

## Effect of Manganous Ions on Thyroidal Iodine Metabolism in the Rat (35022)

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(Introduced by S. Fedoroff)

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In 1932 McCarrison and Madhava (1) reported that manganous chloride, when added to raw cabbage which was the exclusive food of rabbits in their experiment, exerted a definite antigoitrogenic effect. Small doses of the same salt have been reported to increase oxygen uptake in mammals, whereas large doses had the reverse effect (2-4). Furthermore, the thyroid will concentrate manganese very markedly and rapidly, releasing it relatively slowly (3, 5, 6). Reineke and Turner (7) showed that manganese salts catalyzed the formation of thyroxine from casein in an enzyme-free buffered medium. Kaellis and Goldsmith (8) in 1965 hypothesized an antagonistic relationship between manganese and calcium on the formation of thyroid hormones, with the former directly facilitating the formation and the latter inhibiting it.

To study the effect of manganous salts on the proportion of protein-bound radioiodine in the serum, the distribution of radioiodine among the iodinated hormones of the thyroid and their precursors, this experiment was performed.

*Procedure.* Thirteen male CFN rats were used. This strain and sex were chosen to permit application of previously reported results (8). The animals, for at least 1 week prior to any intervention, were all fed normal rat chow and given water *ad libitum*. Seven were injected intraperitoneally under light ether anesthesia with approximately 1 ml of normal saline and 20  $\mu$ Ci of  $^{125}$ I. Six were similarly injected with a freshly-made solution of manganese chloride such that there were delivered 3 mg of manganese for every 100 g of body weight, and 20  $\mu$ Ci of  $^{125}$ I. Twenty-four hr later, the animals were

weighed and killed under ether anesthesia by removal of their neurocrania. Blood was collected and the sera were separated by centrifugation. One ml of serum from each rat was counted for 10-min periods in a gamma scintillation counter. Following that, 3 ml of 10% trichloroacetic acid (TCA) were added, and the resulting precipitate was washed two times with TCA. The precipitate was then counted for 10 min. Meanwhile, the thyroids were dissected out, cleaned, weighed, and placed in a small ampule along with 12 mg of Pronase<sup>1</sup> and 0.2 ml of 0.1 M ammonium bicarbonate buffer saturated with propylthiouracil. The ampules were flushed with nitrogen, sealed, and incubated for 12 hr at 37°. This is the method of Van Middlesworth (9). At the end of that time, the ampules were counted for 1-min periods in a gamma scintillation counter, and 0.1 ml of the hydrolysate was spotted along a line 4 cm from the edge of 3 MM chromatography paper measuring 60 by 480 mm. Other papers were similarly spotted with potassium iodide (I), monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (T3), and thyroxine (T4). These papers were developed by ascending chromatography for 36 hr at 20° using a solvent system of *n*-butanol, ethanol, and 0.5 M NH<sub>4</sub>OH (5:1:2, v/v/v) (10). The radioactive papers were dried, cut into 5-mm strips, and each one was counted for 5 min in a Packard Auto Gamma spectrometer, Model 578. Distance from the origin was plotted against count, and areas corresponding to the standards had their strip counts totalled and divided by the total paper count

<sup>1</sup> Obtained from California Biochemicals Corp., 3625 Medford St., Los Angeles, Calif.

TABLE I.  $R_f$  Values (13).

Compd	Mean value	SEM
T4	0.471	0.005
T3	0.662	0.004
DIT	0.152	0.003
MIT	0.243	0.003
I	0.333	0.003

to derive a percentage of each constituent. The standard papers were treated with suitable reagents for color development and the  $R_f$  values were determined.

*Results.* To demonstrate that the single solvent system in which the chromatograms were developed can provide good separation of the constituents when development is allowed to proceed for 36 hr, Table I is presented. Table I presents the mean of 13  $R_f$  values for iodide and iodinated tyrosines and thyronines along with the standard error of the mean. As was determined, all the  $R_f$  values differed from one another quite significantly. Table II is a summary of the experimental results. Significant differences between the experimental and control groups occurred with the ratio of serum to thyroid radioactivity ( $S/T$ ), in which the experimental group showed an increase of over 1.5 times as compared with the control group. The ratio of protein-bound (or, more precisely, TCA-precipitable) radioactivity in the serum to that of the total serum declined by over 80% in the experimental group. Furthermore, there were significant declines in the percentages of radioactive iodinated thy-

ronines in the thyroid glands of the experimental rats (19.3% decline in the case of thyroxine and 50.6% decline in the case of triiodothyronine), but increases in the percentages of radioactive iodinated tyrosines (7.4% in the case of diiodotyrosine and 10% in the case of monoiodotyrosine). The percentage of iodide did not differ significantly. These data would lead one to infer that the effect of the manganese injection was to cause a release of iodinated thyronines as unbound hormones into the serum. The percentage radioactivity at the origin was always low enough to indicate nearly complete digestion, but was significantly lower, by about one-third, in the experimental animal chromatograms. This may be related to a small radioactive fraction which appeared on the chromatograms between the origin and DIT and which may have increased in the chromatograms resulting from the hydrolysates in the manganese-injected rats. Also, assuming that the expected uptake of manganese ion by the glands took place, the proteolysis of the thyroids of the experimental animals may have been slightly enhanced by the presence of manganese in the mixture.

*Discussion.* The results of this experiment are in accord with what has been reported previously, *i.e.*, one would expect that the decline in the glands of iodinated thyronines and what appears to be an increase in their concentration in the free form in the serum would result in a general increase in physiological effects attributable to thyroid gland activity. Certainly this would explain the atten-

TABLE II. Summary of Results (mean values and SEM).

Parameter	Control (7)		Exp. (6)		$\frac{\text{Exp. cont.}}{\text{Cont.}} \times 100$	$p$	
Body wt (g)	299	23.5	306	14.8	—	NS	
Thyroid (mg/100 g body wt)	6.88	0.72	6.92	0.40	—	NS	
Serum: hydrolysate counts	6.45	0.86	16.34	3.38	153.3	>.01	<.02
Precipitate: serum counts	59.63	4.44	10.03	2.72	-83.2	<.001	
% T4 in chromatogram	18.53	1.24	14.95	0.73	-19.3	>.02	<.05
% T3 in chromatogram	2.47	0.29	1.22	0.14	-50.6	>.001	<.01
% DIT in chromatogram	41.45	1.05	44.50	0.72	7.4	>.02	<.05
% MIT in chromatogram	24.95	0.85	27.45	0.67	10.0	>.02	<.05
% I in chromatogram	8.46	0.47	7.74	0.65	—	NS	
% at origin in chromatogram	1.05	0.05	0.66	0.09	-37.1	>.001	<.01

uation of the cabbage-induced goiter noted previously. In the hypotheses previously developed (8) thyrotropin was alleged to favor the concentration of manganese by the thyroid, and the effect of manganese was thought to be the displacement of calcium in a presumed calcium-thyroglobulin complex which resisted iodination and/or thyronine formation, whereas the manganese complex was either more easily iodinated and/or more easily formed a thyronine. A recent report (11) showed that calcium, in relatively high concentration in the thyroid, was histochemically demonstrable in the follicular cells. The site and state of thyroidal manganese is presently unknown. Indeed, the relative paucity of data, including those derived from this experiment, does not permit a validation (or invalidation) of these hypotheses now.

*Summary.* Injection into rats of manganous chloride along with radioiodide showed, after a 24-hr period, a marked increase in the *S/T* and decline in the PBI fraction as compared with controls. There were also declines in the percentages of iodinated thyronines and increases in the per-

centages of iodinated tyrosines in the thyroids of the experimental animals.

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