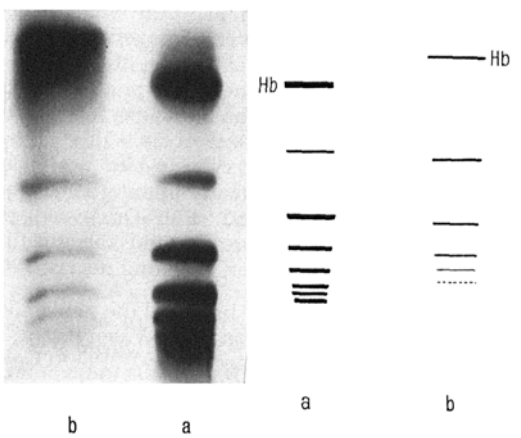
Haptoglobinuria Following Muscular Exercise

Muscular exercise is accompanied by an increase in urinary protein excretion. Though albumin represents the major fraction, we have been able to reveal about fifteen proteins of plasma origin in urine obtained after physical effort[1]. Among these, our attention has been drawn to the haptoglobin, the presence of which has been suggested by PATTE et al.[2] and then established by BERGGÅRD[3] in normal urine. Only the monomeric fraction of haptoglobin has been found in normal urine[4]. In orthostatic, lordotic[5] and pathologic proteinuria[6] the haptoglobin 1-1 form or the more anodic fractions of type 2-1 is found only. As we can consider exercise proteinuria as an intermediate form between normal and pathology, we have investigated the haptoglobin type present in urine after an intense physical effort (9 km cross-country run).

The urine is concentrated within a Visking membrane $^{8}/_{32}$ in. under reduced pressure (see [1]). Not being able to obtain large individual volumes, we have had to combine different samples, and thus it is on a urinary pool (10 g% protein) that the analyses were performed. After addition of haemoglobin and starch gel electrophoresis with the discontinuous buffer of POULIK[7], the haptoglobin-haemoglobin complex is coloured by benzidine. Haptoglobin 2-1 serves for comparison.

The Figure shows the pattern after staining. Urine possesses haptoglobin type 2-1, although in an incomplete form. The monomeric band is the most coloured and therefore quantitatively the most important fraction. Polymers are also present, at least for the anodic first four bands. The last polymers (2 bands), with higher molecular weight, stay absent from urine.



Electrophoretic pattern of haptoglobin 2-1 in urine after physical exercise. a: Serum haptoglobin 2-1, as control. b: Urine.

From this investigation it appears that haptoglobin type 2-1 is present in urine after exercise in a form approaching the complete structure: 5 bands on 7 are visible. It thus seems that the glomerular passage of this glycoprotein is facilitated in exercise proteinuria compared to physiologic urine[4] and nephrotic syndrome[6]. On the contrary, this haptoglobin 2-1 excretion may be compared to orthostatic and lordotic proteinuria[5]. On the other hand, cerebrospinal fluid also contains the lighter polymers of haptoglobins 2-1 and 2-2[8].

The results show that the permeability of the meninges and renal glomerulus plays an important part during the transfer of proteins. The increased glomerular permeability consecutive to muscular exercise permits the heavier haptoglobin 2-1 polymers to pass into the urine. The average molecular weight of haptoglobin 2-1 being about 220,000[9], it appears that the glomerular permeability limit during physical effort is situated around 350,000 molecular weight. The presence of fibrinogen in urine, in small quantity, corroborates this assertion (unpublished results)[10].

Résumé. L'urine recueillie après effort physique intense contient l'haptoglobine de type 2-1 sous une forme proche de sa structure complète. Les cinq bandes les plus anodiques sont visibles dans l'urine d'effort, quoiqu'à un taux inférieur à celui d'un sérum normal.

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Effects of Anoxia on Excitability, Refractoriness, and Contractility in Isolated Rabbit Atria

Introduction. The effects of anoxia on isolated rat atria[1] Purkinje and papillary fibers[2,3] have been shown to include decrease in amplitude and duration of the action potential, prolongation of conduction time and decreased amplitude of contraction. BURN and HUKOVIC[4] have reported that anoxia facilitates the development of fibrillation and hence it is said to increase 'excitability'.

The following experiments were designed to study directly the effects of anoxia on diastolic excitability, refractoriness and contractility in isolated rabbit atria. The results show that changes in neither excitability nor refractoriness can account for increased susceptibility to fibrillation.

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Effect of anoxia on excitability and contractility in the isolated rabbit atrium (mean ± SD for four experiments)

	ARP		TRP		Diastolic threshold	% change in excitability	Tension	
	msec	% of control	msec	% of control	μamps		mg	% of control
Control[a]	119 ± 9	–	147 ± 20	–	70 ± 20	–	1185	–
Anoxia[b]	171 ± 27	+44	210 ± 46	+43	100 ± 40	−30	20	−98
Post-anoxia[a]	120 ± 10	–	167 ± 31	+14	90 ± 40	−22	1097	− 8

[a] Perfusion gas 95% oxygen-5% carbon dioxide. [b] Perfusion gas 95% nitrogen-5% carbon dioxide.

Methods. Rabbit left atria were isolated and prepared for stimulation and recording as previously described[5]. In four experiments a 95% nitrogen-5% carbon dioxide mixture was substituted for the usual oxygen-carbon dioxide used to aerate the Locke perfusion fluid and tissue chamber. The atrium was stimulated at 90/min (630 msec cycle) and perfusion fluid was changed at a rate of 5 ml/min. After control strength-interval studies, used to determine the refractory period, the nitrogen mixture was introduced and contractile responses were recorded and measurements were made on diastolic excitability every few seconds. 12 min after the introduction of nitrogen, strength-interval determinations were repeated. Oxygen was readmitted as the perfusion gas and 30 min later the post anoxia determinations were made.

Results. Substitution of nitrogen for oxygen in the perfusion gas led to an immediate loss of contractility but diastolic excitability was unaltered. The data in the Table were obtained from strength-interval determinations which were made during perfusion with oxygenated Locke solution and 12 min after nitrogen was introduced. With nitrogen, excitability was decreased and absolute (ARP) and total refractory periods (TRP) were prolonged. Strength-interval determinations repeated 30 min after re-introduction of oxygen showed complete recovery of ARP but only partial recovery of TRP and diastolic excitability.

Contractile responses were almost completely abolished in the presence of nitrogen. During the relative refractory period (RRP) a contraction was not seen following an effective electrical stimulus, but the phenomena of post systolic potentiation were frequently observed. Post anoxia, there was recovery of contractility throughout the cycle but the maximum developed tension in the later portion of the cycle (350–630 msec) was slightly less than control (Table).

Discussion. Similar to the observations of WEBB and HOLLANDER[1] and TRAUTWEIN and DUDEL[3] total substitution of nitrogen for oxygen in the perfusion fluid led to a rapid loss of contractility.

Previous reports have shown that anoxia initially shortens the duration of the action potential and eventually decreases the rate of depolarization and resting membrane potential[1–3]. These changes would be expected to produce a shortening in the refractory period and alteration of excitability. However, these studies and the present results indicate that under anoxic conditions there was no correlation between the changes in the duration of the action potential and the refractory period.

BURN has reported that there is an increased propensity toward fibrillation under conditions of anoxia[4] and in the presence of acetylcholine[6]. He has suggested that since both conditions shorten the duration of the action potential there may be a relation between factors which shorten action potential and the development of fibrillation. The interpretation of these results was based on the assumption that shortening of the action potential was associated with shortening of the refractory period[7]. However, as shown herein, this does not obtain in the case of anoxia where the effects on the refractory period were opposite to those reported for the action potential. The situation with acetylcholine is apparently different since both action potential duration and the refractory period are shortened[8]. Although a tendency toward fibrillation has been noted with strength-interval determinations under acetylcholine in the dog atrium *in situ*[9,10] and in the isolated rabbit atria (unpublished observations), confirming the observation of BURN[6], no such tendency was noted in the present experiments under anoxia.

The tendency toward fibrillation as measured in the anoxic heart by BURN[4] may be related to the conditions employed and could be due to changes in the rate of depolarization of the membrane or to the development of ectopic pacemakers[2]. In any event, it is unlikely that they are related to changes in the refractory period or diastolic excitability[11].

Zusammenfassung. An der isolierten Kaninchenherzvorkammer verursacht Sauerstoffmangel Verminderung der diastolischen Erregbarkeit, Verlängerung der refraktären Phase sowie Herabsetzung der Kontraktionsfähigkeit. Herzmuskelflimmern wurde während dieser Anoxie nicht beobachtet. Sauerstoffzufuhr führte zu einer vollständigen Aufhebung der Anoxieauswirkung auf die absolute Refraktärperiode, zu einer teilweisen Aufhebung dieser Auswirkungen auf die Dauer der refraktären Gesamtpause, die diastolische Erregbarkeit und das Kontraktionsvermögen.

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