Table 1. Differential recovery of the first set response to allogeneic and xenogeneic skin grafts in lethally x-irradiated (870 rad) C3D/2 F_1 mice protected with urethan and/or syngeneic bone marrow.

Time grafted post-irradiation (day)	Treatment			Set	No. of mice	Mean graft survival time (days ± S.D.)			
	Urethan	Bone marrow	870 rad			A/HeJ	BALB/c	C57L	Rat
*				1	10	13.8 ± 1.6	14.4 ± 2.2		7.9 ± 0.6
*				2	9	6.1 ± 1.0	6.1 ± 1.4	9.2 ± 1.3†	3.0 ± 0.0
0.2	+		+	1	4	>140‡	>140‡		34.7 ± 3.0
0.2		+	+	1	6	81.8 ± 5.0	89.6 ± 10.0		28.0 ± 5.1
0.2	+	+	+	1	4	89.5 ± 5.0	89.5 ± 5.0		31.5 ± 2.4
62	+		+	1	4	>85‡	>85‡		12.5 ± 4.6
92	+		+	1	5	31.0 ± 13.3	40.0 ± 10.1		8.7 ± 0.6
92	+		+	2	4	6.3 ± 1.2	7.5 ± 1.1	11.7 ± 0.6†	4.0 ± 0.0
240		+	+	1	6	25.1 ± 9.1	30.0 ± 7.5		8.3 ± 1.3
240		+	+	2	6	17.0 ± 10.3	18.0 ± 7.2	22.0 ± 4.6†	
350		+	+	1	7	30.8 ± 3.1	33.4 ± 3.2		9.4 ± 0.4
350		+	+	2	7	12.3 ± 2.8	12.6 ± 2.7	15.4 ± 1.0†	4.3 ± 1.4

* First and second-set norms for nonirradiated adult male C3D/2 F_1 mice. † First-set C57L skin graft. ‡ The grafts were intact at the time the manuscript was prepared.

of a third H-2 type was placed (that is, first-set, C57L).

The mice grafted immediately after irradiation rejected their rat skin grafts in approximately 30 days but retained the allogeneic grafts over 80 days (several allogeneic skin grafts were intact at the time this report was written), regardless of whether they were urethan and/or bone marrow protected (Table 1). The first-set response to rat skin grafts was slightly impaired in the group grafted 62 days after irradiation but normal in the group grafted at 92 days. However, the first-set response to allogeneic grafts was still impaired in the group grafted 350 days after irradiation. The second-set response to allogeneic skin grafts was vigorous in the urethan-protected group grafted 92 days after irradiation but was grossly impaired in the bone-marrow-protected group grafted 240 and 350 days after irradiation.

These data clearly indicate that the cell system involved in the rejection of the xenogeneic (rat) skin graft is functionally distinct from that cell system involved in the rejection of the allogeneic skin grafts, being incapable of recognizing and/or reacting to the transplantation isoantigens of the allogeneic grafts. In addition, the data suggest that, in this strain of mouse, syngeneic bone marrow adds little, if anything, to the immunologic recovery of the lethally irradiated host (*4*).

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24 June 1963

Renal Baroceptor Control of Renin Secretion

Abstract. *Small reductions in renal perfusion pressure to levels still within a physiologic range, which did not reduce renal blood flow, caused the kidney to release renin. Renin appeared in much larger amounts in renal-vein blood than in renal lymph. Release of renin appears to be mediated by a renal baroceptor rather than by ischemia.*

Sixty-five years ago the kidney was found to contain a substance that causes rise in arterial pressure when injected into a vein. It is still not known with certainty whether this substance, renin, may account for some forms of hypertension. The primary difficulty has been lack of adequate methods for its detection in circulating blood.

Renin is an enzyme; it raises arterial pressure by acting on alpha-2-globulin in blood to create a decapeptide termed angiotensin I. The two terminal amino acids of the decapeptide are split off by an angiotensin "activator"; the remaining octapeptide, angiotensin II, is a vasoconstrictor and the most powerful pressor substance known.

There is currently a revival of interest in the renin-angiotensin system as a cause of hypertension. This is due in large part to three recent developments: (i) the finding that human hypertension associated with partial obstruction of one renal artery is accompanied by increased amounts of renin-like activity in blood draining from that kidney (*1*), (ii) the development of techniques to visualize obstructive lesions of the renal arteries in hypertensive patients, leading to surgical repair and cure of the hypertension, and (iii) the discovery that angiotensin causes the adrenal gland to secrete aldosterone.

It has been assumed that if renin is released by the kidney it will pass directly into the renal-vein blood, but recently Lever and Peart (*2*) found renin in dog's renal lymph after partial constriction of a renal artery and in three experiments were unable to demonstrate it in renal-vein blood. This prompted us to examine the importance of this route of release compared to release directly into renal-vein blood (*3*).

In dogs, renal perfusion pressure was reduced by partially inflating a balloon that had been inserted into the aorta by way of a femoral artery and passed upward to just above the origin of the renal arteries. The left renal vein was cannulated by way of the left testicular or ovarian vein in order that renal-vein blood could be collected without obstruction of blood flow or interference with lymphatics. The thoracic duct, which drains renal lymph into the circulation, was cannulated in the neck, and a cannula was also placed in a peripheral artery. All samples of blood and lymph were heparinized and centrifuged for the same time at room temperature and the cell-free supernatants were frozen until the time of assay. Ten to 15 minutes elapsed between collection and refrigeration. Pressor material in plasma and lymph was assayed by intravenous injection of 0.1 to 0.4 ml into rats treated with a ganglion-blocking agent to enhance their pressor responsiveness.

Within 5 to 15 minutes after the balloons were inflated, there was, in all of nine experiments, a slow rise in

arterial pressure. The rise was accompanied consistently by the appearance of considerable pressor activity in renal venous blood and a lesser amount of activity in arterial blood. Pressor activity appeared in thoracic-duct lymph only after 30 minutes or more, and the total amount was small compared with that in renal-vein blood; appearance of activity in lymph did not correlate with change in the dog's arterial pressure.

Thus, pressor material released into lymph appeared not to contribute to the rise in systemic pressure that followed reduction of renal perfusion pressure. To verify this, all renal lymph was drained externally by way of the thoracic duct in one group of dogs and the rises in arterial pressure that followed reduction of renal perfusion pressure were compared with the rises in a group of dogs in which lymph entered the circulation in the normal manner. After 45 minutes of reduced renal perfusion pressure there was no significant difference between the pressor responses of the two groups. Consequently, it is improbable that release of pressor material from the kidney into lymph is concerned in an important way with the early rise in arterial pressure that follows reduction of renal perfusion pressure.

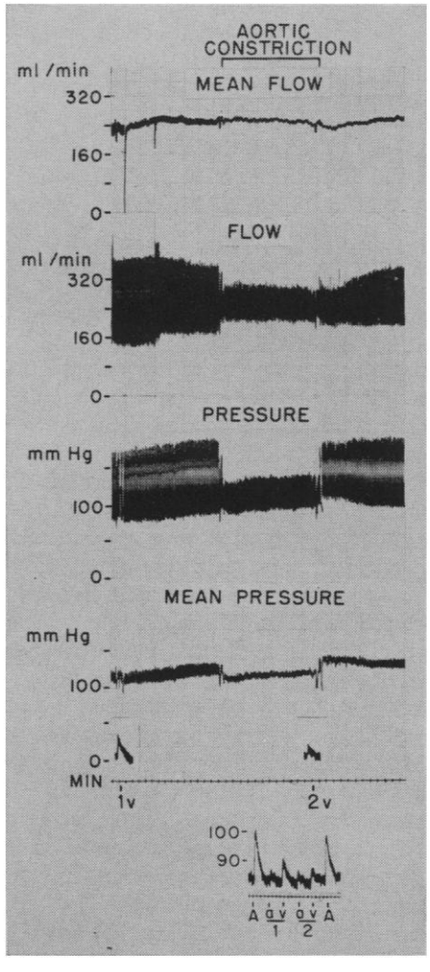

Fig. 1. Changes in renal blood flow and renal perfusion pressure during a 15 min period of aortic constriction above the renal arteries. Pulse pressure was reduced with little effect on mean pressure or mean flow. *1v* and *2v*, Pressor activity in 0.15 ml incubated renal-vein plasma collected at time indicated. Inset at bottom: assay of pressor activity in incubated plasma. *A*, angiotensin 2 ng; *a*, 0.15 ml arterial plasma; *v*, 0.15 ml renal-vein plasma; *1*, *2* indicate times of collection; ordinate, mm-Hg; abscissa, minutes.

The pressor material that appeared in blood and lymph after reduction of renal perfusion pressure is apparently a polypeptide, since it was not inactivated when the proteins were denatured by heat but it was destroyed by chymotrypsin. Pharmacologic blocking agents revealed that it is not norepinephrine or serotonin. Since there is more pressor material in renal venous blood than in arterial blood, it is highly probable that it is angiotensin, made by renin released from the kidney. This is also indicated by the observation that pressor responses to tyramine given to the dog intravenously showed an increase during the period when pressor material appeared in renal-vein blood; this change in responsiveness occurs during intravenous infusion of angiotensin (*4*).

The intrarenal, hemodynamic change that is responsible for the rise in pressure associated with acute release of renin has not so far been identified. After the initial experiments of Goldblatt (*5*) in which hypertension was produced by placing a partially constricting clamp on the renal artery, it was assumed that ischemia was the obvious cause of the hypertension. Doubt was cast when it was found that hypertension could be produced without renal ischemia (*6*). Later, Kohlstaedt and Page (*7*) perfused isolated kidneys at different pressures and obtained results suggesting that a diminution of pulse pressure rather than flow caused release of renin. Unfortunately, these experiments were hampered, as have been all others in this field, by lack of a sensitive and reproducible assay procedure.

During the course of our experiments we noted that pressor material appeared in renal-vein blood after rather slight decreases in perfusion pressure. Whether these small changes in pressure were accompanied by major changes in blood flow was not known. Accordingly, the experiments were repeated while flow was measured with a noncannulating, electromagnetic flowmeter around the renal artery. In these experiments the plasma pressor assay was modified in two ways. The blood was collected [using EDTA (3 to $5 \times 10^{-3}M$) as anticoagulant] in concentrations which inhibit the action of plasma angiotensinase (*8*, and unpublished data) and the plasma was incubated at *p*H 7.4 at 37°C for 60 minutes.

In six of seven normal dogs anesthetized with morphine and pentobarbital, samples of incubated renal-vein plasma showed increased pressor activity within 5 minutes of the time when mean arterial perfusion pressure was lowered to only 80 to 95 mm-Hg from

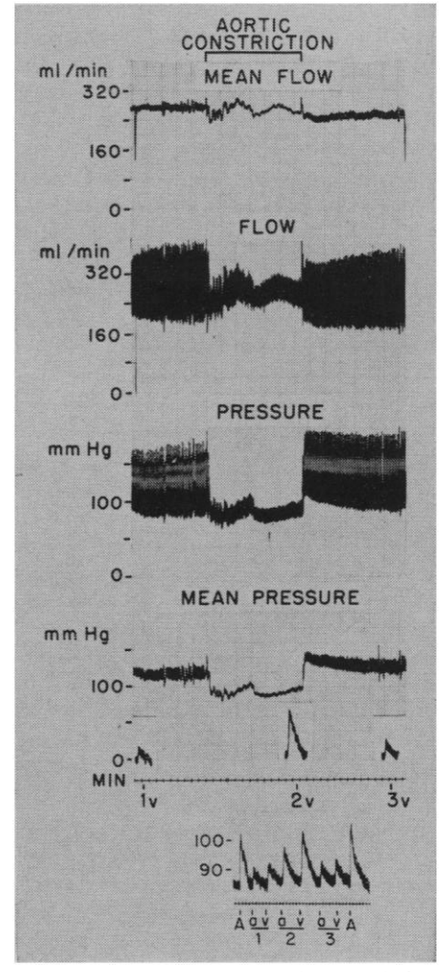

Fig. 2. Effect of further aortic constriction in same experiment as Fig. 1. Mean perfusion pressure was reduced with little change in mean flow. *1v*, *2v*, *3v*, pressor activity in 0.15 ml incubated renal-vein plasma. Inset at bottom: plasma pressor assay as in Fig. 1.

control levels of 100 to 130 mm-Hg by constricting the aorta above the renal arteries. Total renal blood flow remained essentially unchanged with these small decreases in mean perfusion pressure to levels still within the physiologic range. Oxygen saturation of renal-vein blood samples did not alter from control values, being more than 85-percent saturated throughout all experiments.

The first significant change in the character of the arterial perfusion of the kidneys that follows partial constriction of the aorta is a sharp drop in pulse pressure and pulsatile flow accompanied by little, if any, change in mean pressure and mean flow; this alone did not cause increased amounts of pressor material to appear in renal-vein blood. Further constriction by the band around the aorta caused a further decrease in pulse pressure and pulsatile flow and then it began to lower mean pressure but without change in mean flow. A point is reached between 80 to 95 mm-Hg when increased pressor material is detectable. Figures 1 and 2 show the effect of lowering renal-perfusion pressure in stages in one typical experiment. In Fig. 1 a sharp drop in pulse pressure and pulsatile flow with little change in mean pressure or mean flow was not accompanied by any increase in pressor activity in renal-vein plasma. On reducing mean perfusion pressure to 90 mm-Hg without change in mean blood flow (Fig. 2), considerable pressor material appeared in renal-vein plasma and lesser amounts in arterial plasma. As in the previous experiments, chemical and pharmacologic tests indicated that the material in incubated plasma is angiotensin.

It thus appears that release of renin from the kidney involves a baroceptor, but one not sensitive to reduction of pulse pressure alone. Whether superimposing a normal pulse pressure on a reduced mean perfusion pressure will cause cessation of release is not known, but this may occur if renal baroceptors resemble those in the carotid sinus which are more effectively stimulated by a pulsatile than by a steady pressure (*9*).

Since small reductions in renal perfusion pressure to levels still within a physiologic range, but not causing renal ischemia, are sufficient to cause release of renin, the renin-angiotensin system may be a physiologic mechanism that functions in the normal control of arterial pressure. In addition, since angiotensin has been implicated as the trophic hormone for aldosterone secretion, the average pressure level at the renal baroceptor could have an important influence on normal salt and water metabolism as has been suggested by Tobian *et al.* (*10*). Rather than the entirely pathologic system it has generally been suspected of being, it may in the future have to be considered as another physiologic mechanism that, by becoming unregulated, can cause disease (*11*).

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Thyroid Hormones: Control of Terminal Oxidation

Abstract. *Triiodothyronine administered to thyroidectomized rats preferentially increased the total capacity of the electron transport phosphorylation system when isolated mitochondria from the liver were tested with different substrates, but it caused little increase in the activity of the slowest dehydrogenases and no uncoupling. The increased activity appeared to be partly due to direct activation of some component of the electron transport system, although triiodothyronine injection also stimulated the incorporation of amino acids by isolated mitochondria.*

Since thyroxine can uncouple oxidative phosphorylation in vitro (*1*), many investigators believe that the thyroid hormones control terminal oxidation by regulating the efficiency of energy conservation, possibly by inducing mitochondrial swelling (*2*). However, recent work (*3, 4, 5*) has raised considerable doubt about there being any connection between mitochondrial swelling or uncoupling and the influence of physiological levels of the thyroid hormones on the rate of oxygen uptake.

Tata *et al.* (*5*) have suggested two alternative ways in which the thyroid hormones might influence oxygen consumption. They considered it most probable that some process which required energy was stimulated by the hormones, and observed that when triiodothyronine was administered to thyroidectomized rats, there was an increase in the rate of incorporation of amino acids into microsomal protein before the rate of glutamate oxidation was stimulated. However, the results of their experiments also suggested that triiodothyronine increased the electron transport capacity of the mitochondria more markedly than it increased the activity of any of the individual oxidative steps. It is the purpose of this paper to provide evidence that changes in the level of the thyroid hormones affect preferentially the total capacity of the electron transport system without affecting the efficiency of phosphorylation.

Male Wistar rats were thyroidectomized surgically and kept for 5 to 8 weeks prior to use. They were used only if they showed steady weight gains of 0 to 6 g per week for at least 2 weeks (*6*). Suspensions of mitochondria were prepared from liver (*7*) and oxygen consumption was measured with the Clark oxygen electrode (*8*). The suspensions were each incubated with various substrates, and during each incubation period the rate of oxidation of the substrate in the presence and absence of 1 μmole of ADP (*9*), was measured, and estimates of the ratio of P to O (*9*) and the respiratory control ratio (*6*) were obtained.

The results of the experiments are shown in Table 1. Although they are similar to those reported by Tata *et al.* (*5*), they also show that the rate of oxidation of the substrates which have NAD-linked dehydrogenases was affected more markedly when they were mixed together than when they were tested individually. Furthermore, there