In comparison with the normal controls, the testis and seminal vesicles of the chlorpromazinized rats showed atrophic changes. The histological picture of the testis was similar to that seen after hypophysectomy.

Hypertrophy of the adrenal cortex[1], as well as the pseudopregnancy state[2,3], has been noted after the administration of chlorpromazine in rats. Competitive gonadotrophin inhibition by an excessive production of ACTH in reserpinized (tranquilized) rats[4], and the blockade of the pituitary gonadotrophin release after chlorpromazine administration[5] have recently been discussed. Therefore, in this instance, the atrophic changes of seminal vesicles and testis with aspermatogenesis may be concluded to be due to the blockade of the release of pituitary gonadotrophin[6].

Zusammenfassung. Die Wirkung von Chlorpromazin auf die Hodenfunktion der Ratte wurde untersucht und dabei gefunden, dass Chlorpromazin eine Degeneration des Rattenhodens verursacht.

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[6] The author expresses his deep appreciation for the encouragement of Dr. S. R. MAITRA, Head of the Department of Physiology, Calcutta University. – Protovit (Roche) was kindly furnished through Roche Products Limited, Bombay (India).

PRO EXPERIMENTIS

A Technique for Successful 20 h Kidney Storage at 4°C

A technique for storing kidneys for 6 h at 4°C has been reported by DEMPSTER, KOUNTZ, and JOVANOVIC[1]. It was considered that with the same technique an extension of the storing period could be achieved. It was disappointing to find, however, that the technique which was suitable for 6 h storage was not consistently successful for 20 h storage. This communication describes the modifications required in our hands for 20 h storage at 4°C.

It was soon apparent that kidneys stored for 20 h in a cold room at 4°C were much firmer than after 6 h storage. It occurred to us that the problem of overcoming vascular spasm due to prolonged cold required more vigorous attention. Furthermore, it was considered possible that an excess of lactic acid may accumulate over a period of 20 h and accentuate vascular spasm. This obliged us to control the pH of all the fluids used in the technique.

Materials and methods. Greyhounds of weights varying between 20–25 kg were used. Kidneys were autotransplanted to the iliac vessels by a technique described previously by DEMPSTER[2]. The contralateral normal kidney was left in situ and was removed three weeks after the procedure involving storage and transplantation.

The pH of all solutions was measured. All solutions were buffered by bicarbonate to a pH of about 7.4 and contained 0.5 g streptomycin. An aliquot of the flushing solution was stored with the kidney at 4°C for 20 h. The pH of the flushing fluid after 20 h storage fell to 7. The vasodilators used were: 0.01 mg papaverine sulphate, 20 mg procaine hydrochloride, and 5 mg dipyridamole ('Persantin').

7 groups of experiments were performed:

Group 1. Six kidneys were stored for 20 h at 4°C and transplanted using the technique for storing for 6 h (DEMPSTER, KOUNTZ, and JOVANOVIC[1]). Briefly, this consisted of flushing by gravity the isolated kidney with a plasma-rheomacrodex-(Pharmacia Ltd.)-vasodilator solution at 37°C prior to and after the cooling.

Group 2. Five kidneys were stored for 20 h at 4°C and transplanted using the technique for storing for 6 h with the following modification: after the rewarming process and prior to performing the anastomoses, 9 ml of rheomacrodex and 1 ml of Novocaine at 40°C were injected up the renal artery and held in the kidney by placing a bulldog clamp on both the renal artery and vein.

Group 3. Five kidneys were stored for 20 h at 4°C and transplanted using a modification of the method used in Group 2: prior to the anastomoses 12 ml of rheomacrodex and 1 ml each of Novocaine, papaverine, and Persantin were injected up the renal artery and retained in the kidney.

Group 4. Four kidneys were stored for 20 h at 4°C and transplanted using the following modification: the kidney was flushed with fluid in a 20 ml syringe instead of flushing by gravity. The fluid consisted of rheomacrodex, plasma, and Novocaine 12:7:1. On removal from the cold room the kidneys were flushed by syringe with fluid at 4°C.

Group 5. Four kidneys were stored for 20 h at 4°C and transplanted using the same technique as was used in Group 2 but with the plasma omitted. Only rheomacrodex was used for flushing and only Novocaine was used as a vasodilator.

Group 6. Four kidneys were stored for 30 h at 4°C and transplanted using the following modification: as for Group 5 but with the fluids buffered to pH 7.4. The pH of rheomacrodex, Novocaine, papaverine sulphate, and Persantin at room temperature is 4.8–5.8.

Group 7. Ten kidneys were stored for 20 h at 4°C and transplanted using the following modification: all fluids used were buffered to pH 7.4. A total of 150 ml of fluid was used to flush out the kidney, i.e. a 1:3 plasma:rheomacrodex solution with 1 ml of Novocaine at 37°C. By placing bulldog clamps on both artery and vein at the end of the flushing, the kidney was left distended with this fluid. A similar solution was used to re-warm the kidney but with papaverine and Persantin added. Finally 9 ml of rheomacrodex and 1 ml of Novocaine at 40°C were

[1] W. J. DEMPSTER, S. L. KOUNTZ, and M. JOVANOVIC, Br. med. J. *1*, 407 (1964).
[2] W. J. DEMPSTER, J. Physiol. *124*, 15P (1954).

injected up the renal artery and held in the kidney by bulldog clamps on renal artery and vein.

Surgical details. Just prior to the anastomoses, care must be taken to remove a liberal portion of the end of the artery and vein which have been compressed by bulldog clamps during 20 h storage. If this precaution is not taken thrombosis will develop (see Group 7). During the anastomoses a 2% mannitol solution was given intravenously. 2 h after the operation 150 ml of rheomacrodex were given intravenously. Rheomacrodex given in this quantity during an operation on a dog can cause fatal bleeding.

Results. A functioning kidney in these experiments is defined as one which can maintain, on its own, normal health.

Group 1. 6 kidneys cooled and transplanted: 1 functioning kidney.
Group 2. 5 kidneys cooled and transplanted: 3 functioning kidneys.
Group 3. 5 kidneys cooled and transplanted: all failed to function.
Group 4. 4 kidneys cooled and transplanted: 2 functioning kidneys.
Group 5. 4 kidneys cooled and transplanted: 1 functioning kidney.
Group 6. 4 kidneys cooled and transplanted: 1 functioning kidney.
Group 7. 10 kidneys cooled and transplanted: 3 instances of arterial thrombosis, 7 consecutive functioning kidneys.

Functional studies. The general pattern of function from the third week (when the contralateral nephrectomy was performed) is constant and resembles the natural history of the fresh autotransplanted kidney (DEMPSTER et al.[3]). The cooled kidneys take longer to return to normal function. There is a phase of polyuria and hyposthenuria during which the blood urea usually rises for about a week and then starts to fall. The peak level of the blood urea may be about 180 mg/100 ml. By the 8th week after cooling and transplantation the concentrating capacity usually starts to return to the lower limits of normal for the dog (1.030) and the blood urea falls progressively. The blood urea falls to the preoperative level by the 10th week. At this stage the osmolality returns to the range of 1600–2600 mosmol/l and the urine urea concentration to the region of 5000 mg/100 ml; these are normal ranges for unilaterally nephrectomized dogs. In the series of seven kidneys which were successful in Group 7, two failed to regain concentrating power; the blood urea fell to preoperative levels but polyuria persisted. Sometimes, in our experience, even fresh kidneys autotransplanted to the pelvis fail to regain concentrating power and remain polyuric (DEMPSTER et al.[3]). The microscopical features do not suggest an obvious cause of the failure to concentrate but a persistently residual dilatation of the ureter in these cases could account for it.

Discussion. Several storage techniques can produce an occasional successful result. To be of any real value a preservation technique must be consistently successful in the hands of several surgeons. In the present experiments, the only technique which produced consistent results was that used in Group 7.

All the kidneys which functioned after storage for 20 h did so moderately well by the third week and thereafter improved progressively. The dogs were able to eat a normal diet and appeared in every way normal.

The principles to be observed in storing kidneys so as to obtain good function after transplantation appear to be the following: (1) Large volumes of plasma should be avoided because of the possibility of ill-understood immune reactions; rheomacrodex should be substituted. A mixture which avoids deleterious effects is a 1:3 plasma:rheomacrodex solution of 150 ml. (2) Solutions for flushing the kidneys should be buffered to about pH 7.4. (3) Cold solutions should be avoided when flushing the kidneys. (4) The kidneys should be warmed after cooling by flushing with a plasma:rheomacrodex solution containing vasodilators at 37°C. After this a warmer solution of rheomacrodex and Novocaine at 40°C should be injected by syringe and retained in the kidneys by placing bulldog clamps on both renal vessels. The injection should be done with moderate pressure to ensure that all sections of the renal vascular tree are brought into contact with the warm solution and its vasodilator. It is apparent, however, from the scattered foci of calcium deposits in a few preserved kidneys that some areas have been inadequately perfused. Further refinements to achieve this end are desirable. (5) The new blood supply should be established slowly so as not to overload the juxta-medullary circulation (DEMPSTER et al.[1]).

The causes of failure in these experiments were the following: 1. *Arterial thrombosis.* When bulldog clamps are kept on the renal vessels during the 20 h storage, it is essential that prior to the vascular anastomoses a liberal area at the end of the vessels should be cut away. If cadaver kidneys are used, however, damage to the renal vessels can be avoided by cannulating a portion of the aorta and vena cava above and below the origin of the renal vessels.

2. *Immediate failure to perfuse on opening up the new circulation.* This was particularly marked in Group 4. Cold flushing solutions were used. It is reasonable to suggest that inability to perfuse was due to intense cortical spasm.

3. *Medullary damage.* Excluding vascular thrombosis in 3 cases, all kidneys which failed to function suffered medullary damage. This, we believe, is due to overloading the juxta-medullary circulation when establishing the new circulation. Details of this concept were explained in a previous article (DEMPSTER et al.[1]).

It should be stressed that autotransplants were used in these experiments. The main difference between the preservation of fresh and cadaver kidneys is the length of the pre-cooling period. For fresh autotransplants this interval can be reduced to about 10 min. When cadaver kidneys are used the duration of the pre-cooling period can vary enormously and this is crucial to any method of preservation. Only experience can decide how long the pre-cooling period can be extended so as not to interfere with adequate recovery of function after homotransplantation.

An anuric preserved homotransplanted kidney can be rather difficult to manage. It is essential to dialyse patients repeatedly so as to give the best chance for tubular recovery. The administration of anti-metabolic drugs does not appear to interfere with recovery in all cases. However, a rejection process can occur in an anuric homotransplanted kidney and this can be a very difficult complication to manage. To avoid dialysing the animals in our experiments the contralateral kidney was not removed until three weeks after the cooling procedure. These results indicate that consistent return to good function after storage for 20 h at 4°C is not dependent on

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hyperbaric oxygen (MANAX et al.[4]) or perfusion techniques (HUMPHRIES et al.[5]; HITCHCOCK et al.[6]; DUNEA et al.[7]). The low temperature appears to be the important factor[8].

Zusammenfassung. Es gelingt die normale Nierenfunktion des 20 h bei 4°C tiefgekühlten Organs durch Perfusion mit Novocain und Reomacrodex von 40°C wieder herzustellen.

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STUDIORUM PROGRESSUS

The Structure-Antimicrobial Relation for Valinomycin Depsipeptides

We have recently shown by total synthesis that valinomycin[1], discovered in 1955 by BROCKMANN[2], is a cyclododecadepsipeptide (I) comprising 3 fragments of which each contains a D-valine, L-lactic acid (L.Lac), L-valine and D-α-hydroxyisovaleric acid (D.HyV) residue. Having available a general method for the synthesis of such cyclic depsipeptides[1,3–5] we were able to undertake the preparation of various valinomycin analogues in order to expand our investigations into the structure-activity relation of cyclodepsipeptides and also, in cooperation with B. C. PRESSMAN (USA), to attempt to shed some light on the mode of their action.

We synthesized a number of linear and cyclic analogues of valinomycin differing in the chain length or ring size and in the nature, configuration and position of the amino and hydroxy acid residues (II–XXXV in Table I). The antimicrobial activity of the compounds was investigated by the serial dilution technique on Gram-positive and Gram-negative bacteria and on yeasts (glucose-peptone medium), on acid fast bacteria (Soton's medium) and on phytopathogenic fungi (Chapek-Dox medium). All the compounds investigated were dissolved in DMF or Me_2CO, and successively diluted with water until a 2% aqueous DMF (Me_2CO) solution was reached. The results of tests of the valinomycin analogues are given in Tables II and III[6]. Moreover Table III presents for comparative purposes data on the synthetic enniatins A and B (XXXVI and XXXVIII), their analogues (XXXIX to XLI), sporidesmolide I (XLII) and serratamolide (XLIII), all of which had previously not been tested with respect to the aforementioned fungi.

Contrary to valinomycin (I), its linear tetra-, octa-, dodeca- and hexadecadepsipeptide analogues (II–V) are completely inactive. Regarding the activity of the cyclic analogues, this depends upon a number of factors which will be discussed in the following: (1) Similar to the enniatin cyclodepsipeptides[6], the size of the ring is here also of essential importance. All the cyclotetra- and cyclooctadepsipeptides (VI–XV) we investigated were found to be completely inactive, whereas many cyclododecadepsipeptides (XVI–XXIII) displayed considerable activity, which disappears again on passing over to the cyclohexadecadepsipeptide (XXXV). A marked difference between the valinomycin and the enniatin series is that optimum activity in the former is observed with the 36-membered ring, whereas in the latter it occurs with the 18-membered ring. (2) The structure of the radicals and the configurations of the amino acid residues in the valinomycin molecule can undergo considerable changes (true, on a limited section of the chain) without considerable loss in activity. Thus if a D-valine residue in valinomycin is substituted by a D-alanine (XXI), D-leucine (XX) or D-allo-isoleucine (XIX) residue or an L-valine residue is substituted by an L-allo-isoleucine (XVIII) residue, the activity and spectrum of the compound in general change little. Even inversion of the configuration of one of the valine residues (D to L or L to D; compounds XVI and XVII) leads only to partial and not total inactivation. Considerable retention of activity is observed also when, in one of the valinomycin fragments, the D- and L-valine residues are simultaneously replaced by D- and L-leucine (XXII) or D- and L-alanine (XXIII). However, when all the 6 amino acid residues of the valinomycin analogues are either only D- or L-valine (XXX and XXXI), or 3 D-valine residues are replaced by D-allo-isoleucine (XXXII), the compounds

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