TABLE I. Serum Transaminase—Pre-irradiation and at Intervals Post-exposure. Means and standard errors.

	Pre-irradiation		+ 3 hr		+ 6 hr		+ 24 hr	
	N	Units/ml	N	Units/ml	N	Units/ml	N	Units/ml
Sham controls	15	22 ± 1.3	15	35 ± 2.2	15	30 ± 2.2	15	24 ± 1.7
500 r	12	24 ± 0.9	12	33 ± 1.3	12	38 ± 2.7*	11	31 ± 2.7*
750 r	12	22 ± 2.7	12	65 ± 14.1*	9	50 ± 5.3*	6	45 ± 7.9*
1,000 r	13	26 ± 1.7	12	52 ± 5.6*	11	66 ± 13.5*	8	42 ± 3.7*

* Difference statistically significant. Difference between means of sham controls and experimentals exceeds the stand. error for the difference by 2 + times.

TABLE II. Serum Transaminase and Time of Death (625 r Total Body Exposure).

Rabbit No.	Pre-irrad., units/ml	Max value 6-24 hr post-irrad., units/ml	△, units/ml	No. days 'til death
1	36	40	+ 4	12
2	26	50	+24	S* 30 d
3	30	50	+20	S 30 d
4	25	73	+46	21
5	26	51	+25	S 30 d
6	28	87	+59	8
7	29	43	+14	7
8	26	39	+13	S 30 d
9	38	33	- 5	13
10	15	50	35	8

* S = Survived.

irradiation in dosages of 500, 750, and 1,000 r. Serum glutamic oxaloacetic transaminase estimations were accomplished at 3, 6, and 24 hours post-irradiation. A significant increase in G-O-T activity could be identified at 6 hours in the rabbit group subjected to 500 r, and as early as 3 hours post-irradiation in the 750 r and 1,000 r animal groups. For the latter animals, the noted increase at 6 hours served to segregate 75% of the group on an individual basis. In rabbits subjected to 625 r of x-irradiation no relation was found between the serum G-O-T elevation and survival time.

1. Milch, L. J., Stinson, J. V., and Albaum, H. G., *Radiation Res.*, 1956, v4, 321.

2. Karmen, A., Wroblewski, F., and LaDue, J. S., *J Clin. Invest.*, 1955, v34, 126.

Received October 26, 1956. P.S.E.B.M., 1956, v93.

Pathogenesis of Experimental Arteriosclerosis in the Rat.* (22834)

DAVID LEHR AND CONSTANCE R. MARTIN

Department of Physiology and Pharmacology, New York Medical College, Flower and Fifth Avenue Hospitals, New York City.

We have demonstrated that the standardized production of severe arteriosclerosis (Mönckeberg type) and myocardial necrosis by means of renal injury in the albino rat can be completely prevented by prior thyroparathyroidectomy(1). Studies directed towards elucidation of the mechanism underlying this striking protective effect of thyroparathyroidectomy, led to the conclusion that the cardiovascular injury of intact rats was caused primarily by autointoxication with parathyroid hormone. On further analysis it was inferred that excess function of the parathyroid glands following standard renal damage was mediated through the adrenal cortex. This communication describes experiments which formed the basis for these conclusions, and outlines our present concept of the mechanism of experimental arterial mediosclerosis.

Procedures. The method used for production of lesions has been described previously (2). The procedure consists in a single par-

* This investigation has been aided by grant H-890 (C5) of N. Heart Inst., N.I.H., U.S. Public Health Service.

enteral injection of the sodium salt of a poorly soluble sulfonamide in excessive dosage. This induces invariably massive and long-lasting intrarenal deposition of sparingly soluble crystals and results in obstructive nephropathy and permanent kidney damage, followed by severe disseminated cardiovascular and smooth muscle lesions in 4 to 7 days. The pathologic-anatomic changes are readily recognizable with the naked eye. Rats with typical injury manifest enlargement of parathyroids and adrenals as well as atrophy of thymus gland. Animals permited to survive for longer periods (2 to 5 weeks) show apparently no substantial progression of the injury but correspondingly increasing degrees of reparative changes in damaged organs such as scar formation and intense calcification of necrotic areas. Since emergence of these severe cardiovascular lesions could be obviated by prior thyro-parathyroidectomy, and since, in preliminary observations, a similar protective effect was achieved by adrenalectomy(3), further analysis of the mechanism responsible for disseminated muscular necrosis concerned the relative importance of the separate thyroid and parathyroid hormones and their possible relationship to the adrenal cortex. Three hundred adult male and female albino rats were used. In addition to thyro-parathyroidectomy, hypophysectomy and nephrectomy, the following procedures were carried out: Surgical removal of parathyroids and adrenals separately as well as in combination, elective and graded hormone substitution in such animals and in thyro-parathyroidectomized rats, and finally chemical "thyroidectomy," using 0.01% propylthiouracil in drinking water for a minimum of 4 weeks preceding the experiment. For hormonal substitution or treatment, cortisone and desoxycorticosterone acetate were injected subcutaneously in daily dosages of 3 mg each, starting 3 days prior to initiation of renal injury and continuing for a minimum of 3 days thereafter. Similarly, parathyroid extract was injected subcutaneously for substitution or poisoning in daily dosages ranging from 10 to 200 International Units, starting 5 days prior to kidney block and continuing for at least 5 days thereafter. Serial microdeterminations of calcium(4) and inorganic phosphate(5) were done in plasma of rats from most experimental groups. Complete postmortem examinations were performed on every animal. All important organs were fixed in formalin and sectioned for microscopic study using routine and special stains.

Results. Distribution of 9 sub-groups comprising 215 rats with various hormonal deficiencies and type of hormone employed are shown in Table I (Groups 2-11). For conciseness, experimental results are presented only for medionecrosis and calcification of the arterial tree as the severest and most consistent lesion (93% in intact rats, Group 1). However, data on vascular damage are fully in line with those for muscular necrosis in heart and gut.

Table I shows that treatment of thyroparathyroidectomized rats with parathyroid extract in amounts of 50 units daily induced typical vascular damage (Group 3). Since even the smallest dosage (10 units) raised plasma calcium levels to normal and allowed for development of nephrocalcinosis, parathyroid substitution was believed to be more than adequate so that these rats could be considered as thyroidectomized only. It is clear, therefore, that vascular necrosis did occur in complete absence of the thyroid gland. Similarly, chemical "thyroidectomy" did not prevent vascular injury, although it did provide a substantial reduction of its incidence (Group 4). The lowered incidence was interpreted as due to diminution of parathyroid hormone formation in the absence of thyroid hormone.

The singular significance of parathyroid hormone in pathogenesis of arterial necrosis is illustrated by the full protection afforded the 30 rats of Group 5 by parathyroidectomy alone. Moreover, one can readily enlarge this number by adding the 29 rats of Group 2, and, with some reservations, the 14 animals of Group 9. Thus, none of a total of 73 parathyroprive rats showed vascular lesions or other muscular necrosis under careful microscopic examination. Exhibition of parathy-

TABLE I. Hormonal Influences upon Development of Renogenic Arterial Medionecrosis in Albino Rats. (215, 4- to 6-mo-old animals of both sexes, with standard renal injury.)

Group No.	Endocrine status prior to hormone admin.	Hormone treatment or substitution daily	No. in group ♂	No. in group ♀	No. in group Total	No. with arterial necrosis	Incidence of arterial necrosis, %
1	Intact	—	31	15	46	43	93
2	TP X	—	24	5	29	0	0
3	TP X	PE (10-20 units)		5	5	0	
		PE (50 ")		5	5	2	
4	"T X"	—	4	8	12	3	25
5	P X	—	22	8	30	0	0
6	P X	PE (100 units)	18		18	13	72
7	A X	Cortisone	18	6	24	0	0
8	A X	Cortisone & DCA	10	10	20	12	60
9	A X & P X	*Idem*	7	7	14	0	0
10	A X	Cortisone & PE (200 units)	12		12	8	67

X = Removal of gland, T = Thyroid, P = Parathyroid, A = Adrenal.
PE = Parathyroid extract, DCA = Desoxycorticosterone acetate.

roprive rats to excess amounts of parathyroid extract (100 I.U. daily) restored their ability to develop the characteristic lesions, as depicted in Group 6. Of 18 rats in this group, 13 or 72% presented typical vascular damage.

It was postulated, therefore, that autointoxication with hormone from excessively secreting parathyroid glands was responsible for emergence of muscular necrosis in intact rats with renal injury, (Group 1). Further evidence was seen in behavior of plasma calcium and phosphate levels of these animals in the initial stages of renal disease, in which calcium levels were normal or even elevated (9-15 mg%) in the face of marked retention of inorganic phosphate (8-12 mg%).

In further pursuing the role of parathyroid gland, rats deprived of their adrenals and maintained on cortisone did not incur parathyroid hypertrophy, and, like parathyroidectomized rats, failed to develop the characteristic picture of disseminated muscular necrosis (Group 7). However, when adrenalectomized animals were given DCA in addition to cortisone (Group 8), the parathyroid glands became greatly enlarged and typical cardiovascular and smooth muscle necrosis appeared. (Almost identical observations were made with hypophysectomized rats, not shown in Table I.) On the other hand, when adrenalectomized animals were, in addition, deprived of their parathyroid glands, DCA administration appeared to have lost its deleterious effect (Group 9), whereas typical muscular injury could be readily produced in adrenalectomized rats given parathyroid extract (200 I.U. daily) *instead* of DCA (Group 10).

Under our experimental conditions, therefore, desoxycorticosterone administration in itself to rats with standard renal injury does not induce arterial necrosis; rather an ample supply of adrenal steroid of this type seems essential for development of hyperparathyroidism which, in turn, causes vascular injury. Hence a hitherto unknown direct or indirect stimulatory action of mineralocorticoids upon the parathyroid gland must be postulated. Preliminary evidence indicates that, to a certain extent, androgens can enhance this desoxycorticosterone effect, whereas estrogens cannot. In fact, female sex hormone which has been shown to be capable of inhibiting the output of adrenal steroids(6), appears to counteract the development of muscular necrosis.

In line with this observation, the incidence and severity of arteriosclerosis in the young adult rat were found to be substantially higher in male than in female animals. This difference was most pronounced in 2-month-old animals, and gradually decreased with advancing age of the rat. In 2-month-old rats (*not used in this study*), incidence of lesions is around 80% in the male rat and around

TABLE II. Influence of the Parathyroid Gland upon Development of Cardiovascular Necrosis in Nephrectomized Albino Rat. (Male animals, 4-6 mo old.)

Group	Description of procedures	Rat No.	Days of survival post-nephrectomy	Macroscopic pathology: Necrosis of Myocardium	Macroscopic pathology: Necrosis of Aortic media
A	Control, nephrectomy only	1	3	—	—
		2	2	—	—
		3	4	—	1+
		4	3	4+	2+
		5	3	1+	1+
		6	3	1+	—
		7	3	2+	1+
		8	3	1+	1+
B	Parathyroid extract, 100 I.U. s.c., on 1st and 2nd day post-nephrectomy	9	3	3+	3+
		10	3	4+	1+
		11	3	4+	3+
		12	3	4+	3+
		13	3½	4+	2+
C	Parathyroidectomy, 1 wk prior to nephrectomy	14	3	—	—
		15	3	—	—
		16	4	—	—
		17	4	—	—
		18	5	—	—

Definition of lesions: —, none; 1+, mild, localized; 2+, moderate and more extensive; 3+, severe and widespread; 4+, maximal degree.

50% in the female rat. In the 4-6-month-old group, male rats show an incidence of 95% of mostly severe aortic lesions, compared with an incidence of 70% of moderate to severe lesions in female animals.

In our most recent studies, involving 10 adrenalectomized, 15 parathyroidectomized and 50 nephrectomized rats, the following additional facts were established: (1) It is possible to induce marked hypertension and the characteristic picture of disseminated necrosis in heart, arterial tree and gut in parathyroidectomized rats *not* subjected to standard renal injury, by daily administration of 100 to 200 units of parathyroid extract. These animals may also develop marked nephrocalcinosis. (2) Adrenalectomized rats maintained on saline and not exposed to standard renal injury, manifest invariably pronounced systolic hypertension within 24 hours following injection of 100 units of parathyroid extract with pressures ranging from 160-210 mm Hg. Daily reinjection results in maintenance of hypertension except for a transient marked dip in blood pressure following each administration and lasting about 6 hours. (3) Cardiovascular necrosis and hypertension can likewise be produced in the rat by nephrectomy (Table II, Group A), which speaks against the importance of a renal factor in their development. The organic changes are closely similar to those induced by obstructive nephropathy and not unlike the pathologic-anatomical picture observed by Grollman and associates(7) and others in renoprival animals. (4) Severity of cardiovascular necrosis and hypertension produced by nephrectomy can be substantially enhanced by administration of parathyroid extract (100 units) on first and second day following removal of kidneys (Table II, Group B). (5) When parathyroprive rats are nephrectomized, they do not develop any lesions in the myocardium or the arterial tree recognizable under magnifying glass, although they seem to survive somewhat longer than rats with intact parathyroid glands (Table II, Group C).†

Comments. The concept of the mechanism leading to cardiovascular necrosis and calci-

† (The nephrectomy studies, exemplified in Table II, were carried out in cooperation with Dr. Isabelle Wajda. The results have since been fully duplicated and will be published elsewhere in detail.)

fication which emerges from our studies is briefly as follows: The "stress" of obstructive nephropathy causes adrenal cortical hypertrophy. Increased output of desoxycorticosterone-like hormones probably triggered primarily by severe derangement of mineral metabolism, results in pronounced stimulation of the parathyroid glands. Autointoxication with parathyroid hormone, in turn, is responsible for the picture of disseminated cardiovascular necrosis and calcification and substantially enhances renal injury (nephrocalcinosis). Concomitant "renal" hypertension probably contributes to the development of vascular lesions. In the light of this concept, it is understandable that mechanisms or procedures which tend to interfere with the availability of the desoxycorticosterone-like steroids, may result in a diminution of the extent and incidence of cardiovascular necrosis, whereas an increase in supply of such steroids may have the opposite effect.

These animal-experimental findings lead us to believe that in human disease as well the parathyroid gland may play a more important role in pathogenesis especially of renogenic cardiovascular necrosis and hypertension than is commonly assumed. For example, it is well known that rapidly progressive malignant hypertension, usually distinguished by extensive medionecrosis of small arteries, is often dramatically arrested by adrenalectomy(8,9). Credit customarily goes to removal of excess mineralo-corticoids and the consequent decrease of hypertension. The possible presence of a hyperfunctioning parathyroid gland is usually considered disproven by the finding of normal blood calcium levels, although it should be realized that, in subacute and chronic phases of clinical as well as experimental hyperparathyroidism, blood calcium levels may return to within the normal range. Moreover, one cannot exclude a possible inhibitory effect of adrenalectomy upon output of a specific vasoactive parathyroid factor, claimed to be separable from the calcium mobilizing activity of parathyroid extract(10) and responsible for both spasm and necrosis of muscles. Such a vasoactive principle may, in fact, represent the long sought extrarenal pressor principle held responsible for both renal and renoprival hypertension and known to be dependent upon adequate adrenal cortical activity(11). Finally, presence of anatomically normal parathyroid glands should not be relied upon as evidence against hyperfunction since, under physiological conditions, these glands were shown to operate only at a small fraction (5-25%) of their full capacity(12). This wide margin of safety allows for excess secretion without alteration of anatomical structure(13). It is conceivable, therefore, that subtotal parathyroidectomy may be more effective than the far more radical adrenalectomy in arresting rapidly progressive sequellae of severe renogenic hypertension.

Our data do not support the time-honored contention that in hyperparathyroidism, calcium deposition occurs in *normal* tissue, so-called "true metastatic calcification" in contradistinction to dystrophic calcification(14), since in our experimental animals calcium imbibition was invariably preceded by disseminated necrosis. Autopsies performed at predetermined stages of the experimental disease proved beyond doubt that areas of necrosis were those which later became calcified. It is of special interest in this connection that in a recently reported post mortem examination of a patient who died from renogenic hyperparathyroidism, the myocardium was found to contain foci of fresh necrosis, a fact which in the authors' view was the reason for extreme severity of "metastatic calcification" (15). Significantly they state that the cause for necrosis is obscure. In the more chronic phase of hyperparathyroidism when healing predominates, especially following various therapeutic efforts, necrosis may be absent, so that foci of calcification appear embedded in otherwise normal tissue. This is the very situation most commonly encountered at autopsy in human beings. It is probably responsible for the concept of "metastatic calcification."

Finally, it would appear in the light of our findings that a number of experimental lesions ascribed in particular to direct effects of desoxycorticosterone and saline(16) or a com-

bination of these with renal insufficiency(17) may deserve reevaluation.

Summary. 1. Pathogenesis of severe arteriosclerosis and disseminated myocardial necrosis, induced in the albino rat by standard renal injury, was investigated. These cardiovascular lesions are due to autointoxication with parathyroid hormone, and this hormone apparently contains an important hypertensive factor. 2. Evidence was presented that excess production of parathyroid hormone, as a consequence of renal dysfunctions, is mediated through the adrenal cortex and that mineralocorticoids are essential for direct or indirect activation of the parathyroid gland. To some degree, androgens can enhance the mineralocorticoid effect, whereas estrogens seem to inhibit emergence of muscular necrosis. The incidence of cardiovascular injury in intact and adrenalectomized rats was found to be substantially higher in male than in female animals. 3. It was shown further that, in the absence of parathyroid glands, mineralocorticoids in excessive dosage fail to produce cardiovascular necrosis, whereas excess parathyroid hormone assures emergence of typical lesions in complete absence of mineralocorticoids. In fact, by providing abundant supplies of parathyroid extract, cardiovascular necrosis was induced without renal injury and even in absence of kidneys. 4. Implications of these findings upon the concept of "metastatic calcification" and upon pathogenesis and therapy of renogenic cardiovascular necrosis and hypertension in human beings are discussed. It is suggested that subtotal parathyroidectomy be tried in preference to adrenalectomy in attempts to arrest rapidly progressive sequellae of malignant hypertension.

1. Lehr, D., and Martin, C., *Endocrinology,* 1956, v59, 273.

2. Lehr, D., and Churg, J., *J. Mt. Sinai Hosp,* 1952, v92, 32.

3. Lehr, D., unpublished data, 1953.

4. Rappaport, F., *Rapid Microchemical Methods of Blood and CSF Examinations,* Grune & Stratton, New York, 1941, p116.

5. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.,* 1925, v66, 375.

6. Vogt, M., *J. Physiol.,* 1955, v130, 601.

7. Muirhead, E. E., Turner, L. B., and Grollman, A., *A.M.A. Arch. Path.,* 1951, v51, 575.

8. Green, D. M., Nelson, J. N., Dodds, G. A., and Smalley, R. E., *J.A.M.A.,* 1950, v144, 439.

9. Thorn, G. W., Harrison, J. H., Merrill, J. P., Criscitiello, M. G., Frawley, T. F., and Finkenstaedt, J. T., *Ann. Int. Med.,* 1952, v37, 972.

10. Handler, P., and Cohn, D. V., *Am. J. Physiol.,* 1952, v169, 188.

11. Wilson, C., and Hedingham, J. M., *Acta Med. Scand.,* 1956, v154, 86.

12. Rosof, J. A., *J. Exp. Zool.,* 1934, v68, 121.

13. Greep, R. O., *Histology,* Blakiston Co., New York, 1954, p822.

14. Barr, D., *Physiol. Rev.,* 1932, v12, 593.

15. Crawford, R. L., and La Zerte, G. D., *Arch. Int. Med.,* 1955, v96, 818.

16. Selye, H., Hall, G. E., and Rowley, E. M., *Canad. M. A. J.,* 1943, v49, 88.

17. Selye, H., and Pentz, E. I., *ibid.,* 1943, v49, 264.

Received July 19, 1956. P.S.E.B.M., 1956, v93.