longation of survival of homografts is to be attempted by an attack on the cellular factors of rejection, exclusion of host cells must be virtually absolute either by chemically induced leukopenia or by interposing a suitable millipore filter between host and graft(10).

Finally, we must explain the conflict of our findings with those of Levinson and Necheles (11), who reported prolongations of graft survival up to 114 days by administering nitrogen mustard in doses of 0.4 mg/kg every 4 to 6 days for 40 days. The fact that employing their dosage schedule effect no change in outcome of intrastrain homografts (Group IV, Table I) when HN_2 treated animals were compared with simultaneous controls speaks against any profound effect of HN_2 on homograft survival. Certainly, this is the most favorable experimental design with a minimum of factors working against homograft acceptance. The key to the conflict appears to lie in the statement of Levinson and Necheles that "Sprague-Dawley Holtzman rats were utilized" presumably as both donor and recipient. We prevously reported the fact that frequently permanent survivals occur with intrastrain grafts in these inbred animals from this particular source(16). Since the prolongations reported by Levinson and Necheles do not exceed the survival found in reciprocal grafts between untreated Holtzman Sprague-Dawley rats, we can only conclude that prolongation of homograft survival reported by them was due to their selection of experimental animals and the absence of an adequate pairing between reciprocally grafted controls.

Summary. Intensive administration of methyl bis (2 chlorethyl) amine (Nitrogen mustard) to rats and rabbits in doses sufficient to produce and maintain marked leukopenia did not depress host response mechanisms in a degree sufficient to allow significant prolongation of homograft survival.

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Effect of L-3,3',5-Triiodothyronine on Epinephrine-Induced Contraction of Isolated Rabbit Aortic Strip. (25489)

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Thyroxine has been reported to increase sensitivity of isolated perfused swine arteries to epinephrine as evidenced by increase in degree and duration of constriction(1). Increased sensitivity to epinephrine was attributed to inhibition of amine oxidase in walls of the artery. Other experiments* indicated

* Personal communication from R. A. McLean and R. J. Geus of Smith Kline & French Labs., Phila., Pa.

that L-3,3′,5-Triiodothyronine (T_3), a thyromimetic agent closely related to thyroxine, potentiates epinephrine-induced contraction of another vascular smooth muscle, that of isolated rabbit aortic strip. Results of several investigations(2) indicated that ability of various agents to potentiate the effects of epinephrine on isolated organs can quite often be related to chelating or complexing action of these compounds. The purpose of our study was to determine if a similar metal-dependent mechanism was operating in *in vitro* potentiation of epinephrine by T_3.

Method. Spiral strips of rabbit aorta were mounted in 50 ml muscle chambers according to the method described by Furchgott(3). The muscle bath, maintained at 37°C, contained a modified Ringer's solution with 1 g of glucose/liter. 95% O_2-5% CO_2 was bubbled through both muscle bath and reservoir bottle, giving pH 7.4. Contractions of aortic strips were recorded on smoked kymograph by isotonic levers. Distilled, demineralized water was used in making Ringer's and isotonic saline. The stock solution of l-epinephrine hydrochloride (Parke-Davis) was kept frozen prior to use. Working solutions of desired concentration were freshly prepared by triturating the drug in isotonic saline, adjusting the pH to 8.5-9.0 with 0.1N NaOH and stiring until all drug was in solution. Isotonic saline served as vehicle for all other drugs used. The volume of any drug addition to muscle bath was 0.2 ml or less. Drug concentrations refer to the salt except for T_3, where concentration is expressed in terms of free acid.

Results. 1) *Control* responses to standard 1×10^{-7} concentration of epinephrine were determined only after 2½ to 3 hours following the mounting of aortic strips in muscle chamber. According to Furchgott(3), maximum sensitivity to epinephrine is reached at this time, after which the contractile response to epinephrine does not significantly change. This finding was confirmed in the present study. Control responses to epinephrine were of 2 types. In most experiments, epinephrine-induced contractions were quite prolonged, often lasting 1½ hours or more. In a few experiments, however, duration of epinephrine contraction was relatively short (10 to 30 minutes). The height of contraction averaged about 10 mm in both cases, which was well below the maximum possible.

2) *Effect of T_3 on Epinephrine-induced Contractions.* After 2 or 3 epinephrine control responses were observed, with washing after each, T_3 was injected into the bath to a concentration of 1×10^{-6}. No response was evoked in any experiment by T_3 alone. The contractile response to epinephrine was again recorded one-half hour later, and at least once more after this. The bath was not washed during this period. T_3 failed to enhance significantly the epinephrine-induced contraction, in height, in experiments in which the control response was of long duration (Table 1). Determination of possible potentiation in terms of duration was not feasible because of quite prolonged control responses. When control response was of short duration, T_3 significantly potentiated the epinephrine-induced contraction in height and particularly in duration. When copper acetate was added to the bath (2×10^{-7}), the usually prolonged contractions after epinephrine were considerably shortened. T_3 again significantly potentiated epinephrine-induced contraction.

3) *Effect of EDTA on Epinephrine-induced Contraction.* In similar experiments, the disodium salt of ethylenediamine tetracetic acid (EDTA), in concentration equimolar to that of T_3, also potentiated the epinephrine-induced contraction under same conditions as described for T_3. Epinephrine was added to the bath 2-5 minutes after EDTA in these experiments.

4) In other experiments, a 1×10^{-5} concentration of epinephrine was incubated at 37°C in a 50 ml oxygenated Ringer's solution which contained 1 g/liter of glucose, a 4×10^{-7} concentration of copper acetate and a 2×10^{-6} concentration of T_3. A similar incubate without T_3 served as control. Aortic tissue was not present in these incubates. At intervals after beginning incubation, 0.5 ml aliquots from each incubate were injected intravenously into a pentobarbitalized cat and blood pressure changes recorded from carotid artery on smoked kymograph by a mercury manometer. Dose of epinephrine administered was

TABLE I. Effects on Epinephrine Contraction of Isolated Rabbit Aortic Strip.

Treatment	No. of rabbits	No. of strips	Avg % increase in: Contraction height	Duration of contraction
T_3 (1×10^{-6}), short epinephrine contraction—no added Cu^{++} in bath	1	2	54 (27–80)	73 (58–87)
T_3 (1×10^{-6}), long epinephrine contraction—no added Cu^{++} in bath	3	8	14 P >.05	
T_3 (1×10^{-6}), added Cu^{++} in bath	7	10	40 P <.01	61 P <.01
EDTA (5×10^{-7}),* added Cu^{++} in bath	5	8	31 P <.05	>200
EDTA (5×10^{-7}),* short epinephrine contraction—no added Cu^{++} in bath	1	3	32 (20–42)	>200
Ephedrine, no added Cu^{++} in bath	6	14	24 P <.05	
Ephedrine, added Cu^{++} in bath	3	7	10 P >.05	22 P >.05

* Concentration equimolar to that of L-T_3.

usually 2.5 μg/kg body weight.

As can be seen in representative recording in Fig. 1, pressor response to epinephrine from treated incubate was somewhat greater than that from control incubate after 15 and 30 minutes of incubation; at 60 minutes epinephrine from treated incubate produced a markedly greater pressor effect than the control; and at 90 minutes pressor response to control epinephrine had disappeared while there was still a definite pressor response to the treated epinephrine. It was determined in separate experiments that T_3 alone, in dose equivalent to that contained in incubate aliquots, produced no effect on cat blood pressure. Control incubates turned pink much sooner than those containing T_3. In similar experiments in which copper was *not* added to the incubates, there was no difference observed in pressor responses to epinephrine from either control or T_3-treated incubates. Moreover, there was no difference in times in which the incubates turned pink.

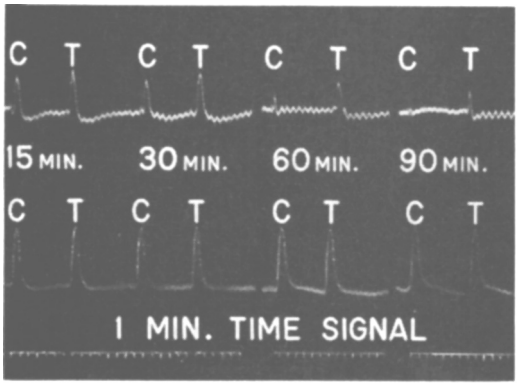

FIG. 1. Cat blood pressure response to epinephrine. Epinephrine incubated in Ringer's solution at 37°C with (T) and without T_3 (C). At intervals after beginning incubation, aliquots equivalent to 2.5 μg/kg epinephrine were taken from the incubates and inj. into a pentobarbitalized cat intrav. and arterial blood pressure recorded. Top recording shows results of representative experiment in which copper acetate was added to incubates and bottom recording shows results of representative experiment in which copper acetate was excluded from incubates.

5) *Effect of Ephedrine on Epinephrine-induced Contraction.* Experiments were designed to determine if amine oxidase inhibition was a possible factor in T_3-potentiation of epinephrine described above. After initial control injections of epinephrine, ephedrine was injected into the muscle bath to give a concentration of 1×10^{-6}. This concentration is one which has been previously reported to inhibit amine oxidase *in vitro* (4). Five to 10 minutes later, the response to epinephrine was again recorded. Ephedrine significantly potentiated the epinephrine contraction in absence of added copper in the bath, but only slightly or not at all when copper was present (Table I).

6) *Effect of T_3 on Barium Chloride-induced Contraction.* Concentration of barium chloride to evoke a contraction of the aortic strip was 4×10^{-4}. Height of contraction averaged about the same as that elicited by 1×10^{-7}

epinephrine, was well below maximum, and was similarly prolonged. T_3 (1×10^{-6}) failed to significantly potentiate barium chloride-induced contractions either in presence or absence of added copper in the bath.

Discussion. The results indicate that T_3 potentiates epinephrine-induced contraction of the isolated rabbit aortic strip in major part by a metal-dependent anti-oxidant effect. Moreover, this mechanism appears to be independent of aortic tissue. The evidence can be summarized as follows:

1. T_3 potentiated epinephrine when copper acetate was added to muscle bath but not in absence of copper. The potentiation in a few experiments in which copper was not added to the bath can probably be attributed to presence of trace metal contaminants in the bath. This is suggested by unusually short duration of epinephrine-induced contractions in these experiments.

2. EDTA, a potent chelating agent, potentiated the effect of epinephrine in similar experiments under same conditions.

3. T_3 delayed appearance of pink color in an incubate containing epinephrine, Ringer's solution and copper acetate. No delay was noted when copper was not added to T_3 treated incubate. The pink color is known to result from oxidation of epinephrine(5). Moreover, the pressor response of anesthetized cats to epinephrine incubated in presence of T_3 and copper (determined at intervals after beginning incubation) was evident long after the pressor response to "unprotected" epinephrine had disappeared. T_3 exerted no effect when copper was not added to the incubate.

The evidence thus indicates that T_3 delays metal-catalyzed oxidation of epinephrine. This explains in major part the potentiation of epinephrine-induced contraction of the isolated rabbit aortic strip. The mechanism of the anti-oxidant effect is probably mediated through the metal chelating action of T_3. Ability of T_3 to chelate copper and other heavy metals has recently been demonstrated by means of an acidometric method.† The strong metal complexing property of thyroxine has previously been indicated by Frieden(6) and Gemmill(7). An alternative explanation of the anti-oxidant effect of T_3 is suggested by more recent work of Frieden(8), who demonstrated that T_3 as well as thyroxine can reduce cupric ions to the cuprous form. This could also result in prevention of copper-catalyzed oxidation of epinephrine.

Amine oxidase inhibition does not appear to be a major factor contributing to T_3 potentiation of epinephrine. The evidence for this was that ephedrine, in concentration known to inhibit amine oxidase, produced effects which were the reverse of those exerted by T_3.

It may also be concluded that the potentiating effect of T_3 can not be attributed to T_3-induced general increase in sensitivity of rabbit aortic strip because T_3 failed to potentiate contraction induced by barium chloride.

Summary. L-3,3′,5-Triiodothyronine (T_3) potentiated epinephrine-induced contractions of isolated rabbit aortic strip only under the following conditions: 1) copper acetate (2×10^{-7}) addition to the bath or 2) when trace metal contaminants were occasionally present in the bath, as suggested by short duration control responses to epinephrine. EDTA, a potent chelating agent, similarly potentiated epinephrine contractions. It was further demonstrated that T_3 delays metal-catalyzed oxidation of epinephrine in separate incubate experiments. This effect appears to be the major factor in potentiation of epinephrine contractions by T_3. It is further concluded that T_3 probably acts by virtue of its metal-chelating effect.

† Personal communication from Dr. Stuart P. Eriksen, Smith Kline & French Labs., Phila., Pa.

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