testosterone concentrations in the plasma of the starved rats are significantly ($p < 0.01$) decreased. Under the maximal and unphysiological stimulation with HCG, both starved and control rats have greatly increased testosterone levels in plasma (table 1). This finding indicates that low plasma testosterone in starved rats is not primarily a consequence of impaired ability of the Leydig cells to secrete testosterone, but of the decreased stimulation by LH, the secretion of which is rather low during starvation[3,9,10].

It is surprising that the increase of testosterone was relatively and absolutely greater in the starved rats (table 1). Although we cannot explain this phenomenon at the moment, 2 factors may be responsible for the higher testosterone values in the plasma of the stimulated starved rats. First, we have observed a longer half-life of tritiated testosterone in the plasma of starved rats[11]. This finding means that the metabolic clearance rate is decreased and, as a consequence, the same production rate of testosterone would result in higher plasma levels. Second, Leydig cells may be more sensitive to stimulation with HCG. Since it is now well documented[12] that gonadotropins regulate the capacity and sensitivity of their gonadal receptors in the way that a higher LH secretion reduces receptor capacity and decreases the activity of the adenylate cyclase connected to this receptor, the low LH secretion in the starved rats may cause an increased sensitivity of Leydig cells to HCG.

The results of the measurement of capillary blood flow are listed in table 1. The data obtained in untreated control animals are in good agreement with the data of Joffre and Joffre[6], who observed an average of 240 µl/min/g, as well as with the results reported by other authors[13,14]. We observed a decrease in capillary blood flow in the starved rats by 33.5%. Stimulation with HCG did not reverse the effect of starvation. A similar observation was made by Setchel et al.[15], who studied the effect of chronic malnutrition on testicular function in the ram. Testosterone output, testicular weight and testicular blood flow were reduced. Oxygen and glucose uptake of testicular tissue was impaired. The explanation for this finding may be that the HCG acts rather specifically on the Leydig cells which occupy only about 4% of the total testes volume in this strain of rat[16]. According to Hardy and Scott[17], the capillary blood flow and the activity of cellular metabolism are closely related. We observed an increase in testosterone production, but not in testicular blood flow after HCG stimulation.

The secondary metabolic effects of testosterone on other testicular tissues, as for instance on spermatogenesis[18], may not become relevant after only a short stimulation with HCG as applied here.

In conclusion: incretory testicular function in starved rats is impaired primarily due to lack of stimulation by gonadotropins. A reduced capillary blood flow in the testes of the starved rats occurs as a consequence of malnutrition and may be responsible for a further impairment of testicular function.

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PRO EXPERIMENTIS

Unsuitability of urethane anesthetized rats for testing potential β-adrenoreceptor blockers

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Summary. Isoprenaline induced tachycardia in urethane, but not sodium barbital anesthetized rats depends upon resting heart rate values. This makes urethane anesthesia unsuitable for testing β-blockers.

Inhibition of isoprenaline-induced tachycardia (IIT) in anesthetized animals is often used to assess potential β_1-adrenoreceptor blockers. Preliminary experiments in our laboratory indicated that urethane-anesthetized rats had lower resting heart rate (RHR) and IIT than sodium barbital-anesthetized rats. In view of the above it appeared worthwhile to determine the influence of these 2 anesthetics on the relationship between RHR and IIT and to develop, if possible, a simple and suitable procedure for screening potential β-adrenoreceptor blockers in the rat.

Methods. Male albino rats, Wistar-Morini strain, weighing 350 g were anesthetized with urethane (1.2 g/kg) or sodium barbital (200 mg/kg) given i.p. In some experiments rats were pretreated with i.p. reserpine (5 mg/kg), 48 h before induction of surgical anesthesia. The right carotid artery and left jugular vein were cannulated for blood pressure recording and isoprenaline injection respectively. Heart rate was measured by coupling a DC Counter MARB 80Cl to a 8805B Hewlett Packard carrier preamplifier connected with the pressure transducer. After a 10-min stabilization period, heart rate was recorded before and after i.v. isoprenaline (0.15 µg/kg). When testing the effects of β-adrenoreceptor blockers, (administered orally 1 h before isoprenaline challenge), the isoprenaline-induced fall in diastolic blood pressure (IFDP) was recorded as a measure of β_2-stimulating properties.

IIT-values were plotted against those relative to RHR and the linear regression calculated according to the method of

least squares. Differences between groups were calculated according to Student's t-test for unpaired data.

Results. RHR- and IIT-values were significantly higher in sodium barbital as compared to urethane anesthetized animals. Reserpine pretreatment of the urethane group abolished the difference in RHR- and markedly increased IIT-values which, nevertheless, were still significantly lower than those of the reserpine-pretreated sodium barbital group (table 1). Analysis of the regression line indicated a highly significant negative correlation between IIT- and RHR-values in urethane (fig.) but not sodium barbital or reserpine pretreated rats. Therefore reserpine-pretreated sodium barbital-anesthetized animals were selected for testing the β-adrenoreceptor blocking properties of propranolol and practolol.

Data in table 2 indicate that while propranolol was equally effective in antagonizing IIT and IFDP, practolol significantly antagonized IIT without affecting IFDP. In addition it was observed that RHR was markedly increased by practolol treatment in a dose-dependent manner.

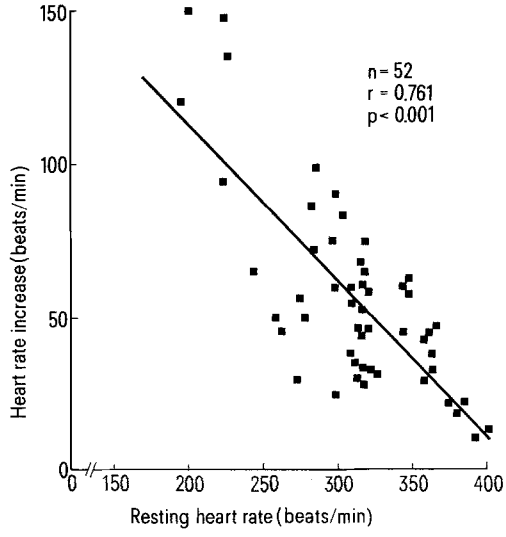
Discussion. Urethane has been reported to reduce catecholamine-induced contractions of vascular smooth muscle[1-6] and its depressant action on blood vessels has been attributed to an inhibitory effect on transmembrane Ca^{++} movements[4]. Since cardiac β_1-adrenoreceptors located on pacemaker cell(s) of the sinoatrial node mediate the chronotropic responses to catecholamines via an increase in transmembrane Ca^{++} movements[7], the depressant effect on IIT of urethane as compared to sodium barbital anesthesia, in both normal and reserpinized rats, might be tentatively attributed to a direct membrane action on Ca^{++} movements, and/or to the hypocalcaemic effect of i.p. urethane in the same animal species[8].

IIT dependence on RHR makes urethane anesthesia unsuitable for testing substances for potential β_1-adrenoreceptor blocking properties. In fact any substance (i.e. a vasodilator or an α-adrenoreceptor blocker) capable of increasing RHR is liable to reduce IIT.

On the other hand the reserpine-pretreated, sodium barbital-anesthetized rats are a very suitable pharmacological test object for assessing β_1- and β_2-adrenoreceptor blocking as well as intrinsic sympathomimetic properties of potential β-adrenoreceptor blocking substances. In fact propranolol is equipotent in antagonizing the effects of β_1- and β_2-adrenoreceptor stimulation[9], whilst practolol possesses a higher affinity for β_1- than β_2-adrenoreceptors[10].

Furthermore practolol but not propranolol exhibits to a certain degree intrinsic sympathomimetic activity[11], i.e. partial agonism, which accounts for the higher RHR of practolol-treated as compared to control reserpinized rats.

Finally the fact that propranolol, although possessing great-



Correlation between RHR and IIT in urethane anesthetized rats. Regression line: $y = 215.6989 - 0.5093x$.

Table 1. Effect of urethane and sodium barbital anesthesia on isoprenalin

Anaesthesia	Reserpine 5 mg/kg/i.p. 48 h before anesthesia	No. of animals	Resting heart rate value	Heart rate increase after isoprenaline (0.15 µg/kg/i.v.)
Urethane (1 g/kg/i.p.)	-	52	306.9±6.3	56.7±4.3
Urethane (1 g/kg/i.p.)	yes	40	279.7±6.2[a]	85.7±4.2[c]
Sodium barbital (200 mg/kg/i.p.)	-	48	336.3±7.5[a]	76.8±3.3[c]
Sodium barbital (200 mg/kg/i.p.)	yes	59	265.7±7.6[a,b]	99.7±2.8[c,d,e]

[a] Significantly different from the urethane group $p < 0.01$; [b] significantly different from the sodium barbital group < 0.01; [c] significantly different from the urethane group $p < 0.001$; [d] significantly different from the sodium barbital group $p < 0.001$; [e] significantly different from the reserpine - urethane group $p < 0.02$.

Table 2. Effect of oral propranolol and practolol on isoprenaline-induced tachycardia (β_1 mediated) fall in diastolic blood pressure (β_2-mediated) and resting heart rate in reserpinized sodium barbital anesthetized rat

Treatment	No. of animals	Dose mg/kg	Resting heart rate values	Chronotropic response to isoprenaline (beats/min)	% inhibition	Fall in diastolic blood pressure after isoprenaline (mm Hg)	% inhibition
Controls	8	-	269.3±9.3	97.5±6.4	-	35.7±3.8	-
Propranolol	8	5	271.2±9.1	59.6±3.6	38.9[b]	23.2±1.2	35.1[b]
	8	10	245.8±7.2	20.1±1.9	79.4[b]	10.5±0.8	70.6[b]
Practolol	7	5	307.5±6.3[a]	32.0±0.9	67.2[b]	37.1±1.7	-
	6	10	336.6±5.3[b]	16.3±1.8	83.3[b]	29.2±2.2	18.3

[a] $p < 0.02$; [b] $p < 0.01$.

er 'in vitro' β_1-adrenoreceptor blocking potency than practolol[12], was less active than this latter in antagonizing IIT, could be attributed to the extensive hepatic metabolism (first pass effect) which propranolol but not practolol undergoes following a single oral dose[13]. Our 'in vivo' procedure supplemented by the 'in vitro' determination of β_1-adrenoreceptor blocking potency could provide useful information on the pharmacokinetic profile of a new drug.

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A microvolume molecular filter

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Summary. A microvolume polymer membrane filter based on Amicon hollow fibers is described which permits separation of low molecular weight compounds from proteins, and can be used for desalting volumes of 100 µl or less, or to separate cellular protein debris from perfusates during release studies. The filter has the advantage of being reusable and having almost no void volume.

During a recent research project, in which we were investigating neurotransmitter release from brain slices[1], it became necessary to separate the amino acid containing perfusate from proteins and cellular debris prior to analysis. Conventional Swinney type filters could not be used because of the small volume of fluid involved, 50–150 µl and centrifugation was also unsuitable.

A series of small diameter tubular polymer filter elements, 'hollow fibers' are made by the Amicon Corporation[2]. These have tightly controlled pore sizes and are available with molecular cut-offs from between 2000 to 100,000 mol.wt (as estimated for globular proteins), and are available as loose fibres (for use as artificial blood vessels etc.). They are intended to be filled, under pressure, with the solution to be filtered, the filtrate passing through the walls and the higher molecular weight substances being retained in the lumen. Thus they appeared to be eminently suited to our purpose.

The filter is constructed as depicted in the figure. A 2.5 cm length of filter element (0.5 mm i.d.), is cut off and a small amount of cyanoacrylate cement applied to one end to seal it off. A disposable 27G (0.47 mm o.d.) hypodermic needle is cut to 0.5 cm length (by filing a nick and breaking off the excess) and the end filed smooth. It is important not to use

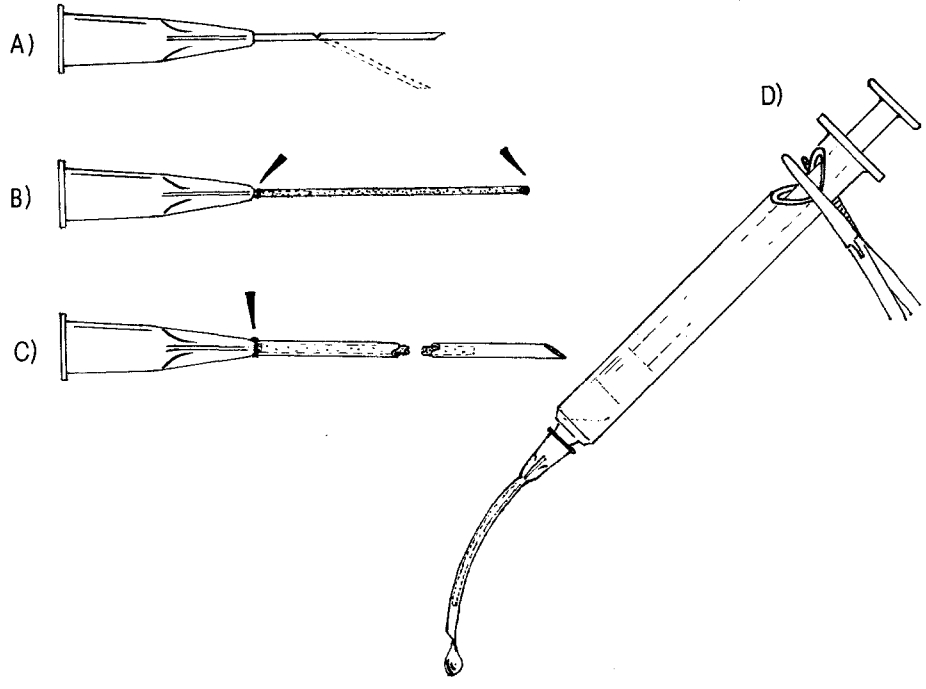

Figure. Stages in the assembly of a microvolume filter. A) the hypodermic needle is cut to length by filling a nick in the tube and bending the excess off, after which the cut end should be smoothed with a file. B) a length of filter element is slipped over the cut needle and a drop of cement used to glue it to the base of the needle and to block the end (arrows). C) polyethelene tubing is then slid over the element and affixed to the base of the needle with cement. D) modifications that can be made to a 1-ml tuberculin syringe to permit it to be used as a combined pressure source and reservoir. The inside edges of the aperture at the top of the syringe should be smoothed, otherwise the rubber seal of the plunger will be damaged. Any suitable haemostat forceps can be used to clamp the plunger in place.