tered intravenously suggested that only lead acetate could induce connective tissue calcification at sites of increased capillary permeability (5,6). The present experiments demonstrate that this effect also is caused by several rare earth metals (Ho, Er, Dy, Tm) when given in small amounts. We have recently observed that small doses of rare earth metals increase, while large amounts decrease the serum concentrations of calcium and phosphorus (10,11). Large amounts of these metals are absorbed by the reticuloendothelial system and induce accumulation of calcium salts in the spleen (7). It is noteworthy that splenic calcification is induced when large amounts of metal are administered, while cutaneous calcification, at the site of polymyxin injection, is obtained only with low levels. The ability of rare earths to induce or to inhibit cutaneous calcification is reminiscent of the dual effect exhibited by different amounts of thrombohemorrhagic "sensitizers" (12).

Summary. In the rat, the intravenous administration of several rare earth metals (Ho, Tm, Dy, Er) induces an accumulation of calcium salts at sites treated with a histamine liberator, polymyxin. This reaction is obtained only with medium doses (3 mg) of metals; smaller (1 mg) and larger doses (5 and 8 mg) were inactive in this respect. The intensity of splenic calcification is directly proportional to the amount of rare earth administered.

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Blood Lactic Acid in Rats and Men: Comparison of Normo- and Hypertensive Individuals*† (33042)

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Demartini *et al.* (1) reported that in patients with either renal or essential hypertension the concentration of lactic acid (LA) in both venous and arterial blood was significantly elevated. Somewhat similar results had been obtained by Gupta and Chakravarty (2). The reason for this finding was not clear.

We have studied the LA concentration in the blood of two strains of rats with an inborn susceptibility (S) or resistance (R), respectively, to experimental hypertension induced by NaCl as well as other techniques

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(3–5). It was assumed that if a derangement of lactic acid metabolism were an inherent part of the hypertensive process, this would be reflected in these strains of rats. Also, a small series of patients with hypertension was studied as well as appropriate normotensive controls.

Material. Rats. All animals came from either the strain genetically sensitive (S) or resistant (R), respectively, to the development of experimental hypertension (3–5). They had been maintained from weaning (21 days of age) on either a low salt chow (0.4% NaCl) or a high salt chow (8% NaCl). At the time of study, they were all approximately 3 months of age and were of both sexes; a pilot study had indicated that sex and age (up to 7 months) were without consistent influence on the results.

Man. The volunteers were employees of Brookhaven National Laboratory; all were working and in apparently good health. From the records of their annual physical examinations in the employees' clinic, we selected 17 males with repeatedly documented asymptomatic hypertension ($\geqq$ 140/90 mm Hg), and 19 presumed normotensive male controls matching the hypertensives for age and weight. Six of the controls, however, were found to have borderline blood pressure elevations (either systolic $\geqq$ 140 mm Hg *or* diastolic $\geqq$ 90 mm Hg) and have been excluded from this study.

Methods. Sample collection—rats. Animals fasted overnight were bled under ether/oxygen anesthesia by nicking the tail. This blood is mainly arterial. Approximately 0.5 ml of blood was permitted to run directly into each of two pre-weighed test tubes from which the exact amount in each tube was found by subsequent weighing within a few minutes of sampling. One ml of 6% perchloric acid was used to stop the production of LA, and was added to tube No. 1 before the first weighing. Tube No. 2 was empty and blood in the latter tube was permitted to clot at 20°C for exactly 45 min at which time the perchloric acid was added, the clot was crushed and stirred with a glass rod. (In preliminary studies with blood from both rats and human beings, samples had been allowed to incubate at 20°C for 15, 30, 60, and 120 min as well: 45 min proved optimal.) The sample was then centrifuged, after which the supernate was separated and immediately stored at −20°C until the LA determination was made. Previously it had been determined that such storage was without influence on the results.

Sample collection—man. Approximately 1.5 ml of blood was collected in a dry syringe between 9–10:00 a.m. from a forearm vein, without stasis, on individuals who had fasted overnight and all of whom, after arriving in the clinic and before the blood sample was taken, had rested in a chair at least 30 min. Two 0.5-ml aliquots were then treated precisely as described for the rats.

Miscellaneous. Rat blood pressures were measured by a modification of the method of Friedman and Freed (6) on the day prior to sample collections. Weights and blood pressures in the study of human subjects were recorded immediately after each blood sample was drawn.

Lactic acid was determined in whole blood extract by an enzymatic method (7).[1] Results are reported as mg of lactic acid per 100 ml of blood fluid; all concentrations were calculated on the assumption that 80% of the sample weight represented the fluid phase.

Results. Rats (Table I). All values obtained in blood without incubation were within the reported normal range, for man, of 5–20 mg of lactic acid/100 ml of serum, (8). Incubation *in vitro* at room temperature increased the lactic acid content. Both at zero-time and after 45-min incubation, there was no significant difference of LA concentration among the four groups of rats, with or without hypertension and on low or high NaCl diet. Subdivision by sex showed no differences.

Man (Table II, Fig. 1). All zero-time values were within the normal limits reported. However, there was a small difference of borderline significance ($0.025 < p < 0.05$) between the average values of the normotensive and hypertensive groups. Incubation at

[1] Boehringer Test Combination in kit form was used.

TABLE I. Blood Pressures and Blood Lactic Acid Concentrations in Some Rat Populations.[a]

Strain[b]	Diet (NaCl %)	No. in group	Age (months)	Systolic BP ± SD (mm Hg)	Lactic acid in blood ± SD (mg/100 ml) Incubation (0 min)[d]	Incubation (45 min)[d]
R	8	61	3	116 ± 10	8.5 ± 4.9	26.9 ± 5.8
R	0.4	48	3	115 ± 12	7.0 ± 2.1	24.5 ± 3.1
S	8	44	3	167[c] ± 5	6.8 ± 3.3	26.7 ± 3.9
S	0.4	53	3	125 ± 12	7.2 ± 3.5	26.1 ± 4.4

[a] Rats were of both sexes; derived from Sprague-Dawley strain, and bred for sensitivity or resistance to NaCl hypertension. Details of sampling procedure and methods in text.
[b] R = strain genetically resistant to developing hypertension; S = strain genetically susceptible to developing hypertension.
[c] BP significantly ($p < .001$) higher than in other groups.
[d] No significant difference between LA concentration of these groups.

room temperature increased the difference between the average values of patient and control groups significantly ($p < 0.001$). Human blood had a lower rate of LA production *in vitro* than rat blood.

Discussion. Our results, in accord with reports of larger and more representative studies on human beings, indicate that as a group, hypertensive subjects have higher blood lactic acid values than normotensive. Among individuals, however, there was considerable overlap between those with and without hypertension, and detailed analysis showed that there was no direct correlation between the level of blood pressure and lactic acid (LA) concentration. This is in contrast to the report of Gupta *et al.* (2), but consistent with the findings of Demartini *et al.* (1).

The results obtained on rats were in contrast with the findings in humans: we were unable to find any significant differences in blood lactic acid values of rats with or without experimental hypertension. These rats were of a genetic uniformity, hardly to be found in man, in that one strain was genetically resistant, the other genetically susceptible, to the development of experimental hypertension from a variety of techniques. Such strains would seem ideally suited to demon-

TABLE II. Lactic Acid Values in Human Blood.[a]

Group[b]	No. in group	Blood pressure ± SD (mm Hg) Systolic	Diastolic	Lactic acid in blood ± SD (mg/100 ml) Incubation (0 min)	Incubation (45 min)
HT	17	172.4 ± 20.7	107.0 ± 12.5	10.2 ± 2.0	18.0 ± 2.2
NT	13	117.3 ± 9.3	72.8 ± 6.4	8.6 ± 1.8	13.9 ± 1.7[c]
	Difference	55.1	34.2	1.6	4.1
	t value	8.9	9.0	2.27	5.41
	p	$< .001$	$< .001$	$.025 < p < .05$	$< .001$

[a] All subjects were male. All patients were ambulatory and had asymptomatic hypertension documented prior to this study. Hypertensive and control groups were matched for age and weight. Nineteen subjects were studied in the normotensive group, but six were eliminated from statistical analysis because at the time of this study, the systolic blood pressure > 140 mm Hg or diastolic blood pressure > 90 mm Hg.
[b] HT = hypertensive; NT = normotensive.
[c] Based on 12 determinations (1 sample lost in preparation).

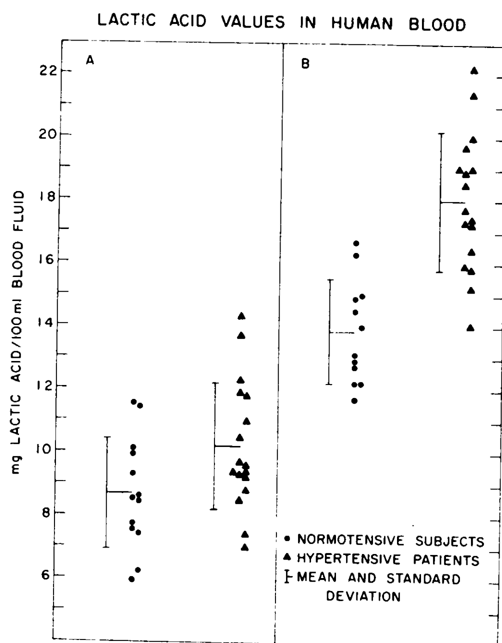

FIG. 1. Individual values obtained in normal and hypertensive subjects. A: Values obtained in blood without incubation. B: Values obtained in blood after 45-min incubation at room temperature.

strate subtle biochemical differences between individuals with and without hypertension. Rats from the susceptible strain can be studied before as well as after the disease is manifest, and the results compared, not only with one another, but also with results obtained on rats which predictably never will suffer from the disease.

The variable occurrence of elevated LA levels in individual human beings with established hypertension, and no such elevations in rats with early hypertension, suggest (if the basic hypertensive processes are similar in the two species) that in man the lactic acidemia is a *result* of the disease, rather than part of the pathogenesis.

Demartini *et al.* (1) reported that "lactic acid was measured in serum obtained from blood that has been allowed to clot by standing for 45 min." Lactic acid is produced by red blood cells *in vitro*, and the length of time during which the blood stands and the rate of red cell metabolism may also influence the results. Since most of the differences occurring in our study developed during *in vitro* incubation, it appears that erythrocyte metabolism was higher in the hypertensive patients. Why there should be a difference between normotensive and hypertensive individuals in this respect is not clear, but the findings indicate that this must be listed as a possible explanation along with those suggested by Demartini *et al.* (1).

Our findings in the rat indicate that although this species has *in vivo* blood lactic acid values comparable to those in man, after *in vitro* incubation at room temperature for 45 min, the blood from rats had a significantly ($p < .001$) higher LA concentration than human blood incubated for the same time. This difference might be explained by the difference in cell size. Although hematocrit values are comparable, rat blood has twice the number of RBC per unit volume; this means that rat RBC has a larger cell surface per volume which requires a more active cation transport, other conditions being equal. Whittam *et al.* (9) have shown that energy production in erythrocytes is partly regulated by the requirement of ATP for the active transport of Na and K. Since erythrocyte metabolism is anaerobic, differences in metabolic rate are reflected in differences in lactate production. *In vivo* the lactate may be utilized by tissues, whereas *in vitro* it will accumulate.

Summary. Among rats from two strains with opposite genetic predisposition to experimental hypertension, lactic acid concentrations in the blood were equivalent. Lactic acid concentration could not be correleated with the presence or absence of overt hypertension. Blood lactic acid values in these rats were comparable to those in man. In confimation of reports by others, among humans the average blood lactic acid value of a small group of hypertensive patients was increased as compared with the average value of an appropriate control group. Lactic acid concentration in all samples increased when the blood was allowed to stand at room temperature. This increase was larger in rat blood than in human samples and, in man, it increased faster in hypertensive patients than in controls. It is proposed that these differences may reflect ion transport activity by

the red cell, and that the difference between man and rat is related to the dissimilar surface-to-volume ratio of the cells.

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Presence of Antibodies to Simian Virus 40 (SV40) T Antigen in Rhesus Monkeys Infected Experimentally or Naturally with SV40* (33043)

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The tumor (T) antigen of SV40 is present in nuclei of all cells transformed by SV40 *in vivo* or *in vitro* (1,2) and it is also produced during the acute cytolytic phase of SV40 multiplication (3,4). Antibodies to T antigen, detectable by complement fixation and immunofluorescence tests, were first demonstrated in sera of hamsters bearing SV40-induced tumors and in this species were found exclusively in tumorous animals (2,5). Recently, it has been shown that T antibodies develop without the production of tumors in African green monkeys infected experimentally with SV40 (6–8).

Relatively little is known about the course of SV40 infection in rhesus, although it is the natural host of the virus. In a study now in progress, nonimmune juvenile rhesus were infected with SV40 by several routes. The present communication reports the development of SV40 T antibodies in the sera of these rhesus and its relationship to routes of infection and viremia. It also records the finding of T antibodies in a proportion of naturally infected rhesus.

Materials and Methods. Juvenile rhesus, 1–2 years old, were obtained from the free-living groups on Cayo Santiago, an islet off the east coast of Puerto Rico. This island is a part of the facilities of the Laboratory of Perinatal Physiology. These rhesus groups appear to be free of active SV40 infection (K. V. Shah and J. A. Morrison, unpublished data). A total of 16 animals were infected as follows: six each by subcutaneous (s.c.) and intranasal (i.n.) routes with 0.4 ml of a $10^{-1.0}$ dilution of virus and four by intragastric (i.g.) route by introduction of 1 ml of undiluted virus by a stomach tube. The gastric acidity was neutralized by 40 ml of 6% $NaHCO_3$ in two of four infected (serum series 103 and 107) by the i.g. route. The animals were caged individually and bled periodically for tests for viremia and antibodies. Two uninfected controls in individual cages were kept in the same room as the i.g. inoculated group.

Strain A2895 of SV40, isolated originally from a hamster tumor produced by inoculation of rhesus kidney extracts (9), was obtained from Dr. B. Eddy. Prior to use in this

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