creased fatty acid oxidation to CO_2 for both acids. However, in contrast to the palmitate data, inhibition of the oxidation of octanoate to CO_2 did not lead to an increase in the recovery of ^{14}C from octanoate in the liver. Ethanol inhibition of CO_2 production from fatty acids did not of itself lead to an accumulation of fatty acids in the liver. Thus, the data suggest that ethanol inhibition of CO_2 production from fatty acids does not contribute to the accumulation of fatty acids (in the form of triglycerides) seen in livers from ethanol treated rats.

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Isolation, Stress, Myocardial Electrolytes, and Epinephrine Cardiotoxicity in Rats* (32642)

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It is known that certain emotional and environmental stresses can provoke or aggravate myocardial necrotization (1).

Prolonged isolation has been found in rats to be associated with a greatly increased catecholamine cardiotoxicity, as manifested by a severalfold decrease of the lethal dose of isoproterenol (2), and a marked intensification of isoprenaline-induced structural lesions (3) after 3 months of isolation: In isolated mice, the toxicity of indirectly adrenergic *d*-amphetamine was likewise augmented (4).

Selye (5) and others (6, 7) have shown that the necrotizing cardiotoxicity of catecholamines is remarkably potentiated by adrenal corticoids especially by 17-hydroxycorticosteroids.

An increased production of 17-hydroxycorticosteroids (8) as well as of total unconjugated corticoids (9) and an increased response to ACTH (9) have been observed in isolated rats. This may account for the isolation-induced exaggeration of catecholamine cardiotoxicity in such animals (2, 3). The mechanism of the catecholamine-"sensitizing" effect (5) of corticoids is not yet clearly understood. However, the myocardial potassium-depleting action of both corticoids (10–

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TABLE I. Electrolytes in the Left Ventricle of Control Rats and of Rats Isolated during 4 Months.

Number of rats	K[a]	Na[a]	K/Na	Mg[a]	Ca[a]
		Controls			
20	342 (SD ± 21)	81 (SD ± 5.3)	4.2	25.4 (SD ± 2.0)	5.0 (SD ± .59)
		4 months isolation			
24	309 (SD ± 13) (p <.01)	79 (SD ± 6.7) (p <.02)	3.9	23.9 (SD ± .90) (p <.01)	5.5 (SD ± .59) (p <.01)

[a] mg/100 gm wet weight.

13) and potentially hypoxiating catecholamine doses (14–16) suggests a combined detrimental influence on myocardial electrolyte balance.

Cardiac structural integrity is largely dependent on the maintenance of sufficient potassium (5, 13, 17) and magnesium (18) stores in the myocardial tissue.[3]

To investigate these complex interrelations, myocardial electrolytes and the effect of injected epinephrine on myocardial potassium distribution and morphological vulnerability were determined chemically, histochemically, and histologically in controls and in rats, exposed to isolation stress for several months.

Methods. Weanling, white, female Sprague-Dawley rats were caged either in groups of 2 to 3 animals as controls, or isolated by metal partitions between cages, each of which contained only one animal. The isolated rats were not excluded from the sight of other rats, kept at a distance of about 4 feet.

For chemical assay of potassium, magnesium, sodium, and calcium in left ventricular tissue, 20 controls and 24 isolated rats were killed after 4 months, and 13 more isolated rats after a total of 14 months isolation. Eleven rats were transferred after 14 months isolation into two community groups of 5 and 6, and killed 14 days later.

Electrolytes were determined in all these animals by atomic absorption spectrophotometry. The results for potassium, sodium, magnesium, and calcium were expressed in mg per 100 gm wet weight of left ventricular tissue.

[3] Raab, W.: Review, to be published (Ann. N. Y. Acad. Sci., 1968).

For evaluation of catecholamine cardiotoxicity in terms of early disturbed topical potassium distribution in myocardial tissue, epinephrine was injected intramuscularly (hind leg, once in each animal) at three dose levels (0.1, 0.25, and 0.5 μg/gm) in pairs of 21 controls and 21 isolated rats after 4–8 months of isolation. These rats were killed 30 min after injection of epinephrine, except for a few which died earlier, and the myocardium was processed for histochemical study of topical potassium distribution by the method of Poppen, (19) applied to frozen sections (fixed for 10 min in 10% acetic acid).

For additional routine histological studies, the sections were post-fixed with formalin and stained with hematoxylin-eosin.

Histochemical and histological changes were graded according to scales indicated underneath Table III.

Results. A. *Left ventricular electrolyte content after isolation and after return to community* (Tables I, II).

Compared with the controls, myocardial potassium and magnesium were significantly decreased after 4 months of isolation, and even more so after a total of 14 months' isolation. Sodium was not significantly altered within 4 months but showed a marked increase after 14 months of isolation. The K/Na ratio fell throughout the isolation periods.

In rats which had been returned to community life for 2 weeks after 14 months of isolation, a partial normalization of the potassium and sodium values and of the K/Na ratio, and a complete normalization of magnesium was observed.

Calcium was found significantly elevated after isolation periods of 4 and 14 months.

B. *Susceptibility of the left ventricle to epinephrine-induced potassium displacement and morphological injury* (Table III). Within the range of administered dosages of epinephrine (0.1–0.5 μg/gm), the myocardial tissue of the nonisolated control animals failed

TABLE II. Electrolytes in the Left Ventricle of Rats Isolated during 14 Months, and in Others, Returned to Community After 14 Months Isolation.

Number of rats	K[a]	Na[a]	K/Na	Mg[a]	Ca[a]
		14 Months isolation[b]			
13	298 (SD ± 15) ($p < .01$)	102 (SD ± 5.2) ($p < .01$)	2.8	22.6 (SD ± 2.3) ($p < .01$)	5.7 (SD ± 1.1) ($p < .01$)
		Two weeks in community after 14 months Isolation[b]			
11	314 (SD ± 12) ($p < .01$)	93 (SD ± 5.3) ($p < .01$)	3.4	25.4 (SD ± 8.8) ($p < .01$)	—

[a] mg/100 gm wet weight.

[b] The plain isolation group was statistically compared with the control group. The "Two weeks in community" group was compared with the plain isolation group.

TABLE III. Vulnerability of Rat Heart to Epinephrine.

Number of rats	Epinephrine i.m. injected dosage μg/gm	Displacement of K (histochemical)[a]		Morphological changes[b]	
		Number of rats showing changes	Degree[c] of changes	Number of rats showing changes	Degree[c] of changes
		Controls[d]			
8	0.1	0	0	0	0
7	0.25	0	0	0	0
6	0.5	4	1.1 ± 0.41	0	0
		Isolated[d]			
8	0.1	2	0.3 ± 0.38	0	0
7	0.25	6	2.3 ± 0.45	3	0.5 ± 0.27
6	0.5	6	3.2 ± 0.34	3	1.2 ± 0.36

[a] Explanation of histochemical data:
0 = no alterations.
1 = loss of K in some isolated fibers.
2 = 1-3 small K-depleted areas.
3 = more than 3 small K-depleted areas.
4 = several large (partly confluent) K-depleted areas.

[b] Explanation of morphological data (hematoxylin-eosin):
0 = no alterations.
1 = hemorrhage without necrosis.
2 = 1-3 small necrotic foci.
3 = more than 3 small necrotic foci.
4 = large, confluent necrotic areas.

[c] Mean and standard error (degrees ranging from 0-4).

[d] Controls and isolated rats were killed in corresponding pairs after 4-8 months.

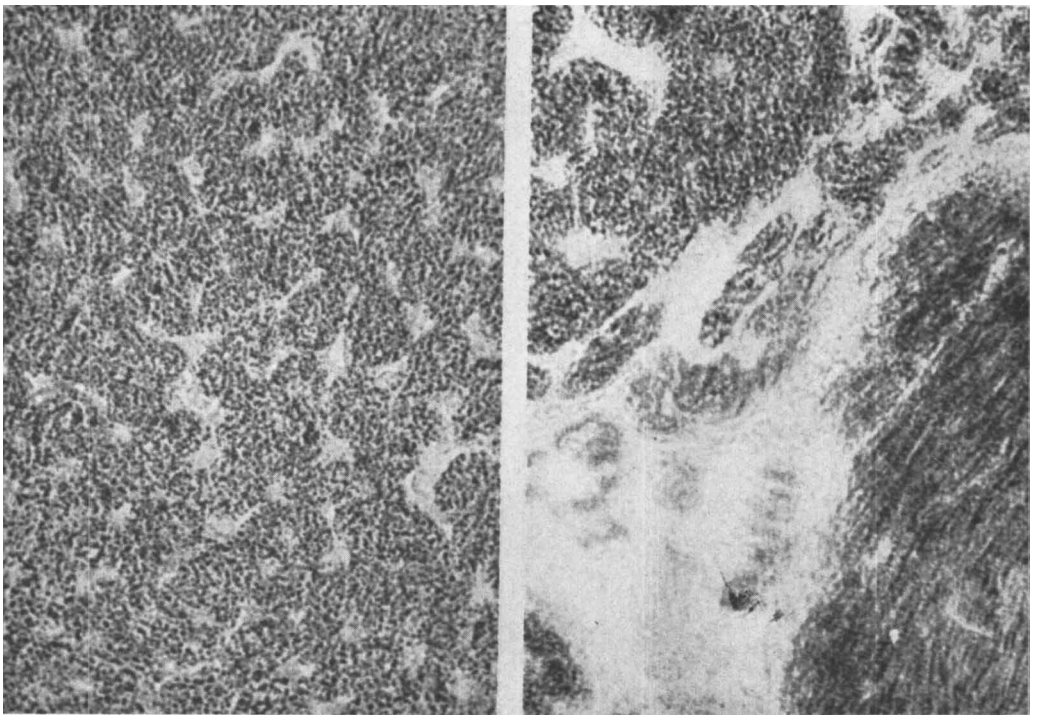

FIG. 1. Left: Normal potassium distribution in the left ventricular wall of a nonisolated control rat. Right: Spotty potassium depletion in the left ventricular wall of an isolated rat, injected with 0.25 μg/gm epinephrine. Note potassium accumulation (darker staining) of some areas around the depleted focus.

to show any detectable alterations, except for a slight degree of histochemically demonstrable focal potassium displacement at the highest (0.5 μg/gm) dose level.

By contrast, all epinephrine dosage levels elicited slight to severe histochemically demonstrable K-displacements in 25–100% of the isolated rats, and to a degree, roughly proportional to the injected amounts (Figs. 1, 2).

No morphological changes (conventional staining) appeared in the control rats after epinephrine injection, while such changes, including focal hemorrhages and necrotic foci of small or large size, were found in about half of the isolated rats after injection of equivalent doses of epinephrine (0.25–0.5 μg/gm) (Fig. 2).

Discussion. In another study[4] we have pointed out the impossibility to detect early, prenecrotic, microfocal displacements and perifocal accumulations of potassium within the heart muscle by mere chemical assay. The latter does not permit recognition of focally circumscribed potassium shifts, caused presumably by vascularly or catecholamine-in-

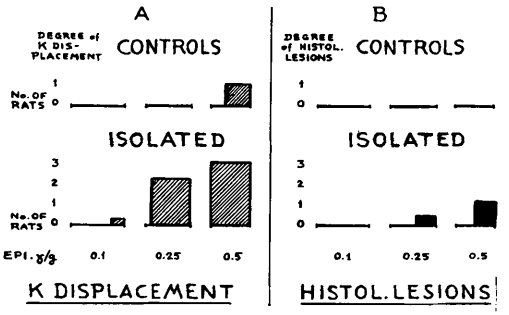

FIG. 2. Augmentation of myocardial vulnerability to injected mounting doses of epinephrine in isolated rats, as evidenced by (A) histochemically demonstrable focal potassium losses, and (B) histologically demonstrable hemorrhages and focal necroses. For grading of potassium displacement (shaded) and of histological lesions (black) see Table III.

[4] Kimura, H., Bajusz, E., Herrlich, H. C., and Raab, W.: to be published.

duced myocardial hypoxia in multiple small areas. This intramyocardial focal potassium depletion, combined with perifocal retention of potassium, extruded from damaged myocardial cell groups, occurs partly before, and partly after the development of actual necrosis (20).

The overall diminution of chemically determined potassium, of the K/Na ratio, and of magnesium in the entire left ventricular myocardium of our isolated rats may be considered as consistent with the prolonged, potassium-depleting, sodium-accumulating, adrenocortical overactivity(8,9) induced by the stress of isolation. It receded after 2 weeks of contact with other animals, in apparent agreement with the disappearance of exaggerated catecholamine cardiotoxicity under such circumstances, as described by Hatch *et al.* (2).

In view of the limited conclusiveness of merely chemical electrolyte assay in the myocardium, more direct information concerning catecholamine cardiotoxicity during isolation could be expected and was obtained from the marked augmentation of myocardial vulnerability to injected epinephrine in terms of both prenecrotic focal potassium displacement and of actual tissue damage (hemorrhage, necrosis).

Exaggerated aggressiveness, a notoriously adreno-sympathetic stimulating feature (1), has been observed in isolated animals by several investigators (2, 4), including ourselves. In human restraint experiments (6–7 hours immobilization in a tank), a slight augmentation of catecholamine production and inceased emotional excitability were described (21).

Multiple myocardial necrotic foci and fibrosis in the absence of coronary vascular lesions were found in a caged baboon with an excessively aggressive temper (22) and in caged ground squirrels (23). The severe cardiodestructive effects of combined emotional and physical stresses (5, 7) and those, produced by anxiety (24) and frustration (24, 25) cannot be directly juxtaposed to the presumably milder cardiometabolic sequelae of mere isolation. However, the complex stress situation of astronauts, and of persons engaged in other isolating occupations, associated with strong elements of responsibility and tenseness, may possibly involve potential cardiac detriments, and deserve specific exploration.

Summary. Prolonged isolation stress of rats was associated with a significant diminution of myocardial potassium and magnesium, and with a marked increase in myocardial sodium, causing a reduction of the K/Na ratio. Restoration of contact with other animals tended to correct these changes within a short period of time. Myocardial calcium was increased during isolation. Histochemical and histological observations revealed a marked aggravation of epinephrine-induced myocardial focal potassium displacement, and the appearance of epinephrine-induced hemorrhages and necroses in the hearts of isolated rats, which could not be elicited in the controls by analogous doses of epinephrine. This type of augmented vulnerability of the heart muscle to catecholamine action is attributed to the well-established principle of exaggerated catecholamine cardiotoxicity under corticoid over-action, in view of the fact that others have observed an increased corticoid production in isolated rats.

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Urinary Procoagulant Excretion in Experimental Renal Disease* (32643)

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Human urine contains a powerful procoagulant (1). It converts prothrombin into thrombin in the presence of factor V, lipid, and calcium (2), a reaction which is the basis for its quantitative assay (3). The procoagulant content of the urine in patients with thrombosis, embolism, myocardial infarction, and hemorrhagic diathesis is the same as that of healthy subjects. There is, however, a statistically highly significant ($p < .001$) decrease of the urinary procoagulant in parenchymatous kidney diseases (4). Numerous patients with nephrotic syndrome and acute tubular necrosis did not excrete it at all. The present investigations were designed to eliminate urinary procoagulant excretion in animals and thereby to obtain information on the origin of the procoagulant.

Material and Methods. Quantitative determination of the procoagulant content of the urine: A modificaiton of a previously described method (3); 0.2 ml urine are required.

Hematuria was graded according to the number of erythrocytes in urine sediment per high power field. —: 0 erythrocytes; +: 1–3; ++: 4–10; +++: 10+.

Glycosuria was estimated with Combistixs (Ames Corp., Elkhart, Indiana).

Protein content of the urine was estimated with Exton's reagent (5) and quantitated with Esbach's procedure (5).

Urine collection. Urine was collected 8:00 a.m. and immediately dialyzed in the cold. In the phlorizin studies, urine was obtained by an indwelling catheter.

Male albino rabbits, 2–3 kg were purchased locally. Male white Sprague-Dawley rats, approximately 200 gm, were obtained from the Simons Lab., Gilroy, California. The animals were fed Purina Chow. They were housed in stainless steel metabolic cages constructed to prevent contamination of urine with feces. After urine collection, all cages were thoroughly cleaned daily.

Nephrotoxic agents. (a) Duck antirabbit-kidney serum (Antibody Incorp. Davis, California). The serum was inactivated for 30 min at 56°C and then adsorbed for 30 min at 0°C with 1/10 vol. washed rabbit erythrocytes. One single dose of 2 ml was given

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