PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 132.
6. Everett, G. M., Richards, R. K., *J. Pharm. Exp. Therap.,* 1944, v81, 402.
7. Jenney, E. H., Smith, R. P., Pfeiffer, C. C., *Fed. Proc.,* 1953, v12, 333.
8. Toman, J. E. P., *Neurology,* 1951, v1, 444.
9. Toman, J. E. P., Swinyard, E. A., Goodman, L. S., *J. Neurophysiol.,* 1946, v9, 231.

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Effects of Administration of L-Thyroxin on Liver N-Demethylating Activity in Normal and Morphine-Treated Rats. (25890)

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Chronic administration of morphine to rats causes a striking diminution in ability of liver microsomal-supernatant preparations from these rats to N-demethylate narcotic drug substrates(1,2). After abrupt withdrawal of drug from rats treated with morphine chronically, complete recovery of this enzyme activity occurs within 12 days(1,3). Recently L-thyroxin stimulated incorporation of amino acids into protein(4), suggesting that it accelerates protein synthesis. Since recovery of N-demethylase activity after withdrawal of narcotic drugs may represent enzyme resynthesis, it was of interest to study the effects of thyroxin on this process.

Methods. Forty-four NIH male black rats, each weighing 200-300 g, were divided into 4 groups so matched that average weight of each group was the same. The 4 groups were treated as follows. For 30 days, Group M received intraperitoneal injections of morphine sulfate in gradually increasing doses from 20 mg/kg to 100 mg/kg twice daily. Group MTh received morphine in same amounts and on same schedule as Group M and, in addition, a daily intraperitoneal injection of 90 μg of L-thyroxin in 0.5 ml of 0.01 N NaOH for 7 days prior to abrupt withdrawal of morphine and throughout period of withdrawal until day of sacrifice. Group Th received only thyroxin in same amounts and on same schedule as group MTh. Animals in group C were untreated controls. At 19 hours, 96 hours, and 8 days after termination of morphine administration, animals from the 4 groups were sacrificed and their livers prepared for enzyme assay by method of Axelrod(5). Enzymatic activity was measured by estimating the amount of formaldehyde formed by the N-demethylation of morphine, as described previously(2).

Results of representative experiment are summarized in Fig. 1. Nineteen hours after withdrawal of morphine and 7 days after initiation of thyroxin treatment in groups receiving the hormone, the N-demethylation of morphine by liver preparations from animals in group M was 6.7% of untreated-control value, and the activity in preparations from animals in group MTh was about one-half that of group M ($p<.01$). Preparations from animals receiving L-thyroxin alone for 7 days did not differ in N-demethylase activity from untreated controls. By 96 hours after withdrawal (and after 10 days of thyroxin administration in thyroxin-treated animals) N-demethylating activity began to recover in both groups M and MTh, and no significant difference between them was observed ($p>.05$). Then, a marked depression of activity was observed in animals treated with thyroxin alone (group Th). Eight days after morphine withdrawal, enzymatic activity of liver preparations from rats in group MTh was still less than one-fourth of control preparations (Group C) and had not increased appreciably from values obtained previously. On the other hand, N-demethylase activity for animals in Group M showed at this time an almost complete return to normal. Administration of thyroxin for 14 days had depressed

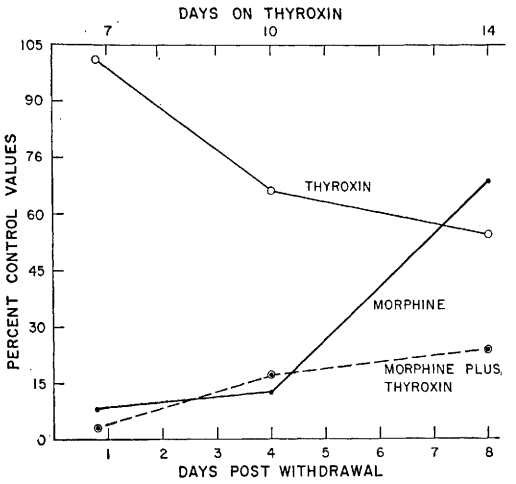

FIG. 1. Effects of administration of thyroxin on recovery of N-demethylating activity after morphine withdrawal.

liver N-demethylase in Group Th even more than previously.

To examine further the effects of chronic administration of thyroxin alone on *in vitro* N-demethylation of morphine, a group of male NIH black rats was given 90 μg of L-thyroxin daily to 29 days before liver enzyme activity was assayed. Table I compares enzyme activity after this treatment with that in untreated control rats of the same age and weight. Again, no effects of thyroxin were observed after only 7 days of administration, but after 10 days a distinct lowering of N-demethylase activity was observed. By 14 days enzyme activity was reduced to approximately half that found in control animals, and it remained at about this level until the end of 29-day period. Thus, thyroxin alone depresses ability of rat-liver-microsome preparations to N-demethylate morphine. This depression reaches a maximum in 10-14 days and remains at approximately the same level for at least an additional 2 weeks.

TABLE I. Effect of *In Vivo* Administration of L-thyroxin on *In Vitro* N-demethylation of Morphine.

Days on thyroxin	Activity (% of control)*†
7	100.9
10	65.7
14	54.4
21	43.0
29	57.7

* Each value represents mean of 7-10 thyroxin-treated animals compared to mean of 4 control animals sacrificed on same day.

† Enzymatic activity was measured by estimating amount of formaldehyde formed by N-demethylation of morphine hydrochloride.[1]

These results indicate that thyroxin administration prior to and during withdrawal neither prevents morphine-induced diminution of N-demethylase activity nor increases its rate of recovery after withdrawal of morphine; instead, it causes a significant decrease in activity beyond that seen with morphine alone early in withdrawal, and a significant delay in recovery of enzyme activity. Under our conditions, administration of thyroxin during withdrawal period seemed to prevent recovery almost completely.

The significant difference between Groups M and MTh during early withdrawal, obliteration of this difference during middle period of withdrawal, and, finally, the markedly retarded recovery of demethylase activity in Group MTh during period when virtually complete recovery in Group M was observed are puzzling but reproducible phenomena. They may indicate a tendency for thyroxin to speed recovery obscured by another action simulating or perhaps potentiating the action of morphine. The nature of the mechanisms underlying the effects on N-demethylase activity by morphine and thyroxin singly and together remains, however, unknown.

Summary. The effect of *in vivo* administration of L-thyroxin on *in vitro* N-demethylation of morphine by rat-liver enzyme preparations from normal and morphine-treated rats has been investigated. Thyroxin given 7 days to otherwise untreated control animals has no effect on N-demethylating activity, but after 10-14 days it significantly depresses this activity. Administration of thyroxin for 7 days prior to and during morphine withdrawal reduces further the enzyme activity already markedly depressed after chronic administration of morphine, and prevents almost completely recovery of activity which occurs some 8-10 days after morphine withdrawal.

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1. Axelrod, J., *Science,* 1956, v124, 263.

2. Cochin, J., Axelrod, J., *J. Pharmacol. Exp. Therap.,* 1959, v125, 105.

3. Cochin, J., Economon, S., *Fed. Proc.,* 1959, v18, 377.

4. Sokoloff, L., Kaufman, S., *Science,* 1959, v129, 569.

5. Axelrod, J., *J. Pharmacol. Exp. Therap.,* 1956, v117, 322.

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Effect of Pre-Immunized Rat Bone Marrow on Lethally Irradiated Mice. (25891)

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Post-irradiation protection of lethally irradiated mice by injection of heterologous (rat) marrow is associated with rapid repopulation of the host's depleted hematopoietic tissue by the transfused marrow cells(1,2). The treated animals initially recover from radiation induced injury, but a significantly large percentage of deaths characteristically occur after third week of irradiation treatment(3,4). Although *direct immunological* evidence is still lacking, it is generally accepted that the pathogenesis of these "late deaths" is immunologic in origin. It is still uncertain whether the host or the transplanted graft initiate the immune reaction, nor is it known whether the pathological foundation of "late deaths" is circulating or fixed tissue antibodies. The experiments to be described were performed to compare time and incidence of deaths in lethally irradiated mice injected with bone marrow from normal rats and with bone marrow from rats immunized against tissues of the recipient strain of mice. On the basis of a graft *vs.* host reaction, it would be expected that both time and incidence of death would be accentuated following injection of pre-immunized rat marrow, resulting from direct restimulation of transplanted cells by the host's antigens. In contrast, if a host *vs.* graft reaction occurred or if colonization of immunologically active donor cells was unsuccessful no difference would result between groups receiving normal or pre-immunized marrow. Our results favor the latter conclusion.

Materials and methods. Sprague-Dawley albino rats, 12 to 18 weeks of age, were immunized against spleen and bone marrow suspensions prepared from normal adult LAF_1 ($(C57LxA)F_1$) mice. The mice were sacrificed by cervical dislocation. Spleens were excised, pooled, minced with fine scissors, then pressed through a 60x60 mesh stainless steel wire gauze. The cells were suspended and washed twice with chilled Tyrode's solution. Large clumps were removed by filtration through surgical gauze, and a cell count made in a hemacytometer. The desired concentration of cells was prepared by addition of Tyrode's solution. Mouse marrow cells were obtained by aspiration from the femurs and a suspension was prepared in Tyrode's solution. Three separate experiments were performed differing only in number of immunizing injections administered to the rat donors and in time after immunization at which the rats were sacrificed for collection of bone marrow and spleen cells. In the initial experiment, 2 intravenous injections of $1x10^8$ nucleated spleen cells were given 9 days apart followed by sacrifice 3 weeks after last injection. Serologic test of pooled sera from 10 rats showed a hemagglutinating titer of 1:512 against washed LAF_1 mouse rbc. In the second experiment, rats were given 2 intravenous injections of $1x10^8$ spleen cells a week apart followed by a third injection 2 weeks afterwards. These donor animals were sacrificed

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