THE ACCUMULATION OF THYROXINE-LIKE AND OTHER IODINE COMPOUNDS IN THE FETAL BOVINE THYROID[1]

J. WOLFF,[2] I. L. CHAIKOFF, AND C. W. NICHOLS, JR.

From the Division of Physiology of the University of California Medical School

BERKELEY

THE allometric constant (k in the equation $y=bx^k$) for thyroid weight as a function of body weight in the fetal calf was shown to be 1.0. This indicates that the growth of the thyroid gland in the fetal calf is almost directly proportional to body weight. Although the above formula was originally used in connection with morphological dimensions, it has been widely applied to chemical magnitudes by Needham (1942) who has termed such studies chemical heterogeny. Thus, in the allometric equation $y=bx^k$, y becomes the chemical entity and x the morphological magnitude (body weight, crown-rump (C.R.) length, or age in days).

In the present investigation, the accumulation of two chemical fractions, thyroxine-like and non-thyroxine iodine, was observed in the fetal calf thyroid from 53 days to term. The amounts found were correlated with body weight, C.R. length, and age of the fetus. The results obtained show that the accumulation of both iodine fractions in the fetal calf thyroid is related to the growth parameters (body weight and C.R. length) in a manner similar to that of the growth of the gland itself.

EXPERIMENTAL

Fetal calf thyroids were collected as described in the preceding paper. A total of 96 thyroids was obtained from fetuses ranging in age from 53 days to term. Fifty-five were males and 41, females. The dams were of the following breeds: Hereford, 82; Guernsey, 2; Holstein, 9; and Jersey, 3.

The thyroids were excised from the fetuses approximately one hour after death of the mothers. Representative portions of most of the glands were hydrolyzed on a steam bath for 12 hours in 2N NaOH (20 ml. per gm. wet weight). A suitable aliquot of the hydrolysate was analyzed for thyroxine-like and non-thyroxine iodine according to the method of Taurog and Chaikoff (1946).

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[2] U. S. Public Health Fellow.

In those cases in which organic and inorganic iodine were separated, the glands were homogenized in an all-glass apparatus with cold 10 per cent trichloroacetic acid (10 ml. per gram). The precipitate obtained after centrifugation was washed twice with cold five per cent trichloroacetic acid. The supernatants, which contained all of the inorganic fraction, were combined and analyzed as previously described (Taurog and Chaikoff, 1946b). The trichloroacetic acid soluble (organic) fraction was hydrolyzed in 2N NaOH, and treated as described in the preceding paragraph.

RESULTS

Thyroid iodine of 16 fetuses was separated into trichloroacetic acid-soluble and trichloroacetic acid-precipitable iodine. The results are recorded in table 1. The trichloroacetic acid-soluble fraction constituted, as a rule, 11 per cent or less of the total iodine. In only one case was a value above 11 per cent found. It is evident, therefore,

TABLE 1. THE ORGANIC AND INORGANIC FRACTIONS OF FETAL CALF THYROIDS

Calculated fetal age	Thyroid weight	Iodine		
		Organic	Inorganic	Inorganic as per cent of total iodine
days	mg.	gamma	gamma	
64	1	0.94	0.10	9.6
68	9	1.2	0.22	18.5
75	9	0.84	0.10	10.6
78	17	1.0	0.08	7.6
80	18	3.4	0.34	9.1
85	24	0.98	0.08	7.5
107	133	14.6	0.24	1.6
108	111	17.0	0.21	1.2
120	255	25.4	2.6	9.3
125	345	55.2	2.2	5.9
143	895	129	12.1	8.5
154	$1.13 \cdot 10^3$	120	14.6	10.8
168	$2.37 \cdot 10^3$	424	33.9	7.4
172	$2.63 \cdot 10^3$	360	34.2	8.6
198	$4.32 \cdot 10^3$	$2.54 \cdot 10^3$	198	7.2
252	$8.00 \cdot 10^3$	$2.59 \cdot 10^3$	76.5	2.8

TABLE 2. NATURE OF THYROID IODINE OF THE ADULT BOVINE
(All females approximately 6 years old)

Thyroid weight	Iodine Contents		
	Organic	Inorganic	Inorganic as per cent of total iodine
gm.	mg.	gamma	
16.2	25.9	450	1.7
17.0	14.9	259	1.7
14.0	15.3	305	1.5
18.0	34.1	345	1.0

TABLE 3. NATURE OF IODINE IN FETAL CALF THYROID

Age range	Number of fetuses examined	Fetal weight		Thyroid							
				Weight		Total iodine		Thyroxine iodine		Non-thyroxine iodine	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
days		gm.	gm.	mg.	mg.	gamma	gamma	gamma	gamma	gamma	gamma
53– 59	3	6.7 –12.7	8.3	—	—	<0.1 – 0.40	—	<0.1	—	<0.1 – 0.35	—
60– 68	6	12.7 –34	24	1– 9	3.4	0.91– 1.2	0.95	0.1 – 0.36	0.15	0.58– 1.0	0.70
70– 80	6	34– 67	52	4– 19	12.5	0.26– 3.8	1.9	0.1 – 0.79	0.44	0.51– 2.9	1.34
82– 88	8	79–156	119	16– 78	38	1.8 – 7.8	4.1	0.42 – 3.5	1.53	0.56– 5.8	2.6
90– 98	7	96–240	179	37– 78	54	3.3 –15.5	6.0	0.5 – 4.0	1.4	2.8 –11.5	4.7
100–105	9	285–490	371	57–183	106	4.6 –20.0	9.9	0.95 – 5.0	2.7	3.6 –15.0	8.3
106–110	7	438–555	491	110–178	131	7.7 –26.6	16.4	1.7 –11.1	4.6	4.6 –18.8	11.8
		kg.	kg.								
118–125	5	0.71– 1.32	0.89	155–435	243	42.3 –63.9	49.7	9.8 –38.3	17.9	25.1 –39.6	31.8
126–138	9	0.14– 1.75	1.44	290–470	379	26.6 –127	96.6	15.8 –36.6	26.5	10.8 –91.2	70.0
				gm.	gm.						
140–160	12	1.80– 3.81	2.54	0.42– 1.13	0.75	135–423	252	28.0 –118	60.6	82.5 –351	189
163–170	6	4.34– 5.50	4.81	1.13– 2.52	1.87	315–805	580	75.4 –300	212	190–550	362
						mg.	mg.	mg.	mg.	mg.	mg.
172–205	6	5.63– 9.60	8.55	2.43– 5.07	3.56	0.40– 2.74	1.95	0.078– 1.01	0.51	0.29– 1.88	1.40
215–218	3	12.5 –15.7	14.3	4.71– 6.95	6.02	3.21– 5.56	4.27	1.07 – 1.54	1.35	2.14– 4.02	3.00
240–255	6	19.9 –26.8	23.7	4.60– 8.00	5.97	2.67– 9.20	6.28	0.69 – 2.63	1.88	1.90– 6.58	4.38
260–265	2	32.5 –40.4	—	10.3 –11.4	—	4.96–17.4	—	1.40 – 5.32	—	3.56–12.1	—

that the major proportion of thyroid iodine in the fetus is organic. The values obtained by trichloroacetic acid separation of the adult bovine thyroid are shown in table 2.

The iodine values for thyroxine and non-thyroxine in the fetal thyroids have been arbitrarily grouped in table 3 according to the calculated ages of the fetuses. The values for the new-born calf are recorded in table 4.

TABLE 4. NATURE OF THYROID IODINE OF NEW-BORN CALVES
(All males)

Thyroid weight	Iodine contents		
	Total iodine	Thyroxine-like iodine	Non-thyroxine iodine
gm.	mg.	mg.	mg.
6.95	6.76	2.08	4.68
7.21	8.48	2.40	6.08
2.63	3.16	0.93	2.23
4.51	6.42	1.60	4.82
4.00	4.42	1.04	3.38

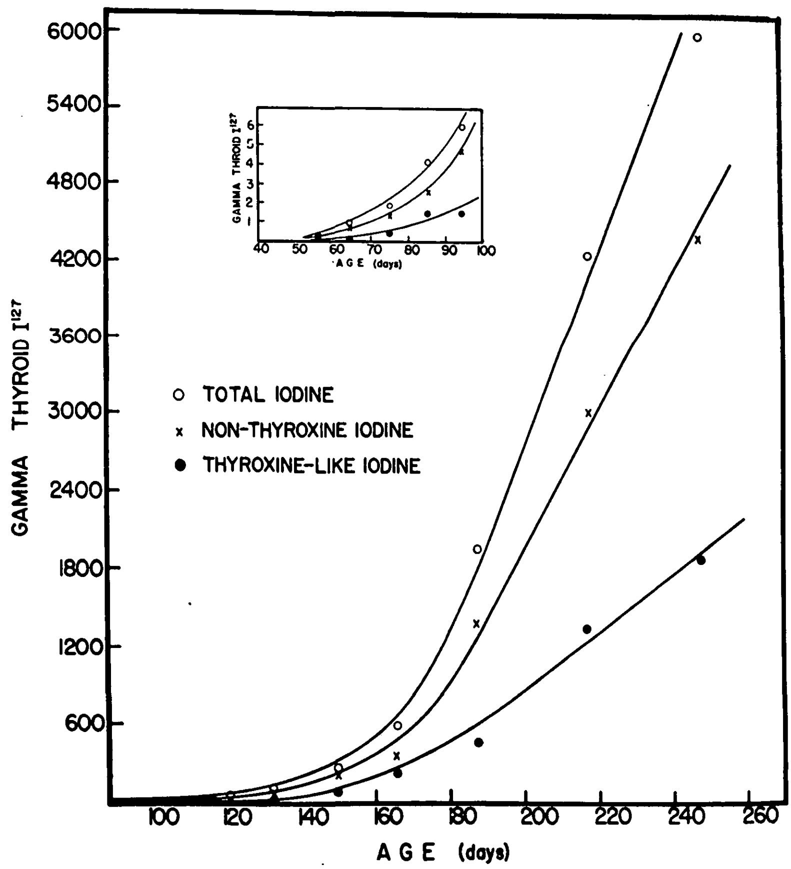

FIG. 1. Plot of fetal thyroid iodine against calculated age.

Despite considerable variation in the individual values for each age group, the means show that the iodine content of the fetal thyroid increases steadily with increasing body weight, C.R. length, and calculated age (table 3).

The average values for thyroxine-like and non-thyroxine iodine are plotted against calculated age in fig. 1. The curves suggest an ex-

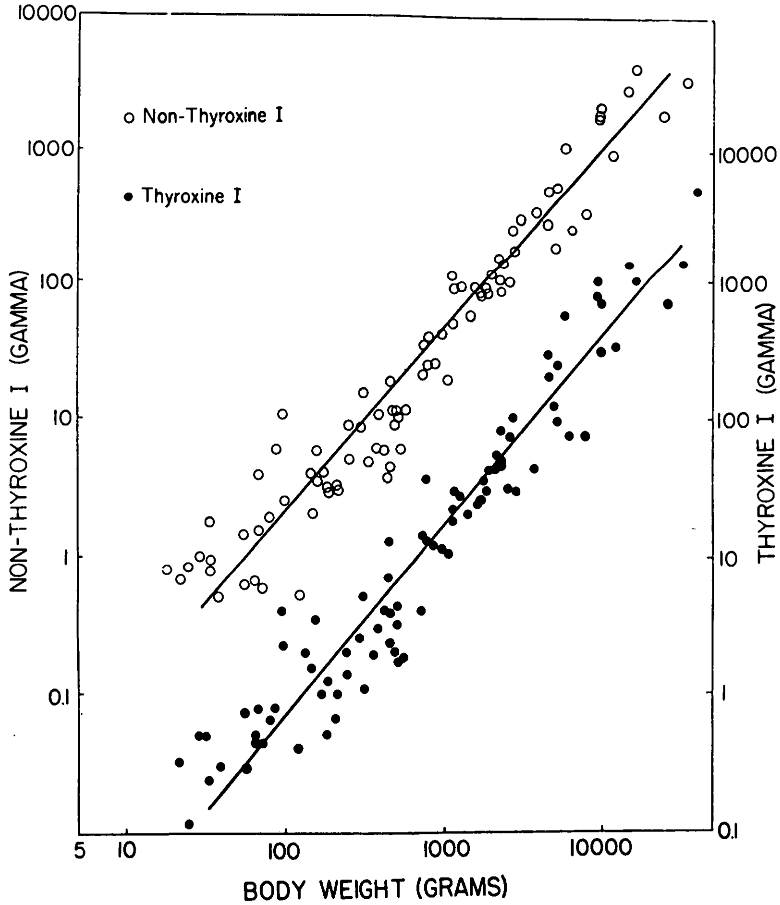

FIG. 2. Double-logarithmic plot of fetal thyroid iodine against body weight.

ponential relation between these iodine fractions and the age parameter. The chemical entities (thyroxine-like, non-thyroxine iodine) were therefore plotted against body weight, C.R. length, and age (figs. 2–4) on a double logarithmic grid. The straight-line curves obtained were subjected to analysis by application of the allometry equation of Huxley (1932), $y = bx^k$.

Relation to Body Weight

The equation for thyroxine-like iodine as a function of body weight (fig. 2) is

$$y_1 = (1.2 \cdot 10^{-3})x^{1.4} \quad (1)$$

where y_1 = thyroxine-like iodine in gamma.
x = body weight in gm.
The constants b $(1.2 \cdot 10^{-3})$ and k (1.4) were derived as described in the preceding paper.

Likewise, the equation for non-thyroxine iodine against body weight shown in fig. 2 is

$$y_2 = (3.1 \cdot 10^{-3})x^{1.3} \quad (2)$$

where y_2 = non-thyroxine iodine in gamma,
x = body weight in gm.

These formulae are valid for a body weight range from 50 gm. to 20 kg.

The constants expressing the "growth" of the chemical entities (1.4 for thyroxine-like iodine; 1.3 for non-thyroxine iodine) indicate that thyroxine-like and non-thyroxine iodine increase at approximately the same rate in the growing calf fetus.

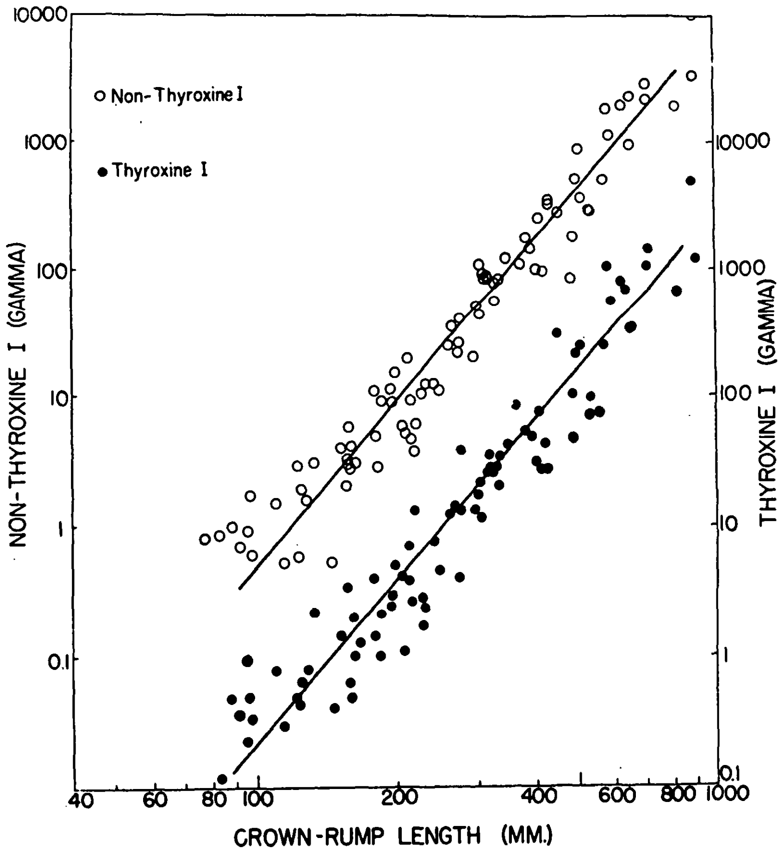

FIG. 3. Double-logarithmic plot of fetal thyroid iodine against crown-rump length.

Grown-Rump Length Relations

The curves for thyroxine-like and non-thyroxine iodine plotted against C.R. length are shown in fig. 3. The following equation fits the data for thyroxine-like iodine against C.R. length:

$$y_1 = (1.3 \cdot 10^{-9})x^{4.1} \quad (3)$$

in which x = C.R. length in mm.
y_1 = thyroxine-like iodine in gamma.

The formula for non-thyroxine iodine plotted against C.R. length is

$$y_2 = (2.1 \cdot 10^{-9})x^{4.2} \quad (4)$$

The above expressions are valid for C.R. lengths extending from 100 to 800 mm.

A comparison of the two equations (where k is 4.1 and 4.2, respectively) indicates that thyroxine-like and non-thyroxine iodine increase at about the same proportion in relation to body length.

Age Relations

A plot of thyroxine-like and non-thyroxine iodine against age yields curves having the following formulae

$$y_1 = (5.7 \cdot 10^{-10})x^{5.4} \quad (5)$$

$$y_2 = (1.8 \cdot 10^{-10})x^{5.3} \quad (6)$$

where y_1 and y_2 represent thyroxine-like and non-thyroxine iodine, respectively, in gamma, and x, the age in days. The validity of the formulae for thyroxine-like and non-thyroxine iodine against age extend from 60 days to term.

The amounts of these two iodine fractions in the fetal calf thyroid increase at approximately the same proportion with increasing fetal age. The values for k_1 and k_2 were 5.4 and 5.3, respectively.

DISCUSSION

As the fetus grows, total and thyroxine-like iodine increase steadily in the gland, and the rate at which each of these iodine fractions accumulates is shown to bear an exponential relation to body weight and body length as well as to calculated age. The accumulation of these two iodine fractions in the fetal gland is not only the result of thyroid growth (which, as shown in the previous paper, is also exponential) but, in addition, appears to be the result of an actual increase in the capacity of thyroid tissue to store iodine with increasing age. This increasing iodine-storage capacity of thyroid tissue, with age, is shown in fig. 5 which shows the *concentration* of iodine fractions in the gland (mg. iodine per 100 gm. of fresh tissue) against fetal age.

It was previously demonstrated, in 11 vertebrates, including the rat, examined under a variety of experimental conditions, that thyroxine iodine, when expressed as a percentage of total iodine of the gland, remains fairly constant (Wolff *et al.*, 1947; Taurog *et al.*, 1946c, 1946d). The values for this percentage ranged from 25 to 32. It therefore became of interest to determine whether a similar relation existed in the fetal calf thyroid.

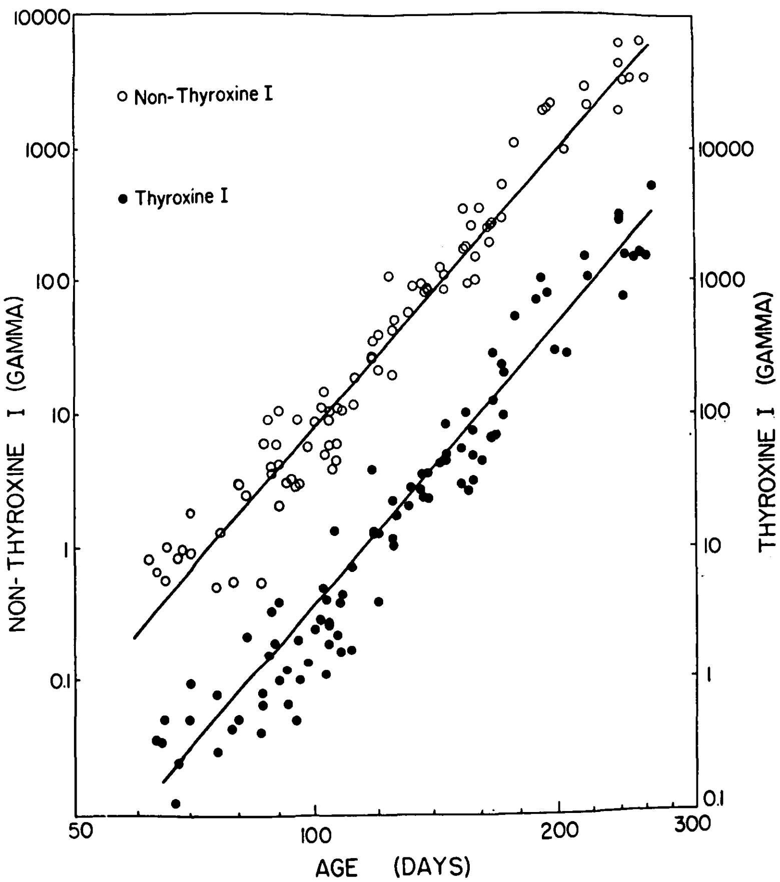

FIG. 4. Double-logarithmic plot of fetal thyroid iodine against calculated age.

The parallelism between the two curves shown in each figure (2, 3, 4, and 5) suggests that in the fetal calf, also, thyroxine iodine comprises a constant proportion of total thyroid iodine. An examination of the ratios for individual thyroids of fetuses obtained during the last third of the period of gestation revealed a constancy in the proportion of thyroxine-like iodine to total iodine. Eighteen specimens that were 170 days or older were studied, and with the exception of a single ratio, all values fell within the range of 24 to 36 per cent. This constancy in the proportion of thyroxine iodine to total iodine was first observed with frequency, in fetuses older than 120 days. But in younger fetuses, the proportion varied considerably, most of the values falling below 20 per cent. The deviations from 24–36 per cent were equally divided between the sexes and did not appear to be related to the absolute amounts of iodine contained in these glands.

Rankin (1941) investigated the appearance of iodine in the thyroid of the fetal pig, which has a gestation period of 114 days. He first found inorganic iodine in the gland between 46 and 50 days of age, and organic iodine at 52 days of age. As shown here, thyroxine-

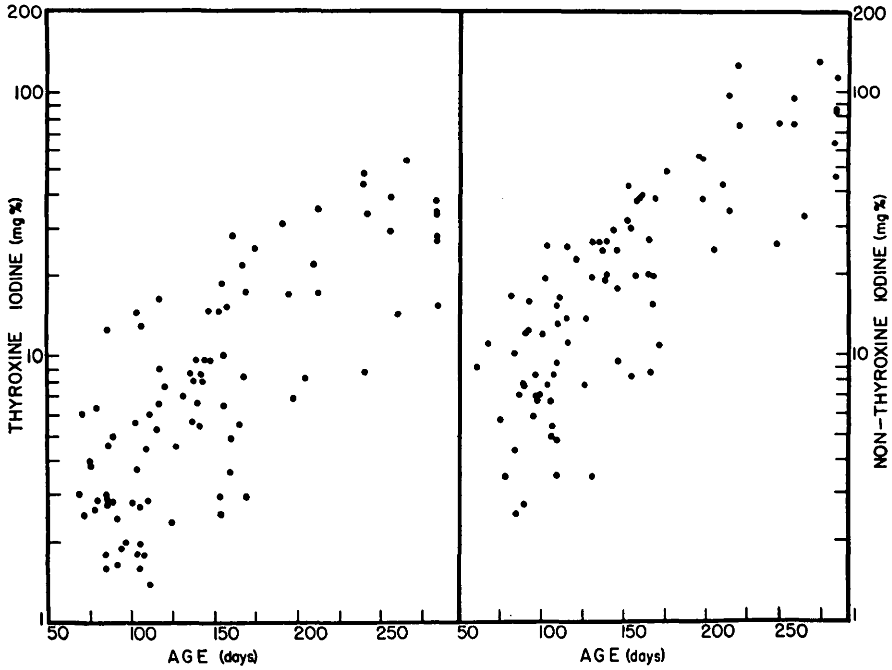

FIG. 5. Semi-logarithmic plot of the concentration of fetal thyroid iodine against calculated age.

like iodine appears at an earlier stage of gestation in the fetal calf, being first detected at 60 days of age (gestation period, 278–285 days).

Although the present investigation clearly demonstrates that thyroxine-like and non-thyroxine iodine increase proportionately with increasing fetal size and age, it provides no information on the nature of the iodine taken up by the fetal thyroid gland. It is unlikely that the iodine was taken up in the form of thyroxine or diiodotyrosine (Leblond, 1942). The iodine probably enters the fetal gland in the inorganic form as it does in the adult. Gorbman and Evans (1943) have demonstrated, in the fetal rat, that functional activity of the thyroid gland (as judged by its capacity to concentrate radioactive inorganic iodine) begins at 18–19 days of gestation. Preliminary experiments with surviving slices of fetal calf thyroid have shown that this tissue also has the capacity to concentrate inorganic iodine and that this capacity appears at a considerably earlier stage in the developing fetal calf than in the rat fetus.

SUMMARY

The amount of inorganic, thyroxine-like, and non-thyroxine iodine were measured in the bovine fetal thyroid from 53 days to term.

Measurable amounts of iodine were first detected in the fetal thyroid at 60 days of age.

The percentages of total iodine present as inorganic were similar to those observed in the adult thyroid gland.

The accumulation of thyroxine-like and non-thyroxine iodine in the fetal thyroid is related to each of the following growth parameters: body weight, crown-rump length, and calculated age. A straight-line relationship was found to exist when these iodine fractions were plotted against each of the parameters on a double-logarithmic grid.

The amounts of iodine found in the bovine fetal thyroid with increasing age were greater than could be accounted for by mere increase in thyroid mass. A progressive increase in the iodine concentrating capacity of fetal thyroid tissue occurred with age.

REFERENCES

Gorbman, A., and H. M. Evans: *Endocrinology* 32: 113. 1943.
Huxley, J. S.: *Problems of Relative Growth.* Methuen and Co., Ltd., London. 1932.
Leblond, C. P.: *Rev. Can. Biol.* 1: 402. 1942.
Needham, J.: *Biochemistry and Morphogenesis.* Macmillan Co., New York. 1942.
Rankin, R. M.: *Anat. Rec.* 80: 123. 1941.
Taurog, A., and I. L. Chaikoff: *J. Biol. Chem.* 163: 313. 1946a.
Taurog, A., and I. L. Chaikoff: *J. Biol. Chem.* 163: 323. 1946b.
Taurog, A., and I. L. Chaikoff: *J. Biol. Chem.* 165: 217. 1946c.
Taurog, A., I. L. Chaikoff and L. L. Bennett: *Endocrinology* 38: 122. 1946d.
Wolff, J., and I. L. Chaikoff: *Endocrinology* 41: 295. 1947.