after instillation therapy the rats were killed.

After ADM instillation (1.5 mg/ml) a necrosis of 50% of the mucosa and the adjacent lamina propria was observed. The necrosis was reversible after 8 weeks. There was reduced tumour growth after ADM instillation but increased tumour growth 2 and 8 weeks after MMC instillation. Combination chemotherapy showed no positive effect. Probably the effect of ADM (1.5 mg/ml) was due to the direct toxicity of the drug to the mucosa. The real effect will be demonstrated after long time follow-up only.

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## HLA Types in Bladder Cancer Patients

P. Puppo, G. Carmignani, E. Belgrano, S. Quattrini, S. Barocci and A. Nocera

Alleles of the HLA system in the major histocompatibility complex appear to be strictly associated with the Ir genes, which play some role in conditioning the host's immunity functions involved in surveillance against tumours.

We report our experience in HLA typing of 47 patients with transitional cell carcinoma of the bladder and 222 healthy controls. We used 117 antisera among those recognized by the VII Histocompatibility Workshop which tested 51 antigens of the loci A, B and C.

The frequency of B5 and BW51 antigens was significantly higher in patients with tumours when compared to the control subjects. The statistical correction introduced by Grumet, that is to multiply the value of chi-square by the number of alleles which were tested, confirmed as significantly higher only the frequency of BW51, with a relative risk of 4.1.

The possibility that individual genetic variability

influences the appearance of bladder cancer and contributes to its differing natural histories is obviously of considerable interest in prevention and early diagnosis of bladder cancer and we think that these investigations, although preliminary, are potentially extremely important.

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## Evidence of Urinary Bladder Carcinogens in the Urine: Experimental and Clinical Studies

R. Harzmann, D. Gericke and K.-H. Bichler

The increase in frequency of urinary bladder carcinoma is currently being related to quite different environmental factors. The carcinogenic effect of beta-naphthylamine, paraaminodiphenyl and benzidine are known; disputed are the effects of cyclamates, tobacco, and such medications as the nitrofurantoins and phenacetin. A procedure was sought which would give evidence of any possible mutagenic effect without recourse to time-consuming experiments on animals. Recent procedures for demonstration of carcinogenic preparations are divided into non-metabolising and metabolizing systems. Since, in carcinogenesis of the urinary bladder, secondary carcinogens must be reckoned with, the salmonella typhimurium test was used. The principle behind this test is the mutation (carcinogenically-caused) of histidine-dependent mutants of Salmonella typhimurium back to a wild type which is histidine-independent. The test strains used were TA-98, 100, 1535, 1537. The carcinogens studied were FANFT, DBNA, PADP and OADP. Urine samples from rats and dogs were analyzed following injection of the compounds. In comparison to the other test strains, TA 100 proved to be very sensitive to FANFT, DBNA and PADP, but showed no mutagenic effect with OADP. Accordingly, animals treated with OADP showed no urinary bladder carcinoma, even after three-year administration of this compound. On the basis of these results, clinical studies of urine samples from chain smokers, phenacetine abusers and urinary bladder cancer patients were carried out. The bacterial Salmonella typhimurium mutagenicity test was found to be an extremely sensitive technique for demonstration of a carcinogen in the urine.

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## Humoral Immune Response in Patients with Bladder Carcinoma

Ch. Chaussy, C. Hammer, Weinfurtner, F.W. Wieland and J. Schüller

There is good evidence that tumour antigens induce antobidy production in patients. Therefore we investigated the humoral immunoreactivity in patients with carcinoma of the bladder.

Single cell suspension of bladder tumours as well as released tumour cells harvested by flushing the bladder pre- and postoperatively were used for this study. After washing, the cells were incubated with autologous and allogeneic serum of patients suffering from bladder tumours and sera of healthy donors. The attached allogeneic and autologous tumour specific antibodies were stained by FiTc-labelled rabbit anti-tumour-IgG. 200 cells were counted. The positive cells were expressed in percent.

In all patients with carcinoma of the bladder, variable levels of tumours specific antibodies could be demonstrated by immunofluorescence. Small levels of specific antibodies could be demonstrated in patients with carcinoma in situ; however in all cases with invasive or metastatica carcinomas the levels were significantly higher. No reactions were found if sera of patients with other malignancies of the urinary tract or sera of healthy individuals were used. There was no significant difference, if cells were incubated with autologous or allogeneic serum. These findings strongly suggest that there is a common tumour associated antigen in bladder cancer.

In conclusion, this method may be helpful as a non-invasive way to diagnose tumour growth or postoperative recurrency of malignancy.

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## Investigations for Immunological Reacting of the Brown Pearce Carcinoma (BPC) in Rabbits

A. Hofstetter, D. Gericke, E. Keiditsch, Chr. Lersch and R. Böwering

The Brown Pearce Carcinoma (BPC) is a very aggressive tumour. Therefore it seemed to be interesting to use the BPC for testing immune responses in immunotherapy, in immunoprophylaxis and in vitro-vivo examination of sera from immunized rabbits or of spleen cells of such animals looking for humoral or cellular bound antibodies resp.

The cell material for immunisation was suspended in HBSS and incubated with 50 µg Mitomycin C pro $10^6$ cells. Then a part of these cells was modified by incubating them 30° at 37°C and pH 7.3 with 20-50 I.U. VCN/$10^2$ - $10^8$ cells.

The immunotherapy with vaccines consisting of Mitomycin C stopped and VCN modified tumour cells was ineffective in so far as all immunized animals at day 18 had bigger tumours than the untreated controls. The number of peripheral metastases was higher in the vaccinated groups also.

On the contrary the immunoprophylaxis was very effective. None of the animals prophylactically immunized with Mitomycin C stopped, and VCN modified cells showed tumours or vital tumour residues at day 30 after transplantation. All control animals have demonstrated progressive and metastasizing tumours. Looking for a possible mechanism of action it could be shown that tumour cells unmodified by VCN have not been attacked by the serum of animals immunized before. However when the tumour cells were modified by VCN before treating them with the "immune serum" the vitality of these cells was depressed. Furthermore it could be clearly demonstrated that the spleen cells of immunized animals were sensitized and killed tumour cells.

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## IX. Urolithiasis

### Influence of Alkaline Salts and Acetazoleamide on Urinary Excretion of Citrate and Oxalate in the Rat

M. Butz and A. Rost

Urinary citrate forms soluble calcium-ion complexes and thus inhibits the precipitation of calcium salts. In the majority of idiopathic calcium stone formers citrate excretion is decreased. A great increase in citrate excretion can be achieved by alkalosis. The effect of alkalizing therapy on urinary citrate and lithogenic compounds should be examined in the rat. 24 male Sprague-Dawley rats were divided into three groups according to the substance which had been added to drinking water: I Na-K-citrate (0.15 m/l), II Na-bicarbonate (0.15 m/l), III acetazoleamide (1 g/l). On the 3rd day of ingestion urine was collected for 24 h and the following parameters were measured: pH, oxalate, citrate and calcium.

In group I and II a 3-5 fold increase of citrate excretion was observed. Calcium and oxalate did not change significantly. In contrast group III revealed a decrease of citrate; calcium and oxalate remained unchanged.

It can be concluded from these data that a great increase of citrate excretion can be achieved by oral ingestion of alkaline salts without negative side effects on calcium and oxalate excretion. The production of alkalosis enhances renal citrate excretion by some unidentified mechanism.

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### In vitro and First in vivo Studies for Dissolution of Calcium Containing Urinary Calculi

R. Hartung, P. Leskovar, M. Simmel, R. Allgayer and M. Hropot

One of the most striking differences between recurrent stone-formers and healthy controls during a 6 week long-term study, was the significantly reduced urinary citrate in recurrent stone-formers. Citrate is the most important but not the only $Ca^{2+}$ - complexor in urine. Other tri- and dicarboxylic acids also lower the $Ca^{2+}$-level by binding the (bio)chemically and lithogeneously active ionized calcium as complex or as contact-ionic pair. Therefore, we were interested to heighten the lithoprotective capability of patients' urine both by stimulation of the tubular citrate secretion as well as by a direct renal secretion of orally given $Ca^{2+}$-complexing organic anions.

During the quantitative in vitro screening of di- and tricarboxylic acids and their salts with respect to their eventual inhibitory effect on growing stone-forming crystals we found that substances like tricarballylate, mesaconate, itaconate and malonate were highly efficaceous in reducing crystals in number and volume.

Our in vivo experiments on Wistar-rats showed that these substances increase - by their direct development in urine or by their stimulatory effect on the tubular citrate secretion - the $Ca^{2+}$-binding capacity. Our preliminary animal experiments show a clear increase of the tubular citrate secretion after the oral application of tricarballylate, malonate and oxalacetate/acetate. Both, the free acids and the corresponding alkaline salts proved to be efficaceous.

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### Breakdown of Oxalate (Ox) to Carbon Dioxide ($CO_2$) in Guinea Pig and Rat

V. Hagmaier, C. Bannwart, E. Truxius, G. Dietsche, K. Schmidt, H. Reber and G. Rutishauser

Oxalate plays an important role in the pathogenesis of calcium oxalate stones. However, only limited data are available on the breakdown of Ox to $CO_2$. Therefore we investigated the fate of Ox to $CO_2$ in guinea pig and rat.

The animals received a single oral dose of $^{14}$C-Ox and were placed over 24 h in a metabolic chamber suitable for collection of $CO_2$.

In the guinea pig the main route of metabolism of $^{14}$C-Ox was found to be respiratory exhalation as $^{14}CO_2$. Within 24 h 72% of the administered radioactivity was recovered as $^{14}CO_2$ whereby almost 70% of the dose was excreted during the first 8 h. Peak exhalation of $^{14}CO_2$ occurred between 3 to 4 h after dosage.

In contrast, the rat was found to excrete only 5.4% of an oral dose of $^{14}$C-Ox as $^{14}CO_2$. In conclusion, evidence is given for the existence of a species-specific difference in oxalate metabolism of guinea pigs and rats following oral application. Whether the metabolism of Ox to $CO_2$ is due to in vivo metabolism after absorption from the intestine or whether it is caused by microbial destruction should be discussed.

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### Investigations for Characterizing Single Crystal Phases in Urinary Stones by Means of an Arrangement of Light Microscopy in Combination with Scanning Electron Microscopy

A. Hesse, W. Hicking, D. Bach and W. Vahlensieck

With modern methods of urinary stone analysis — X-ray-diffraction and infrared spectroscopy - it is possible to characterize the urinary stone composition which will be destroyed with the chemical analysis. The composition of single crystals and crystal aggregates, however, can not be determined with these methods.

Structures and optical properties of single crystals can be studied by means of different light microscopic methods (phase contrast microscopy, polarized light microscopy, interference contrast microscopy) in thin sections and urinary sediment.

For the determination of the chemical composition of these crystals we used an arrangement of light microscopy combined with scanning electron microscopy in our present investigation in combination with X-ray analysis (Fa. Leitz, Wetzlar).

In thin sections of Ca-oxalate-stones with different phosphate portions the distribution of Ca, P and Mg could be coordinated to characteristic crystal structures. Also the composition of single crystals in urinary sediment could be determined with this method.

The investigation of different zones of growth in urinary stones and their exact chemical composition are of great importance for the characterization of the causal factors for stone-formation - supersaturation of the urine, formation of crystals, aggregation, fixation and stone growth.

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### Generation of Shock Waves for the Disintegration of Renal Calculi Using a Linear Electroacoustic Energy Converter in a Closed Reflector System

G. Konrad, M. Ziegler, Th. Gebhart, E. Häusler, U. Kaspar-Sersch, L. Stein, H. Wurster and W. Krauss

Calculi of the kidney may be broken up experimentally into small fragments without direct contact by means of shock waves. These shock waves are generated in a closed, liquid-filled reflector system and are radiated through the intermediate tissue.
Previously, a wire held by a bracket in the focal line of an elliptical-toroidal reflector was used as electroacoustic energy converter. This wire has been replaced by a spark-gap array. A large number of spark-gaps are held in the focal line of the reflector by means of a bracket. This arrangement allows for the generation of a sequence of shock waves. Single impulses of high energy (typically 100 Joule, 60 kV, 1 kbar) as well as sequences of impulses of low energy (60 Joule, 120 kV, 300 bar), separated by 0.8 seconds, can be applied to the kidney stones. With increasing array length higher voltages must be used.

In vitro experiments indicate that a single high-energy pulse has a different effect on the destruction of renal calculi from a sequence of low-energy pulses. The size of the resulting fragments also varies with the chemical composition of the calculi for shock waves of a given energy.

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## X. Prostatic Carcinoma

### Comparing Cell Kinetic Studies of the Effect of Ftorafur and 5-Fluorouracil on L 1210 Ascites Tumour Cells

W. Jellinghaus, G. Weis, V. Müller and B. Schultze

Ftorafur (FT) is considered to be a depot form of 5-Fluorouracil (FU) which is continuously released from FT by microsomal hydrolytic action. FU inhibits the DNA synthesis by blocking the enzyme thymidilate synthetase.

The following questions were presently studied: 1. Does the protracted release of FU from FT lead to a depot-like effect of the drug? 2. Can the FT-dose therefore be reduced? 3. Are these less toxic side effects with FT than FU?

Previously, cell kinetic studies of the effect of FU on L 1210 ascites tumour cells have shown that 3 µg/g FU results in an almost complete inhibition of DNA synthesis and a decrease of the mitotic index (MI) to zero (Urol. Res. 7: 39, 1979).

A comparison of the effect of FU and FT has shown that only beyond a dose of 100 µg/g FT a slight and transitory decrease of the MI was observed. With 300 µg/g FT the mean grain count per nucleus decreased sharply to about zero for 5 to 10 hrs. Strikingly, the MI is less reduced after 400 and 600 µg/g FT (self-limiting effect of FT?). Concerning the side effects a drastic decrease of the temperature of the animals from 39 to 32°C within 1 h after 300 µg/g FT was prominent. This was not seen with FU.

Even a FT dose 60 times equimolar of FU leads to only part of the cell kinetic effect of FU. A depot-like effect of FT could not be confirmed. FT has side effects not related to the release of FU. Thus, cell kinetic studies are suitable to investigate the mechanism of action of cytostatic drugs.

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### An "Indicator" Cell for the Grading of Prostatic Carcinoma

P.J. Spaander, H.J. de Voogt, D.J. Ruiter, J. Hermans, J. Brussee and M.E. Boon

To facilitate objective discrimination between the cytological malignancy grading of prostatic carcinoma a morphometric parameter was tested with regard to prognosis. Smear preparations from 12 patients with cytologically proven and histologically confirmed prostatic carcinomas were graded by Esposti's criteria. Using a graphic tablet the nuclear contours of 20 of the largest carcinoma cells were then measured by several observers. From these measurements the mean nuclear area was calculated. Some of the observers could discriminate cell populations considerably better than others. This was found to be due to the inclusion of large bare-looking atypical nuclei which were considered by some of the observers as degenerated cells and not suitable for counting. The ultrastructural integrity of these cells was subsequently verified by transmission electron microscopy using an open face embedding technique (1).

Occurrence of these cells in the smear preparations of 22 patients with histologically and cytologically proven prostatic carcinomas was then measured. We also considered the age at time of diagnosis, the survival time and the cause of death as being to progressive therapy resistant carcinoma or other causes. We found that death due to progressive therapy resistant carcinoma was confined to grade II and III. There was considerable variation in survival in all of these patients. But with mean nuclear area we were able to divide our patients in two groups with all deaths due to progressive therapy resistant carcinoma in one of these and with less individual differences in survival in both groups.

This pilot study suggests that mean nuclear area in combination with cytological grading of prostatic carninoma could be useful in assessing prognosis. Prostatic carcinoma cells with large bare-looking atypical nuclei are very useful in this respect and can serve as indicators of a diminished prognosis.

### Hormonal Parameters in Plasma of Men with and without Benign Prostatic Hypertrophy

H. Becker, H. Herrmann, W. Bartsch and M. Krieg

The growth of benign prostatic hypertrophy (BPH) starts at about the age of 40. To prove the theory that BPH is induced by hormonal imbalance with increasing age, we measured blood hormone levels (testosterone, 5 α-dihydrotestosterone, 5 α-androstan- 3 α, 17 β-diol, estradiol, FSH, LH, prolactin and Sex Hormone Binding Globulin) in 128 men with and without BPH in different age groups (36 - 45 years, 46 - 55 years and 56 - 65 years).

Endocrine parameters did not differ in men with and without BPH in corresponding age groups. The older men in the group free of BPH had a singnificantly lower 5 α-androstan - 3 α, 17 β- diol level than the younger ones. In the other group with BPH the age dependent decrease was only slight and not significant.

In the BPH group the 5 α-dihydrotestosterone concentration in plasma was significantly higher in men at 60 compared to men at 50.

These findings support the thesis that the growth of benign prostatic hypertrophy is not explained by an alteration of plasma hormones but probably by an intracellular decrease of 3 α-hydroxy-dehydrogenase activity, resulting in an increase of 5 α-dihydrotestosterone. The increase of 5 α-dihydrotestosterone in the periurethral tissue is probably one of the most important factors which stimulates the growth of the benign prostatic hypertrophy.

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## Prolactin-Androgen Interrelationships in Patients with Prostatic Cancer

G. Janetschek, U. Cordes, U. Wenderoth, U. Krause and G.H. Jacobi

In numerous animal experiments it has been shown that prolactin increases androgen production in the testis and adrenal. On the other hand, there is also a direct effect at the prostatic cellular level. The goal of this study was to investigate whether these prolactin effects on the testis were also present in patients with prostatic cancer. In 13 patients with newly-diagnosed untreated prostatic cancer an LH-RH/TRH stimulation test was done initially and after a bromicriptine-induced hypoprolactinaemia. In 6 of these patients the test was repeated after oestrogen treatment. Testosterone, prolactin, LH and FSH were determined. In another group of 9 patients, the effects of a chlorpromazine-induced hyperprolactinaemia and a bromocriptine-induced hypoprolactinaemia on serum testosterone response to HCG was studied after suppression of the adrenal with dexamethasone.

Bromocriptine treatment resulted in a significant prolactin suppression; the response to LH-RH/TRH was decreased. No changes in the plasma testosterone levels could be seen. Subsequent oestrogen treatment produced a significant decline of plasma testosterone; basal prolactin rose and the prolactin response to LH-RH/TRH was potentiated, but no stimulatory effect on plasma testosterone could be seen. After chlorpromozine, the prolactin level doubled. Bromocriptine resulted in a significant decline. Changes of the plasma testosterone levels were not seen in either group. In man, changes of the prolactin level do not alter plasma testosterone. The extraprostatic effects of prolactin observed in animal experiments do not appear applicable in humans.

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## Steroid Metabolism in Patients with Prostate Cancer Treated with Estramustine Phosphate

U. Wenderoth, K. Pollow, J.E. Altwein and G.H. Jacobi

The mode of action of estramustine phosphate (Estracyt) in patients with prostate cancer is thought to be mainly related to the effect of its cytotoxic ligand (nitrogen mustard) after being carried to the prostate by the oestrogen component (oestradiol phosphate). However some oestrogenic side effects have been observed after Estracyt suggesting its degradation prior to uptake into target tissue.

12 patients with advanced untreated adenocarcinoma of the prostate, average age 67 years, were given 300 mg Estracyt i.v. per day over 5 days. The following parameters were determined before and after treatment: testosterone, oestradiol, oestriol, and prolactin in serum by RIA, total oestrogens in a 24-hrs urine specimen, and testosterone plasma kinetic parameters (volume of distribution, metabolic clearance rate, biological half life, production rate).

Serum testosterone dropped from 438 before to 312 ng/100 ml after treatment (n.s.). Oestradiol increased from 51 to 2103 pg/ml, oestriol from 0,9 to 5,3 pg/ml ($p<0,001$). Prolactin rose from 8,7 to 12,6 ng/ml (n.s.). Oestrogen excretion in urine increased to a mean value of 819 µg/24 hrs after Estracyt (normal value 17 to 20 µg/24 hrs). Testosterone plasma kinetic parameters did not change significantly.

Estracyt has no significant short-term oestrogenic action in the dosage applied due to rapid hepatic degradation reflected by high urinary excretion.

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## Determination of Androgen Receptors in Prostatic Tissue

W. Roos, V. Hagmaier, J. Torhorst, G. Rutishauser and U. Eppenberger

Androgen receptor has been measured in the cytosol and KCl-extracts from human benign hyperplastic prostatic tissue. Assays were done with a dextran-coated charcoal technique using 5 concentrations of [$^3$H]-methyltrienolon in the presence of triamcinolon-acetonid. Incubations were carried out for 20 hours at 4°C. Non-specific binding was determined by addition of a 100-fold excess of unlabelled methyltrienolon.

All samples contained androgen receptors. The concentration measured in the cytosol was 8 ± 3 fmol/mg protein (132 ± 65 fmol/mg DNA; n=8). Extraction of the tissue in the presence of 0.6 M KCl results in fourfold higher values (29 ± 11 fmol/mg protein; 568 ± 310 fmol/mg DNA) indicating that the majority of the androgen receptor is in the nuclear compartment. Experiments were undertaken to enrich the epithelial fraction from benign hyperplastic tissue by use of a garlic squeezer. The results indicate that the receptor concentration of the epithelial fraction is higher than in the whole tissue samples.

From this we conclude: The presence of androgen receptors in benign hyperplastic tissue impedes the interpretation of receptor measurements in prostatic carcinomas. This because carcinomas are generally interspersed or surrounded by hyperplastic tissue. Only the definition of normal ranges for benign hyperplastic tissue would give the necessary impact for a clinical evaluation. We propose not to measure the cytosolic receptor but the total receptor content, including the nuclear receptor by extraction at high salt concentration.

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On the Diagnostic Significance of the Histochemical Demonstration of Aminopeptidase in Prostatic Neoplasms

A. Feustel and F. Wohlrab

Kirchheim (1967) described the LAP as a marker enzyme in the diagnostic field on the differentation between malignant and non-malignant neoplasms in the human prostate glands. By reference to this is the absence of LAP-staining in malignant prostate tissue. Our investigations on sections of benign hyperplasia and cancer with the membrane incubating technique and with some coupling salts (pararosaniline, fast blue BB) demonstrated a different staining in hyperplastic glands. The LAP-staining was not observed by the poorly differentiated adenomatous cancers.

Our investigations suggest that the LAP staining reaction seems to depend on some methodological factors. Furthermore we conclude that different LAP enzymes (isoenzymes?) in the glandular cells of the human prostate are present.

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Further Characterization of the Effector Cell Responsible for in vitro Destruction of Cultivated Cells from Human Prostatic Carcinoma

R. Ackermann, L. Hasler and M. Wirth

In earlier experiments it has been shown that cellular cytotoxicity of patients with CaP as measured by cytolysis of EB 33 target cells correlates with the extent of the tumour inasmuch as high grade of cytotoxicity was observed only when CaP was confined to the gland. In order to define a possible immunological host-tumour interaction in vivo further characterization of the type of effector cell responsible for this in vitro killing has been attempted. Effector cells could be either tumour specific sensitized T-cells, K-cells, macrophages or NK-cells. K-cell activity can be excluded, since EB 33 specific antibodies are not present when cytotoxicity is estimated with $^{51}Cr$-release assay. Macrophages do not participate since removel of this type of cell by nylon wool passage results in an increased cytolysis of EB 33 cells. Enhanced cytolysis of EB 33 target cells can be achieved by preincubation of the effector cells even from normal donors for 18 h on EB 33-monolayers. It is concluded that this observation reflects an increased NK-activity. A high grade of NK-activity in patients with localized CaP may therefore indicate that some CaP are capable to induce NK-acitivty. Alterantively NK-activity may be stimulated by all CaP, depending on the extent of the tumour lesion.

Whether sensitized T-cells participate on these reactions is yet unclear.

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## XI. Prostatic Carcinoma and Benign Prostatic Hyperplasia

A New Approach to Purify Prostatic Acid Phosphatase

J.M. Köhler, A. Biber and R. Ackermann

A method is presented which allows a rapid, simple and pure preparation of prostatic acid phosphatase.

Ejaculate was pooled and centrifuged for 10 min at 4000 g at 2°C. The supernatant was used for further purification. Samples of 4 ml were chromatographed on an ULTROGEL AcA 44 column (3,5 x 90 cm). Elution was carried out with ammonium acetate buffer pH 4,8 at 4°C. The flowrate was 20 ml/hr and 6 ml fractions were collected.

Seven protein peaks were recovered from the column and each peak was assayed for acid phosphatase activity with p-nitrophenylphosphate as substrate. The enzyme activity was concentrated only in the second peak. An SDS gel electrophoresis of freezing-dried fractions of this peak showed only one protein band, corresponding with the acid phosphatase activity.

Next aim will be the production of monoclonal antibodies against prostatic acid phosphatase with the purified enzyme as antigen.

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Different Radioimmunoassays (RIA) in Comparison to the Enzyme Test for Prostatic Acid Phosphatase (PAP) in the Diagnosis of the Prostatic Carcinoma

P.R. Huber, V. Hagmaier, A. Scholer, H. Vogt, W. Weber, E. Linder, E. Viollier and G. Rutishauser

The RIA-technique was applied to measure the prostatic acid phosphatase (PAP) concentration using three commercially available kits (Clinical Assays (CA), Mallinckrodt (Mall), New England Nuclear (NEN)) and was compared to the enzymatic test system using thymophtalein monophosphate as substrate. Serum and bone marrow aspiration samples of patients suffering from prostatic cancer and other illnesses requiring a bone marrow aspiration (controls) were tested for detectable PAP. Both types of assay were performed in the same sampling and storing conditions. Depending on the method applied the RIA-tests performed somewhat better in their property to identify elevated levels of PAP in serum and bone marrow. In untreated cancer patients (all stages combined) 13 (27) in the enzyme test, 13 (27) in the NEN-RIA, 7 (11) in the Mall-RIA, 11 (12) in the Clinical Assays -RIA had elevated PAP-concentrations ([PAP]> $\bar{x}$ + 1 SD). Early detection: TO - T2 (all subclasses included) was possible in 11 (11), 6 (10), 2 (14), 2 (14) cases with Clinical Assays, Mallickrodt, NEN, enzyme test (in thar order). Bone marrow-PAP was elevated in 6 (6) Mallinckrodt, 6 (6) enzyme and 4 (5) in Clinical Assays samples.

Comment: Sample number has to be increased to substantiate these preliminary findings of partial superiority of the RIA over the enzyme test.

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## Transplantable Human Prostatic Carcinoma on Nude Mice — A Tumor Model of Clinical Interest: PC 82

W. Hoehn, F.H. Schroeder, J.F. Riemann, A.C. Joebsis, P. Hermanek and R. Walther

Until 1978 there was no suitable tumor model of human prostatic carcinoma. Then two serial transplantable nude mouse tumor lines, maintaining original moderately differentiated histology, prostatic acid phosphatase and androgen dependence, were developed by Reid and co-workers (1) and by Hoehn and Schroeder in Rotterdam.

Tissue fragments of a cribriform prostatic carcinoma were transplanted into Balb c-nude mice in July, 1977. 4 to 6 months after grafting, the harvested tumors consisted of cribriform carcinoma similar to the origin and a serial transplantable line could be established.

Further characterization resulted in a constant slow growth, the tumor diameter doubling in 4 weeks. The histology is very similar to the original carcinoma even in the present 10th passage. Electron microscopy revealed typical structures of prostatic carcinoma. The tumor contains rich amounts of immunologically proved prostatic acid phosphatase. Chromosome studies showed a human diploid pattern. PC 82 did not proliferate on female mice; castration or oestrogen treatment of tumor bearing male mice results in slight volume regression and histologically in flat epithelium, vacuolized cells and widened acinar lumina.

These results suggest that PC 82 is quite similar to the original tumor, and can be used as a tumor model for human prostatic carcinoma, especially for various treatment experiments.

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(1): Reid L, et al., Proceedings of Am. Ass. Cancer Res., AACR abstracts 19: 151 (1978)

## Results of Routine Transplantation of Human Prostatic Carcinomas on Nude Mice Attempting Serial Transplantable Lines

W. Hoehn, P. Hermanek, R. Walther and F.H. Schroeder

Prostatic carcinomas grown on nude mice can represent a valuable model for various experiments.

Tissue fragments were available from operative specimens. Pieces of about 2x2x1 mm size were grafted subcutaneously. The animals were observed until they became weak. In the case of tumour growth, serial transplantation was attempted. If only fibrous tissue was found, we looked for tiny soft masses which would consist of new grown carcinoma, and we tried to graft these on further mice.

In 1977, only the line PC 82 could be established, which grew after 8 weeks without problems and is described above. In 1978, after 9 months of first passage PC 92 reached the size of a pea, the tumour consisting of solid and cribriform masses similar to the original tumour. Up to now PC 92 fragments grew extremely slowly and could not be established. From the 1979 series, 6 xenografts can be reviewed. Two G3-carcinomas were resorbed. 1 G1, 1 G2, 1 G3 ca. kept vital foci in the first or a later passage, but until now have not proliferated. 1 G3/G2 carcinoma showed new grown cribriform masses after the 5th passage within 15 months on the mice.

We concluded to keep even fibrous loocking residual tissue fragments as long as possible on the mice. The number of available established lines may be increased using meticulous techniques. We could not conclude a selection for undifferentiated carcinomas in the nude mice.

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## Androgen-Induced Growth of the Prostate Gland

R. Stiens and B. Helpap

Increase in weight of the accessory reproductive glands of castrated rodents is a sensitive biological test for androgens. However, the hyperplastic and hypertrophic processes involved have been scarcely investigated. Thus, weight determinations, histological, nuclear morphometric and 3H-thymidine autoradiographic studies were performed on the prostate gland of castrated and normal rats of various ages after multiple interrupted application of testosterone. Androgen administration caused cell increase in all prostate lobes and was most marked in the coagulating gland. Following a latent period of 35-40 hrs the 3H-labeling index and mitosis index increased, with a maximum on day 3. Values then fell and were minimally above control values. The increased cell proliferation was essentially due to an increase in the growth fraction. Rhythmically interrupted administration of testosterone resulted in a decreased ability to stimulate cell proliferation. Cellular increase was detected only in previously castrated animals. Epithelial reduction was found in control animals. The prostate gland belongs to the group of stable tissues. The low proliferative activity can be induced by suitable manipulation. This androgen-induced cell proliferation is a useful model for testing drugs to inhibit proliferation and is, along with the determination of the cell loss index, of clinical importance in investigating the hormone responsiveness of the prostate carcinomas.

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## Regressive Change in the Prostate Gland After Withdrawal of Androgens (Studies on Experimental Animals)

B. Helpap and R. Stiens

The individual processes involved in involution of the prostate gland following androgen withdrawal are incompletely understood. The rat prostate was used to investigate cell loss (numeric atrophy), decrease in cell size (simple or cellular atrophy) and altered cell proliferation. The prostate gland was studied at various times after castration using histological, nuclear morphometric and 3-H-thymidine autoradiographic methods, as well as by measuring weight. Cell loss was determined by the proportion of apoptosis-bodies. Following castration a decreased formation of secretions and reduction in interstitial tissue fluid were observed, as well as reduced cell proliferation, which did not affect the weight, as the normal cell proliferation in all lobes of the prostate is extremely small. The nuclear size fell to 30%. The

apoptosis index, i.e. cell loss, was considerable and reached a maximum in the coagulating glands on day 2, and in the other prostate lobes between day 4 and 8. After this time cell loss corresponded to that of control animals. Cell loss measured by apoptosis has proved to be a reliable parameter of hormone-dependent regression of the prostate. The responsiveness of prostate carcinomas to hormone treatment can be tested using alteration in the apoptosis index within 7 days.

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## Stereological Quantification of Antiandrogenic Effects on Epithelial and Stromal Proliferation in Experimentally Induced Canine Prostatic Hyperplasia

U.W. Tunn, Th. Senge, H.U. Schweikert, B. Schüring and F. Neumann

The effect of the antiandrogen cyproterone acetate (CA) on experimentally induced prostatic hyperplasia (PH) in the castrated dog was investigated by stereological analysis.

22 male beagle dogs were used for these studies and, following castration, were split into groups and treated with 3α-, 17β-androstanediol (Adiol) 17β-oestradiol ($E_2$) and CA over a period of 6 months.

The histomorphology reveals group-specific differences at the level of stroma and epithelium. The descriptive morphological findings can be shwon quantitatively by stereological analysis. Two different types of prostatic hyperplasia were established. The administration of Adiol brings about an absolute increase of glandular parenchyma. After combined use of Adiol and $E_2$ stromal proliferation is induced, while glandular epithelium is replaced by squamous metaplasia, which corresponds to the absolute volume of acinar epithelium in the controls. In both types of PH, CA causes a significant reduction of the absolute and relative volumes of the acinar parenchyma compartment. Furthermore, the absolute volume of the stroma decreases significantly in Adiol plus $E_2$ plus CA treated dogs compared to the Adiol plus $E_2$ treated group.

CA prevents the development of PH by means of an atrophying effect on the epithelium. Furthermore, the stroma-proliferative effect of androgens, which potentiates the oestrogenic effect, can be blocked by CA.

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## Effects of Tamoxifen on Experimentally Induced Canine Prostatic Hyperplasia

P.-J. Funke, U.W. Tunn, Th. Senge, F. Neumann and B. Schenck

The effect of tamoxifen (TA) on experimentally induced BPH in castrated dogs was investigated by histological, histochemical and biochemical examinations. 23 pure bred male beagles were used in this experiment. After a three dimensional caliper measurement of the prostate and after castration the dogs were randomized in groups. Group I was kept as a castrated control. Group II received E2, group III E2 and TA, group IV additional 3a-diol, and a last group consisting of three intact animals was used to examine the antigonadotropic properties of TA. After a 6 months treatment with E2 a significant weight increase of the prostate occurred. Histologically a squamous metaplasia and cystic hypergplasia could be seen. Activation of the fibromuscular tissue was obvious. Slight activities of acid phosphatase and zinc staining could be observed in the metaplastic transformed epithelium cells. Biochemically a rise of RNA and a significant increase in the RNA/DNA ratio was measured, while the zinc content of the gland corresponded to values of the castrated control. The weight increase and the histological changes could be suppressed by combined treatment with TA. Biochemical determinations and the histochemical findings revealed an effect simular to castration. In the oestrogenized and androgenized animals the E2 induced alterations could be abolished by TA, the 3a-diol depending glandular proliferation dominated.

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