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Influence of Parental Dietary Iodine Level on Thyroid Activity of Eighteen-Day Chick Embryos.*† (27471)

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An iodine deficiency in the dam's diet has been reported to cause goiter in the chick embryo, retard embryo development and decrease hatchability(1). Furthermore, the level of parental dietary iodine grossly affected iodine-131 uptake by the embryonic thyroid, and this effect was influenced by the length of the trapping period(2,3,4). The fact that, with embryos from iodine deficient hens, relatively short trapping periods (2 hours) gave greater uptake values than longer trapping periods (4 hours) suggested that thyroids of iodine deficient embryos have the ability to synthesize and release thyroxine at a very rapid rate, and that this rapid release can confound measurements of thyroid activity by I^{131} uptake technics. The work reported herein was designed to obtain information on rate of synthesis and release of thyroxine in 18-day chick embryos as influenced by parental iodine.

Methods. The hens involved in this experiment were the same hens used in previous studies(4) and had been on their respective diets for 32 weeks. The parental diets for Groups 1, 2 and 3 contained 17, 75 and 500 p.p.b. iodine, respectively. The earlier work (4) had shown that the embryonic thyroid glands in Group 1 exhibited the characteristic symptoms of goiter (hypertrophy and hyperplasia of the epithelial cells, lack of colloid, increased weight). The embryonic thyroid glands in Group 2 were larger than normal but not nearly as large as Group 1, and showed hyperplasia and less than normal colloid. Group 3 embryonic thyroid glands were judged normal.

The procedure for injection of I^{131} was the same as reported previously(3,4) with the air cell used as site of injection.

Embryos were broken out at exactly the specified time following injection. The heart was exposed, and a sample of blood obtained by heart puncture with a heparinized needle and syringe. The thyroid glands were removed, trimmed and homogenized in a Potter homogenizer with 1 ml of bovine plasma. The bovine plasma was used to insure adequate protein for binding of thyroxine. The remainder of the embryo was transferred along with wash water to a Waring blender and homogenized. The homogenate was filtered

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TABLE I. Effect of Parental Iodine on Thyroid Activity of 18-Day Embryos.

	Plasma I^{131}			Thyroidal I^{131}			Embryo homogenate I^{131}		
	% inj. dose/ml			% inj. dose			% inj. dose		
Group*	Total I^{131}	PBI^{131}	% PBI^{131}	Total I^{131}	PBI^{131}	% PBI^{131}	Total I^{131}	PBI^{131}	% PBI^{131}
				30 min. post-inj.†					
1‡	2.80	.05	1.8	21.81	21.72	99.6	46.89	1.41	3.0
2§	1.94	—	—	13.46	12.96	96.3	52.22	.47	.9
3§	4.01	—	—	1.40	1.17	83.6	54.66	.46	.8
				60 min. post-inj.					
1	1.24	.66	53.2	42.41	38.81	91.5	31.87	2.09	6.6
2	1.97	.08	4.1	26.66	25.48	95.6	40.91	.56	1.4
3	3.59	—	—	4.80	4.24	88.3	63.25	.84	1.3
				120 min. post-inj.					
1	3.54	3.18	89.8	39.27	37.53	95.6	33.07	14.51	43.9
2	1.52	.11	7.2	37.88	37.00	97.7	40.32	1.04	2.6
3	1.86	—	—	11.44	10.80	94.4	66.89	.89	1.3

* Iodine content of the parental diets was: Group 1, 17 p.p.b.; Group 2, 75 p.p.b.; Group 3, 500 p.p.b.

† One-tenth ml of sterile isotonic saline containing 1 μc of I^{131} inj. into the air cell.

‡ Each value for Group 1 represents the avg of 3 observations.

§ Each value for Groups 2 and 3 represents the avg of 2 observations.

through cheesecloth to remove the feathers, the feathers washed with distilled water and the total filtrate weighed. A measured amount of plasma, which varied depending on the sample size of blood, was counted for total I^{131} activity, and adjusted to percent dose per ml of plasma. Similarly, the total thyroid homogenate and a 4-ml aliquot of the embryo homogenate were counted for I^{131} activity. The results were expressed as percent of initial dose by comparing with an aliquot of the injected dose. The activity of the 4-ml aliquot of tissue homogenate was converted to percent dose in the whole embryo.

After counting the samples for total I^{131} activity, the protein-bound I^{131} (PBI^{131}) was precipitated according to Barker(5) using Somogyi's zinc sulfate reagent(6). The precipitate obtained by centrifugation was washed 3 times with distilled water and after the last washing was centrifuged directly into stainless steel counting cups by the procedure described by Comar(7). To obtain uniform counting geometry, the gelatinous precipitate in the counting cups was covered with a disc of filter paper and allowed to dry until it could be pressed into a layer of uniform thickness (4 mm) with a volume of approximately 2 ml. The samples were then counted to determine the amount of I^{131} that was bound to protein, and presumably in the form of thyroxine, by comparing with a 2-ml aliquot containing a known fraction of the I^{131} in the injected dose counted under similar conditions. Appropriate corrections were made for background and decay.

Results and discussion. The results of this experiment are presented in Table I. After only 30 minutes following injection, the thyroids of iodine deficient embryos (Group I) had trapped a considerable quantity of the injected I^{131}. The quantity of radioiodine present in the thyroid was inversely proportional to the iodine content of the parental diet. Nearly all of the I^{131} in the thyroids at this time was in the form of PBI^{131} regardless of parental iodine treatment with a somewhat lower percentage as PBI^{131} on the high iodine level (Group 3). At 60 minutes post injection, the percent of initial dose present in the thyroid had approximately doubled in all groups as compared to 30 minutes post injection. Similar to the 30-minute post injection period, nearly all of the I^{131} was in the form of PBI^{131}. At 120 minutes post injection, I^{131} content of the thyroid had increased in the 2 higher levels of iodine (Groups 2 and 3), but decreased in the lowest level of iodine (Group 1) as compared to the 60-minute post injection. Thus, it would appear that the re-

lease of PBI^{131} from thyroid glands of the markedly deficient embryos (Group 1) equalled or exceeded the trapping of I^{131} between 60 and 120 minutes after injection. The evident difference noted at 30 and 60 minutes post injection on I^{131} "uptake" between the lowest level in iodine (Group 1) and the next higher level (Group 2) had nearly disappeared at 120 minutes post injection.

Only a very small amount of PBI^{131} was detectable in the plasma of embryos on the lowest level of iodine (Group 1) at 30 minutes following injection into the air cell. However, over 50% of the I^{131} present in the plasma at 60 minutes post injection was in the protein-bound form and 90% was in the form of PBI^{131} at 120 minutes post injection in this low iodine group. No detectable PBI^{131} could be found in the highest iodine level (Group 3) at any of the intervals following injection, and only small amounts of PBI^{131} were found in the intermediate level of iodine (Group 2) at the 60- and 120-minute post injection periods.

The data on tissue PBI^{131} paralleled plasma PBI^{131} data for the most part differing primarily in magnitude. At the 3 periods studied, there was always considerably more PBI^{131} in the tissues of embryos from dams on the lowest level of iodine than the other 2 groups. At the 120-minute post injection period, nearly 44% of the I^{131} was in the protein-bound form in this low iodine group as compared to less than 3% for the next higher level.

These data emphasize the importance of the time relationship in use of I^{131} "uptake" as an indicator of thyroid activity. Examination of only the percent dose "trapped" by the thyroid, or preferably the percent dose present in the thyroid, after 120 minutes post injection reveals very little difference between the lowest level of iodine and the next higher level (39.3 and 37.9%, respectively). However, by adding the percent dose present in the plasma and tissues in the form of PBI^{131}, which presumably had been trapped and released by the thyroid, much greater differences between these groups become apparent (55 and 39% for Groups 1 and 2, respectively). These values more nearly represent the true uptake of I^{131} by the thyroid glands.

The results of the PBI^{131} studies clearly show large differences in rate of synthesis and release of thyroxine between embryos from dams on various levels of dietary iodine. Embryos from hens fed a diet extremely deficient in iodine synthesized and released the hormone at a much faster rate than embryos from hens on higher levels of dietary iodine. Thus, PBI^{131} level in the plasma at various time intervals following injection of I^{131} may be used concurrently with I^{131} uptake to provide an additional, and possibly more precise, measure of thyroid activity.

Summary. Thyroid activity in 18-day embryos from hens fed 3 levels of dietary iodine was studied with the use of I^{131}. The thyroid glands of embryos from hens on the lowest level of iodine synthesized and released thyroxine at a much faster rate than embryos from hens on higher levels of iodine. This rapid synthesis and release of thyroxine in more active thyroid glands can confound measusements of thyroid activity by I^{131} "uptake" procedures if care is not taken in selecting proper time intervals between injection and autopsy.

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