was in maturation of epiphyseal hyaline cartilage. Cells in this area appeared to be arrested in development. These findings are compared with cellular changes found in certain nutrient deficiency perosis.

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Influence of Potassium and Calcium on Behavior of Isolated Rat Atria.* (28083)

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The effects of various drugs and environmental conditions on the membrane potentials and contractility of the rat atrium have been studied in this laboratory(1-6). Correct interpretation of the results requires a knowledge of the influence of the medium which is in contact with the atria during the experimental procedure. This paper is an attempt to supply such information as well as to determine the effects of changes in 2 of the components of that solution, the potassium and calcium ions, on various parameters of atrial function.

Methods. The atrial preparation and apparatus. Rats weighing from 205 to 365 g were killed by decapitation and the hearts were removed and placed in a beaker containing chilled oxygenated Krebs-Ringer bicarbonate medium of the following composition (mM): NaCl 120, KCl 6, $CaCl_2$ 1.22, $MgSO_4$ 1.34, NaH_2PO_4 1.21, $NaHCO_3$ 25.3, glucose 5.55. This solution will be called normal in the text. The atria were dissected from the ventricles and mounted as described previously(1). Electrical stimulation, when necessary, was at a rate of 200/minute. The temperature of the bath was 30°C and the medium was gassed throughout the experiment with 95% O_2-5% CO_2. A strain gauge and Dynograph were used to record the semi-isometric contractions. The resting tension was adjusted to 750 mg at 5 minute intervals throughout the experiment by means of a micrometer attached to the muscle holder.

Blotting procedure preparatory to chemical determinations. After dissection in Krebs-Ringer solution, atria were blotted gently on filter paper between the fingertips either 10, 20 or 30 times and weighed. The blotting and weighing procedures took 2, 3 and 3½ minutes respectively. The atria were then dried to constant weight at 110°C for at least 24 hours and reweighed. The per cent water was calculated to be 80.7 after 10 blottings, 77.8 after 20 blottings and 77.6 after 30 blottings. A significant difference was found between the per cent water at 10 and 20 blottings ($p<.001$ "t" test). No significant difference was found between the per cent water at 20 and 30 blottings ($p>.5$ "t" test). It is concluded that 10 blottings are not sufficient to remove superficial fluid adhering to tissue prior to analysis. Twenty blottings removed as much water as 30 blottings and was, therefore, sufficient.

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TABLE I. Potassium and Water Contents of Rat Atria Under Different Experimental Conditions.

	No. of atria	meq K^+/kg dry tissue	% water
Freshly dissected	10	353.7 ± 18.8*	77.8 ± 1.02*
Stimulated in normal solution	10	363.1 ± 21.1	77.3 ± 1.43
Stimulated in K^+-free solution	4	154.5 ± 11.8	75.8 ± .47

* Standard deviation.

Determination of potassium. After drying for at least 24 hours, the atria were placed in test tubes to which 0.5 ml of concentrated nitric acid was added. The tissues were disintegrated and allowed to stand for one hour. Two to 3 ml of water were then added and the tissues were allowed to stand overnight. The extract was then placed in a graduated cylinder to which 5 ml of lithium standard (5000 ppm) had been added. The volume was brought up to 100 ml with de-ionized water and the extract filtered. The potassium content of the sample was then determined with a flame photometer.

Results. Potassium and water content of rat atria. A comparison of the water and potassium contents of rat atria was made under 3 conditions: (A) Freshly dissected atria: (B) Atria stimulated for 3 hours in normal Krebs-Ringer bicarbonate medium; (C) Atria stimulated for one hour in the above medium followed by 2 hours in K^+-free medium. The results are given in Table I. No significant difference was found between either the potassium or water contents of freshly dissected atria and those stimulated in the normal medium. This indicates that the medium used is physiological as far as the K^+ ion and the osmotic pressure are concerned.

Stimulation of the atria in a K^+-free medium for 2 hours resulted in a 57.4% loss in K^+ content which was highly significant ($p<.001$) with no significant change in water content when compared with atria stimulated in normal solution.

Behavior of rat atria in potassium-free media. A. Electrically stimulated. 1) In K^+-free medium, the stimulation threshold is increased. An inverse relationship between external K^+ concentration and stimulation threshold was also noted by Green *et al.*(7) in the cat papillary muscle. Idiopathic rhythmicity, a condition in which contractions out of phase with the stimulator appear, was always encountered within 20 minutes of changing the medium from normal to K^+ free. These extra beats could generally be reduced or abolished by: a) increasing stimulation voltage when the cause was pacemaker escape due to subthreshold stimulation, b) increasing rate of stimulation when the intrinsic pacemaker was discharging close to or above 200/min (60% of the atria which were not electrically stimulated developed intrinsic rates above 200/min when placed in K^+-free media). Occasionally the atria developed a very fast rate, possibly a flutter or fibrillation. 2) Contracture, as measured by a decrease in resting length, always occurred within 20 min. 3) The force of contraction underwent two phases: a) A marked increase in the force of contraction lasting 20 to 90 minutes, and b) a subsequent decrease in contractile force.

B. Self-stimulated. 1) The intrinsic rate underwent two phases: a) An increase of 10-65% above control rate lasting 5-80 minutes. Changes in Ca^{++} concentration influenced the force of contraction and degree of contracture in the expected way, both in presence and absence of K^+, *i.e.*, raising the calcium concentration increased the force of contraction and degree of contracture. Addition of epinephrine (5×10^{-7} M) during this phase resulted in a further increase in the rate (Table II). b) A marked slowing of the rate with less than 10 beats per minute. Addition of epinephrine during this slow phase did not change the rate (Table II).

2) An inverse relationship between the force of contraction and the rate was observed. During the very slow rate phase (phase *b* above) the contractions were very large, amounting to 2 or more times that seen in phase *a* above.

3) A direct relationship between degree of contracture and rate was observed. During phase *a* above, when the rate was increasing, degree of contracture increased. As the rate slowed in phase *b*, the atria relaxed.

4) Within 10 minutes after being placed in K^+-free media, the atria developed irregulari-

TABLE II. Influence of Epinephrine Bitartrate, 5×10^{-7} M, on Intrinsic Rate of Rat Atria in Potassium-Free Medium.

Rat No.	Rate before epinephrine	Rate after epinephrine
1	184 (35)*	260 (3)†
2	128 (25)	176 (1)
3	100 (40)	232 (4)
4	58 (20)	184 (2)
5	56 (25)	7
6	6 (125)	9
7	4 (20)	4
8	4 (60)	4
9	3 (50)	4
10	0 (20)	1

* Figures in parentheses indicate time in minutes in potassium-free medium.

† Figures in parentheses indicate time in min after addition of epinephrine. Where indicated, it is the maximum rate. Where not indicated, it is the rate 5 min after addition of epinephrine.

ties in rhythm. On the basis of the contractile patterns, there appeared to be extrasystoles and bigeminy. Addition of epinephrine (5×10^{-7} M) to atria exhibiting an irregular rhythm usually resulted in a rapid, more regular, rhythm.

5) When atria were placed in K^+-free media in which the Ca^{++} was reduced to ¼ or ⅛ of normal (0.3 or 0.15 mM), the rate-stimulating effect (phase *a*) was delayed or prevented. The reason for this was made apparent by experiments in which Ca^{++} concentration was varied and K^+ was maintained at normal concentration. Under these conditions, the rate was directly related to the calcium concentration (Table III).

Discussion. Under the conditions of a K^+-deficient medium, there appears to be a mechanism for conservation of K^+, since, according to the calculations of Hajdu(8) K^+ leaves the frog heart at a rate of 0.127 mM per beat. If rat atrial cells behaved similarly they would have been completely depleted within 4 minutes. The fact that more potassium was not lost is consistent with the findings of Paradise *et al.*(6) that in a K^+-free medium, the resting potential of rat atrial cells was still elevated above normal after 2 hours of stimulation. Also, Carmeliet(9) has shown that K^+ efflux from sheep Purkinje fibres is slowed in K^+-free medium.

The rate-stimulating effect of placing the atria in a K^+-free medium could be explained by a decreased efflux of K^+ in pacemaker fibres during the period of diastolic depolarization in presence of a normal or increased Na^+ influx. Thus, the rate of diastolic depolarization would be increased and the pacemaker firing rate increased. Evidence in favor of a slowed K^+ efflux has been presented above.

The terminal rate-slowing effect seen when atria are placed in a K^+-free medium is probably a reflection of pacemaker damage due to the loss of K^+ or gain of Na^+ with subsequent alterations in activity. That it is not due to conduction block is indicated by the fact that the atria were able to follow a very fast rate of electrical stimulation.

The rate-stimulating effects of calcium are in contrast to the findings of Webb(10) on rabbit atria, large changes in Ca^{++} having little effect on the rate, and Weidmann(11) who found that addition of Ca^{++} slowed the intrinsic rate of calf and sheep Purkinje fibres. This probably represents a species difference.

Summary. A blotting procedure was developed to remove superficial fluid adhering to rat atrial tissue in a consistent manner prior to chemical analyses. The K^+ and

TABLE III. Effect of Changing Calcium Concentration on Intrinsic Rate of Rat Auricles in Presence of 6 mM Potassium.

	Rate of beating				
Rat No.	Normal calcium (1.2 mM)	¼ calcium (.3 mM)	Normal calcium (1.2 mM)	2× calcium (2.4 mM)	4× calcium (4.8 mM)
11	180 (60)*	153 (120)			
12	161 (60)	139 (120)	161 (30)		
13	195 (60)	173 (120)	206 (30)	226 (30)	
14	171 (60)		179 (120)	212 (30)	253 (30)

* Figures in parentheses indicate times in min at which rates in indicated media were taken.

water contents of rat atria stimulated for 3 hours in Krebs-Ringer bicarbonate medium containing 6 mM potassium were not significantly different from those of freshly dissected atria, indicating that the medium used in the present investigation is physiological with respect to its potassium concentration and osmotic pressure. Atria stimulated for 2 hours in K^+-free medium, after a one hour equilibration period in the normal medium, showed a 57.4% loss in potassium content. No significant change in water content, however, was observed under these conditions. Atria stimulated 200 times per minute in K^+-free medium showed idiopathic rhythmicity, contracture, and a temporary increase in contractile force. Self-stimulated atria placed in K^+-free medium showed a temporary period of increased rate which was sensitive to the chronotropic action of epinephrine. This was followed by a phase of very slow rate and of epinephrine insensitiveness. Arrhythmias were observed in both phases. The degree of contracture observed was directly related to the rate while the force of contraction was inversely related. The intrinsic rate showed a direct relationship to Ca^{++} concentration both in presence and absence of potassium.

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Folate Activity in Rat Tissue Before and After Pteroylglutamic Acid Load.* (28084)

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Human blood has been found to contain a variety of folic acid-active substances(1,2). With simultaneous use of 3 different bio-assays (*Lactobacillus casei, Streptococcus faecalis* and *Pediococcus cerevisiae*) it has also been established that the ratio of the activities of *P. cerevisiae:S. faecalis:L. casei* in normal human blood and serum is 1:2:15. In moderate anemias due to folic acid deficiency, the *L. casei* values were the first to decrease (2), whereas in severe nutritional megaloblastic anemia all 3 bio-assays revealed extremely low activity both in whole blood and in serum(3). It seemed of interest to learn whether the distribution of the various folate derivatives in the major storage organs, such as the liver, is similar to that found in the circulating blood and, furthermore, to what extent this distribution may be affected by loading with pteroylglutamic acid (PGA).

The present paper deals with the results of such an investigation in the rat.

Material and methods. Seventy random-bred female white rats, weighing between 250-300 g, were used. Three animals were kept in a cage and were fed a standard, mixed diet. Food was withheld for 24 hr before, and for an additional 48 hr after the load. Thirty-eight rats received pteroylglutamic acid (PGA) 2 μg/g body weight, by intra-

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