PROTEIN-BOUND IODINE LEVELS IN DAIRY CATTLE PLASMA, MILK, AND COLOSTRUM

By

ROBERT CHARLES LEWIS

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Dairy

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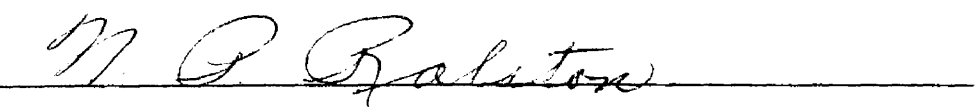
Approved 

ROBERT CHARLES LEWIS ABSTRACT

Induced hyper- or hypothyroidism causes marked alteration in dairy cattle performance, leading one to speculate on the relation of normal variability in thyroid function to growth, reproduction, production, longevity, efficiency, and like factors which determine the value of a dairy cow.

Very little information is available on the thyroid activity of dairy cattle. In other species the plasma level of protein-bound iodine has been found to be closely correlated with thyroid activity. Therefore, in this study an attempt has been made to determine the normal ranges of plasma protein-bound iodine in dairy animals in order to provide a basis for evaluating its relation to performance.

A digestion-distillation technique for the determination of protein-bound iodine in organic material has been used in more than three hundred determinations of this iodine fraction in dairy cattle plasma, milk, and colostrum.

The plasma level of protein-bound iodine in dairy cattle was found to show a marked rise soon after birth and to progressively decrease with age. Newborn calves had an average

plasma level of 8.9 micrograms percent, but within a few hours, after nursing, it rose to 15.0 micrograms percent. This rise appeared to be due to the extremely high protein-bound iodine concentration in the initial colostrum. The thyroid glands of four calves from 12 hours to 6 days old were examined microscopically and showed progressive colloid storage and cellular activity with advancing age. The average protein-bound iodine values obtained on animals of different age groups were 12.8, 7.3, 6.2, and 4.6 micrograms percent for animals under 48 hours, 48 hours to 12 months, 12 to 24 months, and over 24 months of age, respectively.

The suggestion is made that the following ranges of plasma protein-bound iodine concentration be considered normal: calves under 48 hours old, 8.0 to 18.0 micrograms percent; 48 hours to 12 months, 3.5 to 12.0 micrograms percent; 12 to 24 months, 3.5 to 10.0 micrograms percent; and animals over 24 months old, 3.0 to 8.0 micrograms percent.

No breed difference could be demonstrated between Jersey and Holstein cows maintained in the same herd. A group

of Jersey cows recently brought from California were significantly lower in their plasma protein-bound iodine concentrations than either Jersey or Holstein cows reared in the college herd. A number of cows under investigation as sterility cases also had significantly lower values.

No significant seasonal trends in the plasma protein-bound iodine concentration of Jersey cows could be shown although they tended to have lower values in November than during March, June, or August. The seasonal changes were similar and statistically significant in the Holstein cows. No relation of the plasma protein-bound iodine to the stage of gestation or rate of lactation could be demonstrated in the limited production data of this study.

The protein-bound iodine concentration in the plasma of five pairs of identical twin heifers was found to be no more alike than that of more distantly related or unrelated animals.

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INTRODUCTION

The economic situation today demands that we obtain the greatest possible efficiency in the production of milk, butterfat, and meat from our dairy animals. This can not be done unless we thoroughly understand the physiology of our cattle and so select and manage them as to fully utilize their productive potentials.

A long step forward would be taken if some means could be developed whereby the future worth of an animal could be estimated while it was still a calf. An endocrine analysis may offer some possibilities in this direction. No suitable assay method exists for some of the hormones, but the rate of secretion of the thyroid hormone, which has been shown to be related to dairy cattle performance, is closely related to the plasma level of protein-bound iodine in many species. It has been a useful tool in estimating thyroid function in human medicine and is closely related to thyroid activity in rats, mice, dogs, rabbits, and other laboratory animals. Little information is available, however, on the plasma protein-bound iodine concentration

of dairy cattle and its relation to the functional condition of their thyroid glands.

As an approach to this problem, the plasma protein-bound iodine levels of dairy animals of various ages and breeds have been determined in a relatively large number of cases. It was hoped that such data would provide a basis for establishing a normal range of protein-bound iodine values. If such a range could be established, the influence of various environmental factors and the relation of plasma protein-bound iodine concentration to performance could be determined in future studies.

REVIEW OF LITERATURE

The Phylogeny of the Thyroid Gland

In his recent excellent review, Goldsmith (75) traced the phylogenetic development of the thyroid gland as one ascends the evolutionary tree.

The most primitive thyroid gland is found in the adult cyclostome, of which the lamprey is an example. Lower forms, in which no thyroid tissue has been identified, probably do not secrete thyroxine although the possibility has not yet been ruled out. In this connection it is well to remember that even in the higher vertebrates a certain amount of extra thyroidal thyroxine is present (40, 104, 138).

Iodine in organic combination is found in many invertebrates and, indeed, 3,5-diiodotyrosine was first isolated from a species of coral (84). In these lower forms, and in the lamprey, exogenous thyroxine is said to have no effect on maturation although there is some data to the contrary.

The thyroid gland of the adult lamprey has been shown to arise from the endostyle of the larva which itself must possess

some type of thyroidal activity since it is capable of accumulating radioactive iodine. The gland of the adult lamprey is not encapsulated but is made up of a small number of isolated follicles.

In some elasmobranches the thyroid is encapsulated and arises as a solid epithelial bud ventral and caudal to the first and second gill with the follicles developing as they do in the human. The thyroids of the trout and salmon arise from the floor of the pharynx and clasp the upper end of the trachea. In the elasmobranch and in teleosts there is some variation in the degree of compactness of the thyroid tissue. Larger individuals of these species tend to have a more compact, nodular gland.

The thyroid gland of the higher vertebrates arises from the ventral wall of the pharynx and clasps the upper end of the trachea. The gland is divided into a right and left lobe and is at first attached to the pharynx by a stalk, the thyroglossal duct. This duct atrophies during the intrauterine period, although segments may persist, giving rise to thyroglossal cysts during postuterine life.

Iodine in the Thyroid Gland

The amount, concentration, and nature of the iodide compounds in the thyroid gland will obviously be somewhat dependent upon the iodine intake and physiological status of the subject. The factors which affect thyroid activity will be discussed in another section. It is the intent here to describe the conditions which exist in the normal gland. It is, of course, difficult to decide what is "normal." If, however, those animals on a customary diet, maintained under usual conditions, and which show no gross abnormalities, are considered to be "normal," it will be possible to describe the normal situation.

In reviewing the data on the iodine content of the thyroid gland, one is struck by the general agreement among investigators, even when the results of early workers are compared with the most recent reports. It is all the more amazing when one considers the techniques now used which were not available to the early workers. It is best, however, not to compare the absolute magnitude of the findings of the various investigators, since many of them, especially the early workers, were forced to use relatively impure materials and crude procedures. The trends and relationships may be safely compared.

The iodine-concentrating ability of the thyroid gland has been demonstrated many times; Salter (169) has reviewed the older literature in this respect and has summarized the iodine content of various tissues of man, dogs, and rabbits as reported by several workers. In general the thyroid, followed by the anterior pituitary and the ovaries, contains a higher concentration of iodine than the other organs.

Placental Transmission

Hudson (103) has shown that inorganic iodide can pass through the placenta from the mother to the fetus. Thus, the level of iodine in the fetal thyroid reflects the availability of iodine to the mother. The thyroids of hairless pigs, for instance, are very low in iodine, and fetuses aborted by cretinoid mothers are nearly iodine-free (223).

The ability of the thyroid hormone to pass through the placental barrier from mother to fetus is not yet established. Courrier and Aron (47) fed fresh ox and hog thyroids to dogs and guinea pigs throughout gestation. Although the mothers' thyroids were markedly altered, no gross or microscopic change occurred in the fetal thyroids. When the thyroid administration

was continued after delivery the thyroids of the nursing young rapidly changed. From this it was concluded that the hormone can not pass through the placenta. On the other hand, it is well known that pregnant bitches may be thyroidectomized with no ill effects appearing until after they have been delivered. The marked hyperplasia of the fetal thyroid under these circumstances points to transfer of thyroid hormone from the fetus to the mother (169).

Fetal Thyroid

On the basis of limited evidence, it would appear that the fetal thyroid is inactive in many species until about the middle of pregnancy. The apparent lack of thyroid activity prior to this time of course implies, too, that the anterior pituitary is not elaborating the thyrotropic hormone or that the thyroid is not capable of responding to its stimulation.

Schultze and Turner (172) have shown that prior to mid-term the thyroid glands of fetal goats do not respond to the administration of thiouracil or thiourea to the mother. After mid-term, however, the glands were greatly enlarged when these goitrogens were administered.

The bovine fetal thyroid may begin to function at a comparatively earlier age, since it has been found to contain measurable amounts of iodine at 60 days (231). At this time the percentage of the total iodine which was present as inorganic iodide was the same as that found in the adult thyroid. The ability of the thyroid to concentrate iodine progressively increased with advancing age of the fetus. The same workers (139) found, too, that after 62 days' pregnancy the growth of the fetal thyroid, as measured by increased thyroid weight, is nearly directly proportional to the body weight.

According to Salter (169), the total iodine in the thyroid tissue of the human fetus between the third and ninth months varies from 1 to 19 micrograms, or a concentration of 2 to 21 milligrams percent, of which at least a portion is present in an active form. Newborn babies have been reported as containing 2.4 to 48 micrograms of thyroidal iodine, varying from 2.3 to 1,450 micrograms percent in concentration, while the other body tissues contained a concentration of only 12 to 46 micrograms percent.

The thyroids of eighteen newborn infants, most of whom were term fetuses born dead, were analyzed by Palmer et al.

(140), who found a range of 35 to 654, with an average of 254 micrograms of iodine per gram of dry tissue. The average percent of the total iodine which was present in the form of thyroxine was 20.0, which is only slightly less than the adult figure.

The uptake of radioiodine by the hamster fetus has recently been studied by Hansborough and Seay (87). With a gestation period of 15 days and 8 hours, no accumulation of radioiodine could be detected in the fetal thyroids until the thirteenth day of development. The uptake of radioactive iodine did not begin until the appearance of the follicles which occurred between the twelfth and thirteenth day. As the number of follicles increased, the accumulation of iodine also increased.

Uptake and Binding of Iodine

The rate of uptake of iodine by various tissues has been measured by Perlman and associates (144), who used tracer doses of radioactive iodine. Within 25 to 50 hours the thyroid gland had taken up 65 percent of the administered dose. The other tissues studied contained a maximum of 0.605 percent of the administered iodine at this time. Whereas the amount of

radioiodide in the thyroid had steadily increased up to this time, the amount in the other tissues rose to an early peak which was followed by a gradual decline. The situation appeared to be one of diffusion of inorganic iodide into the nonthyroidal cells and then diffusion back into the circulation rather than an exhibition of any especial affinity of these cells for iodine.

The uptake of injected radioiodine by the thyroid occurs very rapidly after its administration. Lein (114) found the most rapid uptake occurred during the first 10 minutes after injection. A small amount of acetone-insoluble labeled iodine could be detected in the gland within 5 minutes after its administration.

The rate of iodine binding by surviving slices of dog, sheep, and rat thyroids was measured by Morton and Chaikoff (137). Within 2 hours after the addition of radioactive iodine to Ringers solution containing thyroid tissue, sheep glands converted 31 to 37 percent of the radioiodine into diiodotyrosine and 5 to 6 percent into thyroxine. During the same time dog thyroid tissue converted 21 to 24 percent of the iodine into diiodotyrosine and 3 to 4 percent into thyroxine while the corresponding rate for rat tissue was 60 to 71 percent and 8 to 12 percent, respectively, for diiodotyrosine and thyroxine.

In a recent review, Chaikoff and Taurog (37) presented data which showed that withing 15 minutes after rats are injected with a tracer dose of radioactive iodine (I^{131}), 95 percent of the radioactivity in the gland occurs in the organically bound protein. Of this amount, 80 percent is in the diiodotyrosine-like fraction and the balance in the thyroxine-like portion. The relationship between the percentages of thyroxine and diiodotyrosine in the gland remained quite constant even though the gland increased in its total iodine content.

In a later study designed to measure the maximum capacity of the thyroid gland to take up and store iodine, Taurog and Chaikoff (194) fed groups of rats at various levels of iodine intake. With a daily iodine intake of 1 to 2 micrograms, the thyroid contained 21.5 milligrams percent of total iodine (wet weight) and 5.9 milligrams percent of thyroxine iodine. When the daily intake was increased to 78 micrograms, the corresponding values changed to 134 and 44.4 milligrams percent. With an intake of 440 micrograms the concentrations were 131 and 36.5 milligrams percent, respectively. Thus, the ability of the gland to take up and store iodine is limited. At its

peak the gland contained an iodine concentration about 10,000 times greater than that found in the blood.

Distribution Between Thyroxine and Diiodotyrosine

The distribution of iodine between the thyroxine and diiodotyrosine fractions has been studied by Perlman et al. (145). Labeled iodine was deposited in the diiodotyrosine fraction in about twice the amount that appeared in the thyroxine fraction. Forty-eight hours after the administration of labeled iodine as much as 16 percent of it appeared in the thyroxine and 32 percent in the diiodotyrosine fractions.

The thyroid glands of fifty-two humans were analyzed by Leland and Foster (115), who extracted an alkaline hydrolysate of the glands and found about 25 percent of the gland's total iodine was in the form of thyroxine. In fifty-four patients they obtained a range of 0.33 to 4.21 milligrams of iodine per gram of dry tissue and a concentration of 0.173 to 5.93 milligrams of thyroxine in the whole gland. These values were believed to be about 15 percent low because of some loss of thyroxine during the hydrolysis. Earlier, Foster (67) had reported that 33

percent of the iodine of thyroglobulin is present as diiodotyrosine and 16 percent as thyroxine.

Blau (24) modified the Leland-Foster technique to eliminate most, if not all, of the thyroxine loss encountered by these workers. He obtained a range of total iodine per gram of dry human thyroid of 0.72 to 4.04 milligrams and a concentration of 0.013 to 0.887 milligrams of thyroxine iodine in a series of six fresh human thyroids. The thyroxine iodine varied from 1.77 to 31.8 percent of the total iodine. These values averaged about 9.7 percent higher than those obtained by the Leland-Foster method on the same glands. In a later trial (25) five fresh human thyroids were found to contain 0.59 to 0.895 milligrams of iodine per gram, of which 23.9 to 29.8 percent of the total iodine was in the form of thyroxine. Two fresh pig thyroids containing 0.615 and 0.695 milligrams of iodine per gram had 29.8 and 31.8 percent of the total iodine in the thyroxine-like fraction. A desiccated human thyroid contained 1.41 milligrams of total iodine per gram and a desiccated pig thyroid, 3.45 milligrams. The corresponding thyroxine concentrations were 19.2 and 31.2 percent of the total iodine, respectively.

In a group of eleven rats, Taurog and Chaikoff (193) found from 4.1 to 7.4 micrograms of total iodine in the thyroid glands, of which 1.1 to 2.0 micrograms were thyroxine: a thyroxine percentage of 23 to 30 percent. In another group of rats, under a wide range of iodine intake, an average value of 31.0 was obtained for the average percent of thyroxine iodine in the gland.

Wolff and Chaikoff (229) have reported that an average of 29.6 percent of the thyroidal iodine was in the form of thyroxine in eleven vertebrates, including fish, reptiles, birds, and mammals.

The changes in the thyroxine concentration of the human thyroid have been studied by Glimm and Isenbruch (73). During the first year the concentration was 24 milligrams percent, and during the first decade it was 25 milligrams percent. The concentration rose to 42 milligrams percent in the second decade, dropped to 29 milligrams percent during middle life, and again rose to 36 milligrams percent after the sixtieth year. Gutman and associates (85) reported that the average total weight of the human thyroid in New York was about 25 grams, which contained 8.85 milligrams of iodine. The range of iodine

concentration was from 0.05 to 0.45 percent of the dried gland. The average was 0.186 percent.

The alteration in iodine content of the thyroid gland with variations in iodine intake has been illustrated by Salter (169), who found 1.2 percent of iodine, of which over 60 percent appeared to be thyroxine in the partially purified, heat-coagulated thyroglobulin of Argentine sheep. Human thyroglobulin in Boston contained 0.22 percent iodine, of which 25 percent was thyroxine-like, while a human colloid goiter contained only 0.006 percent iodine and no detectable amount of thyroxine.

Free Iodide, Thyroxine, and Diiodotyrosine

Recently, Taurog et al (201) have reported that the inorganic iodine of the thyroid gland of rats varied from a concentration of 0.6 to 2.0 milligrams percent and represented about 1.0 percent of the gland's total iodine. This is about 500 times more concentrated than occurs in the plasma. The free (not bound in peptide or other linkages) thyroxine and diiodotyrosine found by Tong (203) amounted to about 0.5 percent of the total iodine of the gland. Even this represents a concentration over 100 times greater than that of the protein-bound

iodine in the plasma. This concentration gradient may be of some significance in the passage of thyroxine into the circulation.

The In Vivo Synthesis of Thyroxine

Nature of the Thyroid Hormone

Thyroglobulin, the stored hormone-containing protein of the thyroid gland, has been reported to have a molecular weight of about 675,000 (Heidelberger and Pedersen [92]). If the value is taken at 665,000, Brand and co-workers (32) have indicated that, according to Bergman's theory, the thyroglobulin molecule contains 5,760 amino acid residues, of which 120 are cystine (240 cysteine), 60 methionine, 60 tryptophane, 110 tyrosine, 10 diiodotyrosine, and 2 thyroxine. There would also be 80 glucosamine residues as well as some other carbohydrates present. The authors suggested that the molecule is composed of ten units of 576 amino acids each of which, aside from the thyroxine grouping, are similar in amino acid composition. Salter (169), however, quoted Svedberg as stating that he had evidence that the thyroglobulin as originally determined is an aggregate.

Thus, we have no definite knowledge as to the actual structure of thyroglobulin.

Recently, Tong and associates (203) have identified three major iodized constituents of the thyroid: thyroglobulin, thyroxine, and diiodotyrosine, which exist in both the free and combined states. Earlier, the same workers (198) found about 15 percent of the iodine in a thyroid hydrolysate to be present as monoiodotyrosine. Gross and Leblond (83) have presented evidence for the existence of three other as yet unidentified iodine compounds labeled 1, 3, and 5. Compound 5 has been identified as elemental iodine by chromatographic techniques, but it may be an artifact resulting from the manipulations (83). Unknowns 1 and 3 appear to be organic iodide-containing compounds resulting from thyroglobulin breakdown. These authors also indicate that some of the monoiodotyrosine and diiodotyrosine may result from thyroglobulin breakdown. Since most of these compounds have not been identified in the blood plasma, it appears that with the exception of thyroxine they are largely metabolized in the gland and are recombined into thyroglobulin or are excreted as noniodized compounds.

Precursors and Synthesis of Thyroxine

It has been mentioned previously that the thyroid gland has many times been shown to possess extraordinary iodine-concentrating ability. This iodine has been assumed to be utilized for iodination of tyrosine and the resulting diiodotyrosine converted to thyroxine. The administration of labeled inorganic iodine and its consequent appearance in the diiodotyrosine and thyroxine fractions of the thyroid confirmed the hypothesis that inorganic iodide is the source of the organic iodine but the sequence and process of conversion has only recently been shown experimentally.

Harrington and Barger (88) suggested that diiodotyrosine is the precursor of thyroxine because of the similarities in their structures. In 1940, Block (27) was able to form thyroxine from a synthetic diiodotyrosine in vitro. Morton (137) has demonstrated the conversion of iodide to diiodotyrosine and thyroxine by surviving slices of thyroid glands from sheep, dogs, and rats. In this case as much as 21 and 37 percent of added I^{131} was incorporated into diiodotyrosine and 4 and 6 percent into thyroxine when dog and sheep thyroids, respectively, were incubated for 3 hours. Mann (122) has presented evidence that

the conversion of inorganic iodide to diiodotyrosine takes place at the level of the cell membrane.

From the data of Leblond and Gross (113) it appears that circulating iodide is continuously bound to protein in the cytoplasm of the cell and the thyroglobulin formed is simultaneously deposited in the follicle.

Specific activity studies have shown that the monoiodotyrosine found by Taurog (198) and Gross (83) and their associates is not a breakdown product of diiodotyrosine, but is most likely a precursor of it. Specific activity-time curves have also shown (196) that diiodotyrosine is indeed the precursor of thyroxine and not the result of cleavage of the thyroxine molecule. In the same manner it has been indicated that free thyroxine in the gland does not represent a step in the synthesis of thyroglobulin but, rather, is a product of colloid breakdown and is the precursor of the circulating hormone (203).

Little can be definitely stated regarding the mechanism by which iodinations occur in the thyroid gland and the manner in which the stored hormone is released. Dempsey (52), in 1944, found peroxidase activity in thyroid cells. Since peroxidases catalyze the release of iodine from iodides, they may be

related to biological iodinations (Keston [110]) and thyroxine synthesis (Westerfeld and Lowe [224]).

The release of colloid to the blood and lymphatic systems can not be explained on the basis of simple diffusion through the cell membrane because of the size of the molecules involved. Furthermore, little, if any, thyroglobulin is found in the blood stream.

Salter and Lerman (168), in 1936, synthesized iodoproteins, using total thyroid extracts, which resembled thyroglobulin in activity. These authors suggested that an enzymatic mechanism involved in both synthesis of the hormone and destruction of the thyroid protein might be present. In 1940, Gersh and Caspersson (71) suggested that follicular colloid is digested enzymatically and the products taken up by the cells. Since that time DeRobertis and his associates (54, 56) have demonstrated proteolytic activity in thyroid colloid which varies in intensity with the pH of the medium and physiological activity of the gland. As will be discussed later, these alterations in proteolytic activity may explain the known changes in thyroid activity produced by administered thyrotropic hormone, iodine, and various goitrogens.

The physical and chemical cytology of the thyroid gland were poorly understood prior to 1940. Since that time the work of DeRobertis, summarized in his recent review (58), has somewhat clarified the picture. Through use of a freeze-drying technique he (53) was able to show the product of thyroid secretion which he designated as intracellular colloid and, with Gersh and Caspersson (71), presented evidence that this material contains organic iodine, which is presumed to represent the thyroid hormone.

Administration of the thyroid-stimulating hormone of the anterior pituitary causes rapid increase in the intracellular colloid (DeRobertis [55]). The colloid droplets are formed near the nucleus and, increasing in size, move toward the apex, where they are excreted into the follicular cavity. Expulsion of the colloid into the lumen is accompanied by rupture of the cytoplasm.

Later, the cells stop secretion toward the lumen and begin to secrete toward the base, reabsorbing the colloid present in the lumen.

Gersh and Caspersson (71) suggested that the follicular colloid might be digested by enzymatic action and the products

absorbed by the cells prior to release of the hormone to the circulation. The presence of proteases in the follicular colloid has been demonstrated by DeRobertis (54). The increased colloid loss in toxic goiter and colloid storage in simple goiter may be explained by increased or decreased levels of protease activity. That the enzymatic activity does change under these conditions has been shown by DeRobertis and Nowinski (56).

The Circulating Hormone

Rate of Thyroxine Secretion

Dempsey and Astwood (51) developed a method for estimating the rate of thyroxine secretion based upon the amount of hormone required to restore the thyroid weight of thiouracil-treated rats to normal. Using this method, these workers found the secretion rate in rats at 25° C. to be 5.2 micrograms of l-thyroxine per 100 grams of body weight daily. Similar results were obtained by Reineke and his associates (161), who reported that 4.75 micrograms of thyroxine per 100 grams of body weight daily were necessary to restore the basal metabolic rate of thiouracil-treated rats and that 4.8 micrograms daily restored the gland to normal weight. Somewhat lower results

were obtained by Griesbach and Purves (78), who found a daily secretion rate of 2.25 micrograms of d,l-thyroxine per 100 grams of body weight.

When Chaikoff and Taurog (37) applied the method of Zilversmit et al. (234) to their data, the calculated time required for complete renewal of the thyroxine in the gland (turnover time) was about 24 hours. Since the average thyroxine content of the thyroid glands of the rats was 3.3 micrograms, the rate of thyroxine iodine secretion was calculated to be about 1.5 micrograms of d,l-thyroxine per 100 grams of body weight per day. By specific-activity-time studies these authors (196) demonstrated a daily secretion rate of about 2 micrograms of thyroxine per 100 grams of body weight.

Wolterink and Lee (232) compared the thyroid activity as assayed by the thiouracil-thyroxine method with the results obtained from the rate of radioactive iodine turnover and found the two techniques gave comparable results. By the Dempsey and Astwood method a group of rats secreted 5.36 micrograms of d,l-thyroxine per 100 grams of body weight per day. The turnover rate calculated on the same rate was 5.16 micrograms

of d,l-thyroxine daily. This was a 4-percent difference which was not significant.

The quantity of d,l-thyroxine required to restore the thiouracil-induced enlargement of the thyroids of White Leghorn cockerels to normal size was determined by Schultze and Turner (171). The requirement for cockerels 2, 6, 7, 9, and 12 weeks old was 1.95, 7.55, 11.35, 14.4, and 16.5 micrograms daily. If these amounts are assumed to represent the normal rate of thyroxine secretion, the rate of secretion per 100 grams of body weight decreased with age during this time. Very similar values were obtained by Reineke and Turner (163) when they measured the rate of thyroxine secretion in groups of two-week-old White Plymouth Rock chicks at intervals throughout the year. Females varied from a d,l-thyroxine equivalent of 0.75 to 2.7 micrograms and males from 0.9 to 2.45 micrograms daily, depending upon the season of the year. Since d-thyroxine has little or no activity (162) the l-thyroxine values would be one-half those listed.

The thyroid glands of chicks were destroyed through administration of radioactive iodine by Winchester et al. (227), who then administered thyroxine in graded doses to ascertain

the amount required to give normal growth. This occurred when thyroxine was administered at the rate of 3.8 micrograms per 100 grams of body weight daily.

Turner (209) has shown that the rate of thyroxine secretion decreases in older hens. Two-year-old White Leghorns secreted the equivalent of 12 micrograms of d,l-thyroxine daily, or 0.6 of a microgram per 100 grams of body weight, as determined by the method of restoring thyroid weight after thiouracil administration.

Male White Pekin ducklings were shown to secrete 13.9 micrograms of d,l-thyroxine, and females, 14.0 micrograms daily at 3 weeks of age, by Biellier and Turner (23). This amounted to 2.85 and 2.67 micrograms per 100 grams of body weight. At 12 weeks the males were secreting 60 micrograms daily, and the females, 61.4 micrograms. These values correspond to 3.18 and 3.39 micrograms of d,l-thyroxine daily per 100 grams of body weight. Slightly higher secretion rates were found by Hoffman (100). In his study, 1- to 3-week-old White Pekin ducks secreted 18.7 micrograms of thyroxine daily, or 3.8 micrograms per 100 grams of body weight. In both cases the rates of

secretion calculated for ducks was considerably higher than those which have been reported for the chick.

The hourly rate of thyroxine secretion in dogs was found by Mann and co-workers (122) to be about 1.55 percent of the hormone contained in the gland. Taurog and his associates (195) found a turnover rate of 50 to 100 micrograms each 24 hours. This represented a rate of hormone secretion capable of completely replacing the protein-bound iodine in the circulation every 4 to 7.5 hours.

Using the Dempsey and Astwood method (51), Schultze and Turner (172) have reported the changes in thyroxine secretion rate with increasing weight of goats. At two months of age goats weighing 22 pounds secreted 1.80 micrograms of d,l-thyroxine daily per 100 grams of body weight, while at 45 pounds the rate was 3.13 micrograms, and at 76 pounds the secretion rate was 2.69 micrograms per 100 grams of body weight daily.

In 1935 the requirement for thyroxine to restore the normal basal metabolism of human myxedematous patients was reported by Thompson et al. (202) to be 0.25 to 0.35 milligrams daily.

It is well known that the rate of hormone secretion may be influenced by many factors. However, under standardized conditions the gland is thought to show little diurnal variation in its rate of secretion. The uptake of iodine and the release of hormone to the circulation appear to be simultaneous, continuous, and to occur at a constant rate (59).

Nature of the Circulating Hormone

It was at first believed that thyroglobulin is the circulating form of the thyroid hormone (15, 93). This view was discarded as a result of the work of Trevorrow (204), who found that the iodine of blood possesses alcohol solubility properties similar to iodide and thyroxine but unlike thyroglobulin of the gland, and Lerman (116), whose immunological studies failed to show any thyroglobulin in the serum or urine of hyperthyroid, normal, or hypothyroid patients.

Until recently, however, investigators have been reluctant to state that thyroxine per se is the circulating form of the thyroid hormone. The reasons, according to Taurog and Chaikoff (197), were: "(1) the failure of some investigators to account completely for the biological activity of thyroglobulin by its

thyroxine content, (2) the delayed response of animals to injected thyroxine, and (3) the failure of thyroxine to act in vitro."

In 1935 Harington (89, 90) postulated that the circulating hormone is a peptide containing both thyroxine and diiodotyrosine but later he concluded that the peptide hypothesis unnecessarily complicated the picture and that the circulating hormone is thyroxine (91).

The development of more sensitive methods for the chemical analysis for iodine (10, 12, 13, 43, 192), the use of radioactive iodine in fractionation studies in conjunction with chemical analysis (197), and the recent use of filter-paper chromotography (80, 112, 199) have aided materially in establishing the nature of the circulating thyroid hormone.

Trevorrow (204), Mann and her co-workers (121), and Bruger and Member (35) demonstrated that "organic" iodine in plasma can be precipitated with the Smogyi zinc sulfate precipitating reagent (183), while the "inorganic" fraction remains in the supernatant liquid. Many workers had previously attempted to fractionate blood on the basis of its solubility in methyl alcohol, ethyl alcohol, or acetone. Trevorrow (204) and Boyd and Clarke (31) have demonstrated that fractionations based

on this solubility are invalid since the amount of iodine extracted varied with the conditions of the extraction.

For the sake of clarity, and in conformity with present usage, the terminology used here in referring to the blood iodine fractions will be those suggested by Salter (169). He has proposed that the blood iodine should be separated into an inorganic and a precipitable (or protein-bound) fraction. The precipitable fraction may then be separated into a thyroxine-like and a diiodotyrosine-like fraction.

Leland-Foster (115), in 1932, showed that n-butyl alcohol completely extracts all of the thyroxine from thyroid protein which has undergone strong alkaline hydrolysis. Taurog and Chaikoff (197) have employed this technique to fractionate the iodine of plasma. Since this iodine is easily extracted with butyl alcohol, while thyroglobulin must first be hydrolyzed, it is apparent that the circulating hormone differs from that of the gland. It does not mean, however, that the circulating hormone is necessarily free from attachment to protein. As a matter of fact, the results of Trevorrow (204) and Riggs and his associates (167) would indicate that the circulating hormone is attached to protein. Riggs et al. concluded from dialysis

and sedimentating rates that circulating thyroxine must exist in a molecule of about the same size as the plasma albumin or in smaller molecules which are attached to the albumin. Taurog and Chaikoff (197) confirmed these findings and those of Bassett, Coons, and Salter (18) that most of the organic iodine of plasma is associated with the albumin fraction. The concentration of organic iodine, however, was highest in the quantitatively smaller gamma-globulin fraction.

These authors also added to the evidence for circulating thyroxine through showing that crystalline thyroxine carrier exhibited a constant specific activity upon repeated recrystallizations after addition to the butyl alcohol extract of the plasma of rats injected with I^{131}. In addition, radioactive iodine in the butyl alcohol extract was distributed between two unmiscible solvents almost exactly as added thyroxine carrier was, but quite unlike an added thyroxine peptide carrier. Wilmanns (226), however, is reported as having treated whole blood with hot butyl alcohol and found that only 65 percent of the iodine could be extracted, and of this only 28 percent could not be re-extracted with 1 N. sodium hydroxide. He concluded, therefore, that blood contains two organic fractions: free thyroxine

(28 percent) and a stable protein-bound iodine fraction (34 percent) (197).

Since Harington in 1935 (91) postulated the presence of both thyroxine and diiodotyrosine, the presence of a diiodotyrosine-like fraction has been confirmed by Trevorrow (204) and Morton and associates (135). More recently, using a chromatographic technique in conjunction with tracer doses of labeled iodine, Taurog and his co-workers (199) have presented further evidence that thyroxine is the major organic-iodine-containing fraction in the plasma. In some cases a faint band corresponding to diiodotyrosine and a faint darkening at the solvent front could also be seen. Taurog questioned, however, whether this technique could distinguish thyroxine from one of its small peptides. Thus, the possibility of a circulating thyroxine peptide has not yet been ruled out. These results were confirmed by Laidlau (112).

Gross et al. (80, 83) were able to show only thyroxine and iodide in rats. In iodine-deficient rats, compound 1, to which reference has previously been made as a component of the thyroid gland, was also found. These workers believe that free diiodotyrosine does not normally appear in the circulation.

In the hypophysectionized rat Morton et al. (136) have shown that about 80 percent of the radioactive iodine taken up by the gland appeared in the diiodotyrosine-like fraction. The diiodotyrosine-like fraction of the blood plasma was likewise greater than normal while the thyroxine portion of the thyroid and plasma decreased.

The exact nature of the circulating iodide compounds must thus remain a question. It is well established that inorganic iodide and thyroxine are found in the circulation. The evidence indicates that the thyroxine is loosely combined with the plasma protein, but not through a peptide linkage. The identity of other organic iodides in the plasma must await further work. Under certain pathological conditions, diiodotyrosine may be excreted by the thyroid gland. Whether this occurs in the normal animal is an open question. Undoubtedly if it does occur the amount is small. Recent work has indicated that other organic iodides may be present under certain conditions but their existence has not been confirmed.

The distribution of iodine between the blood plasma and red blood cells has also been questioned. Silver (182) found very little protein-bound iodine in the erythrocytes, while

McClendon and Foster (128) reported that fully half of the protein-bound iodine of the blood is present in the red blood cells; however, the analytical methods used were subject to error through impurities. Trevorrow (204), on the other hand, studied a series of analyses and found the total iodine of blood and plasma to be distributed in proportion to the water content of the cells and plasma.

Recent work by Rall and co-workers (150) has shown that, in vitro, chlorides and iodide pass rapidly across the red cell membrane and there is no reason to expect the situation to be altered in vivo. Scott (176) has shown through radioactive studies that inorganic iodide rapidly penetrates the red cell. However, the cell membrane appears to be impermeable to large protein molecules containing labeled iodine.

It is interesting to note that the initial red cell-plasma iodine ratio varies from 0.42 to 0.52 and is the same in hyperthyroid, euthyroid, and hypothyroid patients immediately after the oral administration of 100 to 150 microcuries of radioactive iodide. When the time required for the ratio to drop from 0.5 to 0.25 is calculated, a significant difference between the three thyroid states may be found. (A ratio of 0.25 suggests that

one-half of the iodine in the blood stream is protein bound and can not penetrate the red blood cells.) Human hypothyroid patients required 100 hours, euthyroid patients, 40 hours, and hyperthyroid patients needed 12 hours to reach this ratio. Thus, the red blood cell-plasma iodide ratio after administration of radioactive iodide appears to be a good indicator of thyroid status.

At any rate, Salter (169) has recommended the use of plasma values rather than those obtained from whole blood because of the greater spread of values and consequent greater sensitivity when plasma is used. Furthermore, whole blood is unsuited for certain methods of iodine determination because of the presence of certain substances, such as iron, which may interfere with the final colorimetric determination.

There is some evidence that the thyroid may elucidate some substance or substances other than the iodinated compounds previously discussed. Truesdell (205) has reported that gastric secretion of dogs with the Pavlov pouch may be reduced through feeding whole thyroid. Katz is reported to have correlated hypothyroidism with peptic ulcers in the human. Watman and Nasset (220, 221) have reported that thyroidectomy

significantly reduces the survival time of guinea pigs injected with histamine. Thiouracil administration has no effect on the survival time and thyroxine or diiodotyrosine are ineffective in combatting formation of the peptic ulcer which forms in the stomach or duodenum, its perforation, and the resulting peritionitis. Since the animal is hypersensitive when made hyperthyroid either in the presence or absence of the gland, it would appear that the effect is not one of antagonism or detoxification of histamine.

The actual presence or nature of a thyroidal secretion affecting gastric secretion is not confirmed, but an area for future investigation is indicated.

Iodine of the Blood and Plasma

Literally thousands of determinations have been made of the iodine fractions of the blood, plasma, or serum of human thyroid patients since 1920, when Kendall and Richardson (109) established that normal blood contains iodine in a characteristic concentration. The early results establishing the relation between the level of blood iodine and thyroid status has been reviewed by Curtis et al. (48), who concluded that there is good agreement between the blood iodine level and thyroid function.

The total iodine content of the whole blood of normal human patients was found by Perkin et al. (142) to range from 2.4 to 18.5 micrograms per 100 grams of blood, while a somewhat narrower range was obtained by Davis and co-workers (50) on patients in Chicago. In thirty-four determinations on twenty-eight people, they obtained a range of total iodine of 8.5 to 16.2 micrograms percent. The average was 11.9 micrograms percent. A slightly greater variation was obtained with women than with men. The eighteen women varied from 8.5 to 16.2 micrograms percent. The range in men was 9.5 to 14.5 micrograms percent. Since none of the patients showed evidence of thyroid disease and none received iodized salt, the greater variability of the women was attributed to their changing menstrual state.

The effect of various thyroid conditions on the blood iodine level was studied by McCullagh and McCullagh (129). The blood iodine of ten patients hospitalized with nonthyroidal diseases averaged 10.2 micrograms percent as compared with the normal level 10.0 micrograms percent. Violent exercise reduced the level to 6.8 micrograms percent within 2 hours. Ten hyperthyroid patients varied from 11.1 to 49.8 micrograms

percent and six cases of hypothyroidism averaged 7.5 micrograms percent, while of six cases of hypometabolism not related to the thyroid, two patients showed normal levels and the remainder varied from 6 to 11.2 micrograms percent. In six cases of hypermetabolism with no evidence of thyroid disease, the level was between 6.1 and 10.3 micrograms percent.

The blood iodine in thyroid, cardiorenal disease, and leukemia was investigated by Turner and associates (210) in New York City. Twenty males ranged from 3.8 to 8.6 micrograms percent, averaging 5.9, while a like number of females averaged 6.8, with a range of 3.5 to 10.4 micrograms percent. In twenty hyperthyroid patients the blood iodine was elevated in fourteen and normal in six. The level was normal or low in five hypothyroids. The blood iodine was low in eight of twelve cases of myeloid leukemia and normal in the other four. The range was 1.3 to 7.4 micrograms percent, with an average of 3.4. In lysophoid leukemia the level was normal or elevated.

The age differences in blood iodine have been reported, for children, by Fashena (64). Those under 24 hours old averaged 4.7 micrograms percent with a range of 1 to 11.0 micrograms

percent. Up to 13 years the average was 6.6 micrograms percent, ranging from 3.0 to 12.0 micrograms percent.

Perkin and Brown (143) studied the effects of iodine administration and sex difference on the blood iodine of dogs. When 72 milligrams of iodine daily were fed, the blood iodine varied from 75 to 2,000 micrograms percent. The blood level of males was greatly depressed following complete thyroidectomy but there was no apparent effect on the females. However, following bilateral oophorectomy of the thyroidectomized females, the level fell to one comparable with the males.

As indicated by the work of Turner (210) and Salter (169, 170), the total blood iodine, under certain circumstances, fails to reflect the actual thyroid status. Clarke and Boyd (41), for instance, were unable to show any seasonal variation in thyroid activity of pigeons and chickens through the blood iodine, although it is known that the thyroid activity does vary in these birds.

Because of this occasional lack of sensitivity, in recent times the protein-bound iodine fraction of the plasma, serum, or blood has been used. Salter and co-workers (170) correlated the relation of protein-bound iodine of the blood to the final

diagnosis of thyroid status in one hundred of their cases. In about one-third, in which thyroid status had been suspected, the basal metabolic rate was not compatible with the bedside diagnosis. In every case the protein-bound iodine level confirmed the diagnosis. In mild hypothyroidism, where the metabolic rate had not fallen to an abnormal level, this measure was especially useful in establishing the need for thyroid therapy. Total iodine levels were not sufficient in these cases. For example, one patient showed a total iodine level of 7.1, but a protein-bound iodine level of 2.2 micrograms percent, as compared to the normal averages of 6.3 and 4.8 micrograms percent, respectively. In the one hundred cases the basal metabolic rate and protein-bound iodine agreed in seventy-one, and in twenty-nine the protein-bound iodine was more reliable.

The range of protein-bound iodine values of blood does not differ greatly from total iodine of the blood in human patients on a low iodine diet. In livestock receiving iodized salt or feed which contains variable amounts of iodine the difference between the protein-bound iodine and total iodine may be more variable. In any case, however, the protein-bound iodine is somewhat more sensitive to changes in thyroid secretion rate

than is the total iodine. Taurog and Chaikoff (194), for example, have found the correlation between the plasma protein-bound iodine and thyroxine iodine to be 0.84. The plasma protein-bound iodine is dependent upon and limited by the gland's ability to produce thyroxine. The level of the protein-bound iodine in plasma falls rapidly after thyroidectomy. A noticeable decrease occurs within 4 hours and a minimum value is reached by the third day. Injection of the thyrotropic hormone raises the plasma protein-bound iodine (36).

Representative plasma protein-bound iodine levels have been reported by several investigators. McClendon and Foster (128) found the level in two cows to be 6.4 and 6.8 micrograms percent, respectively; humans ranged from 5.8 to 9.4; a horse, rabbits, and a cat gave values of 9.2, 6.6 to 11.1, and 7.6, respectively. Connor et al. (43) found the level in human serum to range from 4 to 6 micrograms percent, with an average of 4.8. The nonprotein-bound iodine in these cases ranged from 1 to 3 micrograms percent.

Long and co-workers (120) have recently studied the plasma protein-bound iodine of a large number of dairy and beef cows. They averaged 3.15 micrograms of iodine per 100

milliliters of serum. The values, arranged according to breed, were: Jersey, 4.11; Guernsey, 3.51; Brown Swiss, 3.37; Ayrshire, 3.19; Holstein, 2.73; and beef breeds, 2.19 micrograms percent. Some of these differences were significant. There were age differences also. Calves averaged 4.8 micrograms percent, 3- to 4-year-old cows averaged 3.1, and 7- to 8-year-old animals averaged 2.6 micrograms percent.

A considerable amount of data on patients at the Iowa State University Hospital has recently been presented by Barker and his collaborators (13). The range of values of 942 determinations of plasma protein-bound iodine on 694 patients clustered between 3 and 7 percent. There was no sharp line separating the normal and hyper- or hypothyroid patients. Determinations made on sixty-eight apparently euthyroid students, technicians, and staff and faculty members ranged from 3.4 to 8.0; the mean was 5.1 micrograms percent. At that institution the normal range of values has been considered to be from 4.0 to 8.0 micrograms percent. If, however, the range had been lowered to 3.5 micrograms percent, of eighty-nine patients on whom basal-metabolism and iodine-uptake data were available, only one hypothyroid and one thyrotoxic patient would have been

included in the normal group. In contrast, several BMR's were at considerable variance with the general clinical opinion. These results are supported by a previous report by Talbot et al. (191).

The relation of obesity to thyroid function, as determined by the plasma protein-bound iodine, was studied by Williams (225). Of twenty-four obese patients, the plasma protein-bound iodine was less than 4 micrograms percent in eleven and above this amount in thirteen. Thus, the relation between obesity and the plasma protein-bound iodine is not great.

Care must be exercised in interpreting the results of plasma protein-bound iodine determinations, since recent iodine therapy or topical application of iodine to the patient will cause abnormally high results (11).

Evidence of a nonthyroidal iodine fraction in the plasma protein-bound iodine of cattle has been presented by Reece and Man (156), who found the serum-precipitable iodine of nonpregnant, lactating Jerseys averaged 4.6 micrograms percent, while the butanol-extractable iodine averaged 2.5 micrograms percent. Similar results were obtained with Brown Swiss cattle, although the serum-precipitable iodine concentration was somewhat less for the latter breed.

The Metabolism and Excretion of Thyroxine

The metabolism of thyroxine remains nearly as much of a mystery today as it was 20 years ago. Although the evidence just discussed points toward thyroxine as being the circulating thyroid hormone, even this fact is not established with certainty. The metabolic effect of thyroxine is well known, but the reactions by which the cellular oxidations are increased have not been demonstrated. The paths by which thyroxine is excreted have been shown, but the excreted metabolites have yet to be identified.

Recent work by Gross and Leblond (81), which confirms previous reports by Monroe and Turner (134), Kellaway et al. (107), Taurog and co-workers (200), and the early work of Barnes (14), has thrown some light on the paths by which the circulating hormone is transported to the body cells and finally excreted, but does not attempt to show how the metabolic effects of thyroxine are produced.

Tracer amounts of radioactive iodide injected into rats were found in the stomach in a butanol-insoluble form within 2 hours after administration. After the iodide had time to be taken up by the thyroid and released again as thyroxine (24 to

72 hours) the activity was found distributed throughout the plasma and tissues of the body.

The radioactivity of both the plasma and tissues decreased with time, but the rate of decrease was greater in the plasma. This can perhaps be explained by the conversion of thyroxine into its metabolites by the tissues. The radioactivity was initially in a butanol-soluble form, but the butanol-insoluble activity increased with time.

The tissue decrease in activity was paralleled by a fall in the activity of the whole body. Thus, in this case it was calculated that the thyroxine content of the body was completely renewed each 25 to 45 hours.

The liver was particularly rich in its thyroxine content, probably because of its role in the deactivation of thyroxine. The mechanism by which the liver deactivates thyroxine is not known. That simple deiodination of thyroxine (Salter [169]) with elimination of the iodide by the kidney and inactive residue through the bile is not sufficient explanation is indicated by recent reports (82, 200). The deamination of thyroxine followed by reamination to form the inactive d-thyroxine is a possibility

(60), or a breaking of one or both of the tyrosine rings may occur (20).

In the light of this and other work it appears that thyroxine is normally destroyed in the liver and the degradation product excreted through the bile, although some thyroxine may be excreted through the intestinal wall (79). When abnormally large amounts of thyroxine are present, some may appear in the bile in active form, but once the level falls to normal the degradation process converts all, or nearly all, to the usual metabolites.

The elimination of thyroxine and its metabolites from the body appears to be largely through the urine and feces: the urine containing iodide and the feces carrying off the metabolites of thyroxine, iodide, and under some conditions, thyroxine itself (82). Very little thyroxine is excreted in the urine (62, 79, 81), and apparently the kidney has no significant role in the excretion of the hormone per se.

No doubt, too, a certain amount of the iodide is returned to the circulation and carried to the thyroid gland where it is reincorporated into thyroxine.

While no explanation of the ability of thyroxine to produce a sustained rise of oxygen consumption can be given, it appears to be the end result of a series of reactions. This is deduced from the fact that the hormone is rapidly eliminated from the body, but its metabolic effects continue for an extended period.

Endocrine and Other Relationships of the Thyroid Gland

Relation to the Anterior Pituitary

It is well known that the anterior lobe of the pituitary produces a hormone which stimulates the thyroid gland to secrete its hormone. Albert (2), in his recent review, defined the thyrotropic hormone as "a substance from pituitary tissue which, when given parenterally in proper dosage to various vertebrates, induces specific effects on the thyroid consisting of secretory alterations of the cytological components of the follicular cells, hypertrophy and hyperplasia of the epithelium, vacuolization and resorption of colloid, loss of hormonal iodine, and increase of vascularity and of the size of the gland."

Although the stimulating effect of anterior pituitary extracts on the thyroid had been known for many years, it was

not until 1929 that Aron (7) and Loeb et al. (117, 118) demonstrated the thyroid-activating principle of the pituitary gland. The site of the production of the thyrotropic hormone in the pituitary has not yet been well established. Purves and Griesbach (149) have recently presented evidence that two types of glycoprotein-containing cells are present in the anterior pituitary, one of which they believe secretes the thyrotropic hormone. Earlier, these workers had presented evidence that the basophilic cells of the anterior pituitary were involved in the secretion of thyrotropin (78); others, however, have shown good reasons to believe that the acidophila may also be involved (74, 104, 178, 179).

The process by which the thyrotropic hormone activates the thyroid gland is unknown, but the effects of its presence or absence are well known. Hypophysectomized animals have a reduced thyroidal ability to take up inorganic iodide from the blood (72, 216), and decreased conversion of inorganic iodine to thyroxine (4, 216), but the primary effect seems to be one of preventing the release of thyroxine by the gland (155, 216). These effects of a deficiency of thyrotropic hormone are then

manifested by lowered levels of circulating hormone (72) and lowered metabolic rate (66).

Although the thyroid gland is generally said to be controlled by the anterior pituitary gland through the thyrotropic hormone, in a manner of speaking, it is self-controlling. The rate of secretion of the thyroid-stimulating hormone is determined by the titer of thyroid hormone in the circulation (78). When the amount of circulating thyroxine is low, through reduced activity, thyroidectomy, or iodine deficiency, the pituitary responds with typical changes in the basophilic and acidophilic cells and increased weight (74, 78). In addition, thyroxine administration reduces the activity of the pituitary (16, 69, 178).

It has been postulated, too, that the thyroid gland may be partially self-regulatory in that thyroxine and, to a lesser extent, inorganic iodide are capable of depressing the rate of thyroxine secretion directly as well as through its action on the pituitary gland (3, 230). DeRobertis and Nowinski (57) have indicated that this effect may result from inhibition of the proteolytic enzyme system by the iodine.

The metabolism of the thyrotropic hormone once it has performed its normal task of activating the thyroid gland is not

well understood. Obviously it must be eliminated per se or inactivated and then eliminated. Although the methods of thyrotropin assay are crude, the evidence points towards the second conception as indicating the fate of this hormone. In vitro studies by Rawson et al. (153) and Albert et al. (1) have definitely shown that thyroxine and iodine can inactivate the thyrotropic hormone under these conditions. In addition, in vivo, thyroxine and potassium iodide have been shown to depress the response of the thyroid to thyrotropin (5, 45), although inorganic iodide does not prevent the cellular proliferation of the thyroid produced by this hormone.

The mechanism by which the thyrotropic hormone is inactivated is not known. It has been suggested that a prosthetic group may be removed from the hormone or that thyrotropin may somehow be bound (153) or that a chemical change, probably oxidative in nature, takes place (1, 154).

It has been suggested that the control of the pituitary secretion of thyrotropin may depend upon nervous impulses. This, however, has been disproven by the work of Hektoen et al. (94), Marine and Rosen (126), Gorbman (76), and Barrnett and Greep (16), who, through attempted nerve stimulation, thyroid

transplants, pituitary transplants, and pituitary stalk section, were unable to show any nervous relationship in thyroid activation.

In spite of the role of the thyrotropic hormone in thyroid control, secretion of thyroxine does not entirely cease on hypophysectomy. The thyroid is able to respond to the stimulus of low iodine intake through increased cell height and vascularity (39) and thyroxine secretion remains at about 12 percent of its former level (152) even after hypophysectomy.

Relation to the Gonads

Little is known regarding thyroid-testes relationship in comparison to the thyroid-ovarian relations.

In the rat, thyroid feeding increases the testis weight (95), while testosterone causes thyroid hypertrophy (96) and increases the uptake of iodine (133). It is likely, however, that in the male the thyroid hormone affects testis development only through its relation to cellular metabolism.

In females the relation of the ovary to the thyroid is essentially one of antagonism as indicated by the well-known fact that goiter occurs much more frequently in women than men.

Furthermore, thyroid enlargement and increased hormone secretion takes place during pregnancy and lactation (49, 169). Iodine excretion is increased during menstruation (42) and blood iodine is not greatly lowered in thyroidectomized females unless they are simultaneously castrated (143).

Turner and Cupps (206) found the thyrotropic hormone content of the female albino rat to be only about 50 percent as high as in the male during growth. The concentration rose during the latter part of pregnancy and increased further during lactation.

The rise in plasma neutral fat, calcium, and phosphorus which is caused by estrogen administration is prevented by simultaneous injection of thyroxine (130). In 1910 Hoskins (101) reported that feeding thyroid to mother guinea pigs resulted in reduced ovarian weight of the offspring. Supporting evidence comes from Tyndale and Levin (213), who observed that thyroxine prevented ovarian response to menopausal urine. Since the response was prevented in hypophysectomized animals, the inhibiting effect was direct and not mediated through the pituitary. Herring (95), on the contrary, reported increased ovarian size in rats fed thyroid material.

An effect of estrogen on thyroid activity was shown by Karp and Kostkiewicz (106), who caused colloidal goiter by folliculine administration. Similar results were recently reported by Gardner (70). Small doses of estrogen increase the output of thyroxine by the thyroid of rats and mice, while massive doses depress iodine turnover, according to Wolterink et al. (233). According to Money et al. (133), some estrogens increase and others decrease iodine accumulation in the rat thyroid. Diet seemed to have an effect on whether enhanced accumulation took place. Pregnancy urine extracts as well as the follicular hormone have also been successfully used in treating human hyperthyroidism (186).

The relations between the thyroid and ovary are complicated and reciprocal. Some of the inhibiting properties of the thyroid on ovarian function are mediated through the pituitary and others are direct repressions. It is believed, too, that the ovary may depress thyroid function through preventing the formation or release of the thyrotropic hormone. The effect may also be a direct one produced by release of the ovarian iodine into the blood stream in quantities sufficient to depress thyroxine release. Under normal conditions, however, there is little

evidence that physiological concentrations of female sex hormones affect thyroid status.

Effect on Reproduction of Farm Animals

The relation of hypo- or hyperthyroidism to reproduction in farm animals has not been thoroughly studied, particularly as far as the female is concerned. Thyroidectomized cows do not show visible symptoms of estrus (33, 185); however, ovulation does occur and fertilization and pregnancy can take place in myxedematous animals.

Only casual observations have been made regarding the effects of thyroprotein administration on dairy cattle. Van Landingham et al. (219) have reported that cows made hyperthyroid in this way are slow to come into heat.

The effects of thyroid deficiency or hyperfunction are better known in male than female farm animals, but even here there is no agreement.

Thyroidectomized bulls are entirely lacking in libido (147), but spermatogenesis is apparently normal and semen obtained by massaging the ampullae is unaffected. Reineke (164) fed thyroprotein to fourteen aged bulls and obtained increased

vigor and speedier ejaculation from this treatment in ten of the sires. Schultze and Davis (173) obtained better conception rates with five of seven bulls treated with thyroprotein.

This effect of thyroprotein is probably a result of the increased metabolic rate, since metabolism declines with age (34); and dinitrophenol, a metabolic stimulant, has the same effect on male sexual behavior as thyroprotein (147). In addition, thyroidectomy, and perhaps naturally occurring thyroid deficiency, reduces the pituitary gonadotropic hormone level in goats (158) and response to gonadotropins in rats (132). Furthermore, added thyroxine increases the metabolism of semen (123, 174, 175) and perhaps the conception rate. Unfortunately there is no known information available on the normal thyroxine titer of semen and its fluctuations with thyroid function.

In rams, the temporary infertility found during hot summer weather (131) appears to be identical with that caused by thiouracil (29), and may be prevented or cured by administration of thyroactive substances.

Thyroidectomy, Berliner and Warbritton (19) observed, causes a decrease in sperm numbers and an increase in abnormal forms. The testes were edematous, the interstitial

tissue decreased, and the seminiferous tubules sloughed and were pyconocic. These observations, and the fact that thyroxine secretion falls during hot weather (51) point towards a direct relationship between the thyroid hormone and reproduction in rams.

The effect of thyroid status on egg production of poultry is not clear. The Missouri workers (207, 208) have reported increased egg production when thyroprotein was added to the poultry ration. However, neither the experimental nor control groups did well, as evidenced by the fact that in one experiment the experimental group averaged 40.6 percent and the control group 22.6 percent production during their second year. One wonders if the effect of thyroprotein would have been the same with high-producing birds. As a matter of fact, Hutt and Gowe (105) were unable to perceive any particular effect of thyroprotein beyond a slight initial decrease in egg production.

Relation to Quantity and Composition of Milk

Since the development by Reineke and Turner (159) of an artificial thyroprotein containing thyroidal activity in good amount, a great amount of interest has developed regarding the relation

of thyroid activity to the quantity and composition of milk from dairy cattle.

Swett et al. (190) have reported that the thyroid gland weight of the breeds of cattle varies inversely with the quantity of milk produced, at least in so far as the Holstein, Ayrshire, Guernsey, and Jersey breeds are concerned. The thyroid is not essential for the initiation of lactation or milk production, but, following thyroidectomy, lactation ceases about six months after parturition (184). Incomplete thyroidectomy temporarily lowered production, but it gradually returned to normal. The milk content of fat, lactose, and nitrogen, and its specific gravity appeared to remain unchanged. Oral administration of thyroid material prevented these effects of thyroid deficiency.

There have been many reports on the effects of feeding thyroactive material to cattle, a number of which have recently been reviewed by Blaxter and his collaborators (26). In general, cattle respond to thyroxine administration by increased milk and butterfat production, especially after the normal peak of lactation has been reached. The increase in butterfat comes about not only as a result of increased quantities of milk, but also through a rise in the percent of butterfat.

The effects of continued administration of thyroidal material is not yet known, although this type of experimentation has been underway for a decade. The Bureau of Dairy Industry of the United States Department of Agriculture, however, has advised against this type of stimulation since cows fed in this way required more nutrients and did not respond to the stimulation as well in succeeding lactations as they did in the first. In addition, calf losses were somewhat higher in their experimental groups (157).

The feeding of thyroidal or other iodine-containing feeds increases the iodine content of the milk; thyroxine, however, is not secreted per se in the milk (160). Matthews and co-workers (127) have reported an average milk iodine content of 80 micrograms percent from cows receiving 3.2 milligrams percent of iodine as potassium iodide in the ration. This amount was seven to twenty-six times that of milk from cows on a similar ration which did not include an iodine supplement. The effect of iodine supplementation was least during the late spring and early fall. Bartlett et al. (17) found the normal iodine content of milk to be 1.5 micrograms percent, but when 1 gram of iodine

in the form of iodinated protein was fed daily for 8 days the value rose to 125 micrograms percent.

Remington and Supplee (165) studied pooled milk from several points in South Carolina and reported a variation in iodine content with the region. Milk obtained from the Piedmont area was considerably higher than that from the coastal region. Samples from New York and Wisconsin were lower than those from the southern state. Little seasonal variation was found in the Carolina samples, although they were significantly lower in April and May than at other times. The seasonal variation was somewhat greater in the New York and Wisconsin samples.

The iodine content of skimmed human milk and human and dairy-cow colostrum was investigated by Turner (212). Skimmed human milk ranged from 6 to 23 micrograms percent, with an average of 12.4 micrograms percent. The average value was higher in goitrous than nongoitrous areas, possibly because of compensatory enlargement of the mother's thyroid. The iodine concentration tended to decrease after the third month of lactation.

The iodine content of both human and cow colostrum is considerable higher ~~in iodine~~, particularly the first day or two

after parturition than later in lactation. This initial high iodine content may be of importance to the young in meeting the stresses of its new environment.

The effect of hyperthyroidism or thyroprotein administration on milk and butterfat composition is not well established. The composition of the milk fat appears to be unchanged, although there is a tendency for a rise in the unsaturated fatty acids and a fall in the saturated acids for a short time after treatment begins (99). The solids-not-fat increase (65).

The percentage of lactose was reported to increase by Ralston et al. (151) and several other workers, but Archibald (6) found a decrease.

Many workers have studied the protein content of milk from hyperthyroid cows, but again there is disagreement as to what effect, if any, this condition has on the milk nitrogen. Ralston (151) and Archibald (6) and their associates reported declines in the nitrogen or protein content; however, Van Landingham and his co-workers (218) and Hibbs and Krauss (97, 98) reported no change in this constituent.

No large amount of work has been carried out on the vitamin content of milk from hyperthyroid cows. The indications

now are that Vitamin A and carotene are not affected (97); Vitamin C may be decreased (17, 217), although Hibbs and Krauss found it unchanged; thiamine content may be unchanged or decreased (98, 108); and riboflavin is lowered (108). No information is available on the other vitamins.

It may be seen, then, that the changes in milk composition are not great and its nutritional value is on the whole unchanged by thyroxine administration.

Relation to the Adrenal Cortex

Hoskins (102) first showed an antagonism between the adrenal cortex and the thyroid gland by demonstrating that hyperthyroidism in guinea pigs caused adrenal hyperplasia. The effect of hypothyroidism produced by thiouracil and thyroidectomy on adrenal activity has recently been studied by Freedman and Gordon (68), who found that thiouracil caused pronounced atrophy of the adrenal glands and an initial increase in their ascorbic acid concentration which soon returned to normal. Thyroidectomy, on the other hand, produced less atrophy but a greater adrenal inhibition, as indicated by a final decrease in ascorbic acid concentration. This effect of

hypothyroidism has been shown to be primarily one of failure of the pituitary to release the adrenal corticotropic hormone, although its rate of production may also be reduced (86).

Perry (146) has demonstrated that cortisone and the adrenal corticotropic hormones reduce the thyroxine content of the thyroid probably through direct reduction of the rate of iodine uptake. The effect did not appear to involve the thyrotropic hormone since no atrophy of the thyroid occurred.

Paschkis et al. (141) were unable to show any significant effect of desoxycorticosterone acetate and adrenal cortical extract on radioactive iodine uptake by the thyroid. Severe stress, which is also probably mediated by the adrenals, has been shown to reduce iodine uptake by the thyroid. Bogorach and Timira (30) suggested that this may be caused by reduced secretion of the thyrotropic hormone which decreases the thyroid's ability to trap iodine. Since, however, the primary effect of the thyrotropic hormone has been demonstrated to be on the release of thyroxine from the thyroid, it would appear that the stress mechanism is more likely to be one of direct inhibition of the thyroidal concentration of iodine.

Relation to the Adrenal Medulla

The adrenal medulla appears to have only a minor effect on thyroid activity. Adrenaline administration produces a temporary rise in blood iodine followed by a fall to a subnormal level. The source of this iodine has not been proven, although there is some evidence that it comes from the thyroid gland. Its clinical or physiological significance, if any, is not known (169).

Relation to the Thymus

The antagonism between the thyroid and thymus is well known, although its significance is not. In 1924, Marine et al. (125) showed a relationship between the two glands. Thyroidectomy hastened the involution of the thymus in rabbits, while hyperthyroidism caused hypertrophy of the thymicolymphatic tissue. Likewise, thymus extract inhibits the response of rabbits to thyroxine administration (169).

Boatman and Campbell (28) were recently unable to show any effect of thymus feeding on radioactive iodine uptake or histological appearance of the thyroid glands of rats. Cosma

(46), however, found increased thyroid activity in thymectomized animals.

Relation to the Parathyroids

When large doses of thyroxine are administered to an animal the parathyroid glands tend to hypertrophy, and calcium excretion is greatly increased. In hypoparathyroid animals thyroxine induces tetany. Thyroidectomy, on the other hand, reduces calcium turnover and causes involution of the parathyroids in rabbits (169). The effect of thyroxine on calcium and phosphorus metabolism thus appears to be a reflection of the activity state of the endocrine glands involved, and not a direct relationship between calcium and iodine balances.

Relation to the Pancreas

The aggravation of diabetes in hyperthyroidism and its alleviation by thyroidectomy in man establishes a link between the thyroid gland and carbohydrate metabolism. This effect of the thyroid is due to (1) increased oxidation of carbohydrate and (2) increased rate of hepatic gluconeogenesis (21).

Ligation of the dog pancreas causes an increase in colloid and iodine content of the thyroid (189). Blood iodine is high in diabetes (211) unless the plane of nutrition is low. Intravenous administration of insulin causes a temporary drop in the circulating iodine. The pancreas itself has little affinity for iodine (169).

Relation to Nutrition

The relation of nutrition to goiter has recently been reviewed by Greer (77), who has classified the early work into four divisions: "(1) The effects of high protein diet; (2) high fat diet; (3) high carbohydrate diet; (4) vitamin deficiency and excess." Later work is referred to as cabbage goiter, since most recent work has been with cabbage and related foods.

High protein diet. Early reports from England and Germany, and later in this country, have shown that diets high in meat, especially liver, tended to produce enlarged thyroids. Whether such glands are over- or underfunctional is not known, and since such factors as temperature are not known to have been controlled, further studies utilizing present knowledge might well be undertaken.

High fat diet. The results on high fat diets have been conflicting. Enlarged thyroids in dogs and reduced growth of tadpoles has been attributed to the large amount of fat injested. On the other hand, rats and rabbits have given no response to fat. Reduced food intake on high fat diets with consequent iodine deficiency could, in some cases, account for any thyroid enlargement observed.

High carbohydrate diet. The last report is over a quarter of a century old, but a few experiments tended to show thyroid hypertrophy on high carbohydrate diets. These reports are difficult to evaluate, however, since the iodine content and palatability of the rations varied markedly.

Vitamin deficiency. Much of the early work showed deficiency of Vitamins A, B, and C caused thyroid enlargement but, as with most of this work, too little was known about thyroid physiology and the vitamins themselves for the results to be conclusive.

More recently, Sherwood and Luckner (181) found that administration of Vitamin A causes an increase in the stroma and epithelium of the acini, and that colloid is reduced. Carotene

produces similar, but not identical, changes. Cooper and co-workers (44) reported that hyperthyroid chicks have a higher-than-normal requirement for Vitamin A.

Sure and Buchanan (187) have presented data showing that crystalline Vitamin B_{12} counteracts the toxic effect of thyroxine on rats. Vitamin B_{12} has also been reported to protect rats against massive doses of thyroxine (188), although Ershoff (63) could not confirm this. Liver concentrate rich in the antipernicious anemia factor was effective in this case, but whether the active principle is Vitamin B_{12} alone is not certain. Betheil and Lardy (22) indicated that Vitamin B_{12} is a growth factor for hyperthyroid rats.

Cabbage goiter. Cabbage, spinach, carrots, and various related vegetables have shown marked goitrogenic properties for many animals, including goats, sheep, and man. The active principles appear to be related to the thioureas and their action on the gland resemble this goitrogen. Soybeans are also goitrogenic (180), but iodine administration counteracts the condition.

On the other hand, legume and fresh-cut green grass, carrots, and oats have been claimed to have antigoitrogenic

properties which prevent the action of the vegetable goitrogens. It has been suggested that these materials may contain some thyroxine-like material. However, the presence of these anti-goitrogens has not yet been proven. More recently, Remington et al. (166) found the antigoitrogenic activity of dried milk, oysters, and haddock to be proportional to their iodine content, while Irish moss is less so than its iodine content would indicate.

Relation to Temperature

The decrease in thyroid secretion rate at elevated temperatures was first described by Dempsey and Astwood (51). The effects of such declines on farm animals have already been mentioned in connection with summer sterility in rams. It is also evidenced by the well-known decline in egg production during the summer months.

Seidell (177), in 1913, reported the iodine content of the thyroid gland to be higher from June to November than during the winter and early spring months. This high iodine level in the gland presumably indicates a lower rate of hormone secretion into the circulation.

Relation to Light

Light and darkness are known to affect thyroid activity in many species. Puntriano and Meites (148) recently reported thyroid atrophy and reduced activity when mice were exposed to continuous light. This effect of light is thought to be mediated by way of the pituitary and does not represent a direct action of light on the thyroid gland.

It is probable that the observed seasonal variations in metabolism and breeding patterns of domestic and wild animals are largely due to the interaction of temperature and light on the thyroid and pituitary glands.

Antithyroid Compounds

The modes of action of various antithyroid compounds have recently been reviewed by Astwood (8), who classified them into four groups: "(1) Thyroid hormone; (2) Iodine (3) Thiocyanate ion (4) Antithyroid substances proper, compounds which interfere with thyroid hormone synthesis."

Thyroid Hormone

It has previously been mentioned that the thyroid hormone can, to some extent, regulate the rate of hormone release by the gland. This is accomplished through reducing the rate of thyrotropin secretion by the pituitary gland and perhaps through a direct effect by desensitizing the thyroid gland to the action of the thyrotropic hormone.

Iodine

Iodine has long been utilized to reduce thyroid activity in Graves disease, although the mechanism of its action is not known. It has been suggested (54) that its effect may result from iodination of the proteolytic enzyme which breaks down thyroglobulin and releases thyroxine to the circulation. On the other hand, Marine and Lenhart (124) showed that iodine administration to goitrous dogs had the same effect as thyroxine injections. Hypothyroid rabbits became hyperthyroid when iodine was administered to them (222). Thus, iodine may act differently under different circumstances of thyroid activity. These effects of iodine are unexplained, but may be related to the

observations that iodine reduces the thyrotropic hormone of the normal pituitary (119), and that iodine reduces the respiratory metabolism of thyroid tissue when exposed to the thyrotropic hormone (214).

Astwood (8) has suggested that, in Graves disease, a normal diet is iodine deficient. Iodine administration would then act to reduce the goiter simply by supplying a normal amount of iodine, which would be followed by inhibition of the hyperplasia that occurs in iodine deficiency.

Thiocyanate

Thiocyanate administration produces all of the symptoms of myxedema, but they can be prevented through thyroxine administration (9). It has been shown that the thiocyanate ion is unique in that the uptake of iodine is reduced (228), whereas most goitrogens affect the conversion of iodide to thyroxine or the release of the hormone. In addition, Vanderlaan and Vanderlaan (215) have shown that inorganic iodide already present in the thyroid is discharged upon thiocyanate administration. The mechanism of the effect of thiocyanate is not known. At first it was thought that thiocyanate might be selectively adsorbed

by the thyroid. However, no accumulation of this ion has been found (8).

Antithyroid Substances

The antithyroid substances include a large number of compounds, the most active of which contain either a thiocarbonamide grouping (thioureas) or aminobenzene grouping (sulfonamides). Both of these compounds are thought to exert their influence through preventing the oxidation of inorganic iodides which is necessary before they can be converted to diiodotyrosine and thyroxine. Again, the mechanism is not known. DeRobertis (58) suggested that thioureas inhibit the peroxidase system of the thyroid and that sulfonamides have a competitive action by which the iodine combines with it rather than with tyrosine.

EXPERIMENTAL MATERIALS AND METHODS

Materials

Plasma, milk, and colostrum samples used in this study were for the most part obtained from cows and calves of the Michigan State College dairy herds maintained at East Lansing. A limited number of plasma samples were obtained from cows in farm herds which were examined as sterility cases by Dr. J. A. Williams, of the college Department of Pathology. Dr. L. O. Gilmore, of the Ohio State College Agricultural Experiment Station, also kindly supplied plasma from cows after calving, and their calves before and after first nursing.

The blood samples were drawn from the jugular vein directly into a 15-milliliter centrifuge tube containing 4 drops of 20-percent sodium oxalate as an anticoagulant. The blood was centrifuged as soon as possible after withdrawal and placed in a refrigerator until the determination of iodine could be made.

Methods

The protein-bound and inorganic iodine determinations were made according to the method described by Barker (10, 11), with slight modifications. The reagents and procedure will be described in detail for convenient reference.

Reactions

This method involves the separation of the protein-bound iodine fraction from the inorganic iodine by precipitation and washing. The precipitate is then digested and oxidized with chromic oxide and sulfuric acid, leaving the iodine in a highly oxidized inorganic state. The exact form of the iodine is not known, but is thought to be iodic acid.

The iodic acid is then reduced with phosphorous acid and the volatile iodine (hydrogen iodide or elemental iodine) is distilled off and collected in a sodium hydroxide-arsenious acid solution. The colorimetric determination of iodine is based on its catalytic effect on the decolorization of ceric sulfate by arsenious acid.

Reagents

Distilled water: Once-distilled water was used in making up all solutions, as well as in other phases of the work. Barker (43) used water once redistilled over glass, while Taurog and Chaikoff (42) took the precaution of redistilling over glass from alkaline solution. Satisfactorily low blanks were obtained in this laboratory, however, when once-distilled water taken from a Stokes still was used with no further purification. Tap water itself gave very low iodine values.

70-percent sulfuric acid: 780 milliliters of concentrated sulfuric acid (C.P.) is slowly added, with cooling, to 600 milliliters of water.

7 N. sulfuric acid: 196 milliliters of concentrated sulfuric acid (C.P.) is added to 600 milliliters of water and the volume made up to one liter.

3.5 N. sulfuric acid: 49 milliliters of concentrated sulfuric acid (C.P.) is added to about 300 milliliters of water and the volume made up to 500 milliliters.

0.25 N. sulfuric acid: 7 milliliters of concentrated sulfuric acid (C.P.) is added to 600 milliliters of water and the volume made up to one liter.

60-percent chromic oxide: 600 grams of chromic trioxide (CrO_3) (C.P.) is dissolved in water and the volume brought to one liter.

50-percent phosphorous acid: The contents of a one-pound jar of phosphorous acid (C.P.) is weighed and dissolved in an equal weight of water.

Sodium hydroxide-arsenious acid: 1.5 grams of arsenious acid (As_2O_3) (C.P.) is dissolved in 20 milliliters of 5 N. sodium hydroxide (4 grams of NaOH in 20 milliliters of water) and the volume made up to 100 milliliters with water.

Arsenious acid: 3.71 grams of arsenious acid is dissolved in 50 milliliters of 1 N. sodium hydroxide (2 grams of NaOH in 50 milliliters of water). Add 200 milliliters of water and neutralize with 70-percent sulfuric acid (about 2.5 milliliters). Add 54 milliliters of 70-percent sulfuric acid and make up to 500 milliliters. Dissolve 3.125 grams of sodium chloride (C.P.) in the above solution.

Ceric sulfate: 12.65 grams of ceric ammonium sulfate is stirred into 500 milliliters of water plus 230 milliliters of 7 N. sulfuric acid. Make up to one liter.

Somogyi precipitating reagent: 12.5 grams of zinc sulfate ($ZnSO_4$ - $7H_2O$) (C.P.) is dissolved in 125 milliliters of 0.25 N. H_2SO_4 and the volume made up to 1 liter.

The Somogyi reagent and the 3-percent sodium hydroxide are so balanced that 50 milliliters of the zinc sulfate solution requires 6.7 to 6.8 milliliters of the sodium hydroxide to show permanent pink to phenolphthalein.

Sodium iodide stock: Dissolve 1.181 grams of desiccator-dried sodium iodide (C.P.) in 1 liter of water. Dilute 100 milliliters of this solution to 1 liter. This solution will contain 118.1 milligrams of sodium iodide per liter, or 100 micrograms of iodine per milliliter. This stock solution is stored in a brown bottle and kept under refrigeration. Standard iodide solutions containing 0.02 to 0.10 microgram of iodine per milliliter (or other concentrations as required) were obtained by proper dilutions of the stock solution.

Procedure

Precipitation and washing. A 2-milliliter aliquot of plasma, milk, or colostrum was pipetted into a 50-milliliter round-bottom pyrex centrifuge tube with an Ostwald-Folin

pipette. Sixteen milliliters of Smogyi's acid zinc sulfate reagent (185) was added from a burette, and to this 2 milliliters of 3-percent sodium hydroxide was slowly added from a pipette while the centrifuge tube was gently shaken. It was found that dropwise addition of the sodium hydroxide and constant agitation of the centrifuge tube helped to obtain complete precipitation of the zinc-protein combination.

With each series of protein precipitates, a blank precipitation was made for use in preparing the standard curve described later. The blank precipitate was made by adding 2 milliliters of sodium hydroxide to 16 milliliters of the precipitating reagent.

The zinc-protein precipitates were centrifuged for approximately 10 minutes to obtain good compaction of the precipitate. The supernatant liquid was poured off and washed three times by resuspending the precipitate in 10 milliliters of water and centrifuging after each washing.

The blank precipitate and similar precipitates used in the determination of total iodine were at first washed three times in the same manner. Tests showed, however, that the washings did not alter the blank value so, to conserve time,

the washings were later omitted on blank and total iodine determinations.

Digestion. Three milliliters of chromic oxide were pipetted into a 250-milliliter flask. The precipitate was dissolved in 5 milliliters of 70-percent sulfuric acid and poured into the flask. The centrifuge tube was rinsed with four additional 5-milliliter portions followed by one rinse with 5 milliliters of distilled water. The blank precipitate and the precipitates for determination of total iodine were similarly transferred to the flask.

In the case of total-iodine determinations, the 2 milliliters of plasma, milk, or colostrum were now added directly to the flask.

Considerable difficulty was encountered in digesting the colostrum and some milk samples, especially Jersey milk, due to the large amount of organic material present. At first the milk and colostrum samples were centrifuged and determination made on the skimmed portion. Very low iodine values were obtained on these samples and it appeared that some of the iodine was lost in the fatty portion which rose to the top. Later it was found that satisfactory results could be obtained by increasing

the amount of chromic acid used in the digestion. In obtaining the data reported here on milk and colostrum, 3 milliliters of chromic acid were used on most milk samples and 6 milliliters were routinely used with colostrum. In the few cases where these amounts were not sufficient for crystalization of the chromic oxide after digestion, the amounts of chromic oxide were further increased. No evidence was found that increasing the amount of chromic oxide had any untoward effect on the reaction.

A few glass beads were added to the flask, a thermometer inserted, and the contents heated to 165° C. The flame was immediately removed when the desired temperature was reached, and the flask was set aside to cool. When the temperature fell below 100° C., 15 milliliters of water and a few more glass beads were added and the digest reheated to 165° C. Then the flask was set aside to cool.

Distillation. Distillation of the iodine was carried out in Barker's modification of the Chaney-Riggs-Talbot still. The digest was transferred to the 250-milliliter distillation flask by 25 milliliters of water added in 5-milliliter portions. A few glass beads were added and the still assembled. One-half of

a milliliter of the arsenious acid-sodium hydroxide solution added through the top of the still so that it filled the region of the stopcock in the trap and 5 milliliters of 50-percent phosphorous acid was pipetted into the bowl of a side-arm thistle tube. A Bunsen burner with a moderately low flame was placed under the distilling flask and the condenser inserted.

In assembling the distillation apparatus it is essential that all glass joints be well lubricated with water to prevent freezing.

Heating of the digest was continued until the condensed vapor started dripping into the return tube. The stopcock on the side-arm thistle tube was turned to permit the phosphorous acid to drip into the reaction flask. It was not found necessary to blow the phosphorous acid out of the thistle tube, as described by Barker and Taurog and Chiakoff. Care was taken, however, to close the stopcock immediately after passage of the last of the phosphorous acid. Heating was continued for 7 minutes after the addition of phosphorous acid was completed. At this time the flame was removed and the distillate immediately drawn into a test tube graduated to 25 milliliters. This distillate normally amounted to 7 to 9 milliliters.

The condenser was raised and the still rinsed with three 4-milliliter aliquots of water added in 2-milliliter portions. These rinsings were added to the original distillate and the total volume made up to 25 milliliters. The test tubes were sealed with parafilm and placed in a refrigerator until the colorimetric determination of the iodine.

Colorimetric determination of iodine. Four milliliters of the distillate were pipetted into an Evelyn colorimeter tube; 1 milliliter of water and 0.5 milliliter of arsenious acid were added. Standard tubes for preparation of a standard curve were prepared by pipetting 4 milliliters of the blank distillate into each of five colorimeter tubes. One milliliter of water was added to the first tube and 1 milliliter of sodium iodide solution containing 0.02, 0.04, 0.06, and 0.08 microgram of iodine per milliliter were added to the other four tubes.

These iodine dilutions were prepared by appropriate dilution of the stock solution and were found to be stable for periods of several months when kept refrigerated in tightly closed brown bottles.

One milliliter of ceric sulfate was added to the colorimeter tubes on a 30-second time schedule and the tube was

immediately placed in a water bath at 36.5° C. The tubes were incubated for variable lengths of time, depending upon the number in the series. No incubation time of less than 10 minutes or more than 20 minutes was used.

About 20 seconds before the expiration of the incubation time the tubes were removed, wiped dry, and read in an Evelyn colorimeter at the same 30-second interval. The standard curve was plotted on two-cycle semilog paper and iodine values of the unknowns were determined from this standard. It was necessary to multiply the results by the factors 6.25 and 50 to convert them to micrograms per 100 milliliters of plasma.

Notes on the Procedure

Two general techniques have been recently used for the determination of iodine in plasma or tissue. One, described here, is a digestion-distillation procedure, while the other is an alkaline-ashing method.

Both methods were studied while the writer was attempting to master the technique of the microdetermination of iodine. It became apparent, however, that the ashing method could not be easily utilized in this study.

The ashing procedure of Barker and Humphrey (12) has much to recommend it. It appears to be simpler and less time-consuming than the distillation method and is said to give essentially the same results. However, in order to utilize an Evelyn colorimeter it is necessary to increase the dilution of the dissolved ash. Whether due to this or some other factor, the author was unable to obtain satisfactory or consistent iodine values by this method in a long series of experiments. Two contributing difficulties were the lack of an ashing furnace which could be used only for this work, and the difficulty with which the transference of carbon particles into the colorimeter tubes was avoided. While no implications as to the accuracy of the ashing method for iodine determination are intended, it is the author's opinion that any deviation from the procedure as described by Barker et al (12, 13) will markedly affect the results.

The distillation method which was used in this work is not difficult to use after it has once been mastered. It is, however, a difficult technique to learn unless someone who is familiar with the procedure is present to oversee the details.

Barker (10) and Taurog and Chaikoff (192) have described the digestion phase as ending when the sulfuric acid fumes start to form. Chaney (38) and Riggs (167) used digestion temperatures of about 200° C. Neither of these were very satisfactory in our hands. It was soon found that the heating time required for producing visible fumes varied with the type of digestion flask used. When a digestion temperature of 200° C. was used, the chromic oxide-sulfuric acid-protein precipitate mixture turned green and, on cooling, no crystals of chromic oxide formed. The recovery of iodine from this digest was very low.

In order to standardize the procedure, various temperatures from 140° C. to 200° C. were tried and the recovery of added iodide measured for each. The percentage recovery increased to 170° C. and fell after a temperature of 180° C. was reached. Since the percentage was a little less than 100 at 160° C., and above 100 percent at 170° C., it was decided to use a digestion temperature of 165° C. The recovery of sodium iodide, thyroxine, and radioactive iodine added to plasma, as described in the next section, were very satisfactory at this temperature.

In general, the digestion method appears to be much less sensitive to alterations in the amount or concentration of the reagents used than the ashing procedure.

It has been observed above that the distillation technique is difficult to learn without instruction from someone who is well versed in the procedure. However, the mechanics of making the determinations have been successfully taught to several students in our laboratory who had little chemical background. Thus, for routine work, where some supervision can be given, skilled technicians are not necessary.

Previous workers (38, 192) have mentioned encountering lots of phosphorous acid which appeared to be incapable of releasing iodine from the digest. On one occasion in this study a lot of phosphorous acid was used in which some bottles were active while the others were inactive. Acting on a suggestion by Dr. G. M. Curtis, of the Ohio State University, various amounts of manganese dioxide were added to the digest before the distillation step. It was thought that the manganese dioxide might catalyze the reduction of the digest by the inactive phosphorous acid, but it was found that the apparent iodine recovery varied with the amount of manganese dioxide added. No solution

of the problem of inactive phosphorous acid is known except that less trouble is said to occur if technical instead of C.P. grades of reagent are used.

EXPERIMENTAL RESULTS

Test of Method

Recovery of Added Iodine

The efficiency of this procedure in recovering iodine from the plasma of dairy cattle was estimated by determining the recovery of inorganic and radioactive iodine (I^{131}) and thyroxine added to precipitated and washed plasma protein.

Table I shows that the recovery of the added iodine and thyroxine was accomplished very efficiently. In a series of experiments the recovery varied between 97.5 and 105.0, with an average of 100.9 percent.

The percent recovery was checked with radioactive iodine added to the washed precipitate in the same manner. The range of recovery which was somewhat greater than that determined colorimetrically can be (Table II) attributed, in part at least, to mechanical difficulties in preparing the distillate for measurement of its radioactivity. Even so, the recovery of radioactive iodine averaged 98.5 percent.

TABLE I

RECOVERY OF SODIUM IODIDE AND THYROXINE ADDED TO PLASMA PROTEIN

No. of Trials	Micrograms of Added Iodine	Micrograms of Recovered Iodine	Percent Recovery
1	.04	.044	105.0
3	.06	.061	101.7
3	.08	.078	97.5
2	.10	.102	102.0
1	.067 (thyroxine)	.066	98.5
Average			100.9 ± 2.7

TABLE II

RECOVERY OF RADIOACTIVE IODINE ADDED TO PLASMA PROTEIN

Trial No.	Sample	Number in Series	Average Counts per Second	Percent Recovery
1	Standard Distillate	1[a]	81.0 83.4	103.0
2	Standard Distillate	6	63.3 58.2	91.9
3	Standard Distillate	4	71.9 72.3	100.6
Average				98.5 ± 4.8

[a] Each series was determined in triplicate.

Repeatability of Results

During the course of this work, it was the practice to occasionally insert duplicate or triplicate plasma samples into a series in order to provide a check on the repeatability of iodine recovery in routine analysis. The results of one such check, more extensive than most, are shown in Table III. Even during routine iodine determinations, the results were reasonably consistent and the variation less than 10 percent.

It has been our experience in several hundred analyses of plasma protein-bound iodine that the method used gives accurate and repeatable results and does not require the services of a skilled laboratory technician. However, untrained personnel require constant supervision until they learn the mechanics of the procedure. Furthermore, they must be impressed with the need for accuracy in every step; particularly in the colorimetric determination of iodine.

TABLE III

RESULTS OF NINE CONSECUTIVE PROTEIN-BOUND IODINE DETERMINATIONS ON ONE LOT OF PLASMA

Sample No.	Colorimeter Reading	PBI γ %
1	18.5	5.1
2	18.6	5.2
3	17.3	4.3
4	19.0	5.5
5	17.9	5.0
6	18.9	5.3
7	18.6	5.2
8	18.1	5.0
9	19.0	5.5
Average		5.1 ± .37

Plasma Protein-bound Iodine of Dairy Cattle

Age Changes

Between June 12, 1951, and March 3, 1952, 296 determinations were made of the plasma concentrations of protein-bound iodine of 177 cows and calves in the Michigan State College dairy herds. The results, arranged according to the ages of the animals, are shown in Table IV.

The over-all average of the 296 determinations was 6.8 micrograms percent. An inspection of these data reveals, however, that an average value, not qualified by a consideration of age, has little meaning. These data show that the organic iodide fraction of plasma tends to decrease with age. However, for convenience, the age changes can be grouped into four periods, each with a characteristic iodine concentration. Thus, in this study, calves averaged 12.8 micrograms percent of plasma protein-bound iodine during the first 2 days after birth. During the rest of the first 12 months the average concentration was 7.3 micrograms percent. From 13 to 24 months the average was 6.2 micrograms percent, while the level in cows over 24 months old was 4.6 micrograms percent. It is therefore pointless

TABLE IV

AGE CHANGES IN THE PLASMA PROTEIN-BOUND IODINE CONCENTRATION OF DAIRY CATTLE

Age	Number of Animals	Number of Determinations	Average γ %
Under 24 hours	22	22	14.8
24 - 48 hours	8	8	10.8
3 - 4 days	8	9	7.9
5 - 7 days	9	9	7.6
8 days - 1 month	24	34	6.9
1 - 3 months	18	18	6.2
4 - 6 months	11	17	8.1
7 - 12 months	21	27	7.3
13 - 18 months	10	27	6.8
19 - 24 months	16	33	5.7
25 - 36 months	12	31	4.7
37 - 48 months	4	7	4.6
49 - 72 months	8	33	4.6
73 months or over	6	21	4.4
Total or Average	177	296	6.8

to give an average plasma protein-bound iodine value without specifying the age of the animal concerned.

Although these averages show distinct age differences in the plasma iodine concentration, there is a considerable amount of overlapping in the range of values one obtains in the different age groups. Figures 1 through 5 show the range of values and the frequency of each in the 177 animals tested and in each age division.

In Figure 1 the range of 248 plasma protein-bound iodine concentrations obtained on 130 dairy animals over 1 week old are given. Although the values varied from 2.1 to 17.2 micrograms percent, more than 80 percent of the concentrations were under 8.0 micrograms percent.

When the distribution of values is broken down into the four age groups mentioned above, the effects of age can be seen. The values of calves under 48 hours old seldom fall below 8.0 micrograms percent (Figure 2) or rise above about 18.0 micrograms percent. Within these limits the distribution is fairly regular. An abrupt decrease in the average plasma protein-bound iodine concentration occurs soon after the second day. This is illustrated in Figure 3, where it is shown that 84.0

Figure 1. The distribution of 248 plasma protein-bound iodine determinations on 130 dairy animals over 1 week old. The values ranged from 2.1 to 17.2 micrograms percent with 80 percent of them under 8.0 micrograms percent.

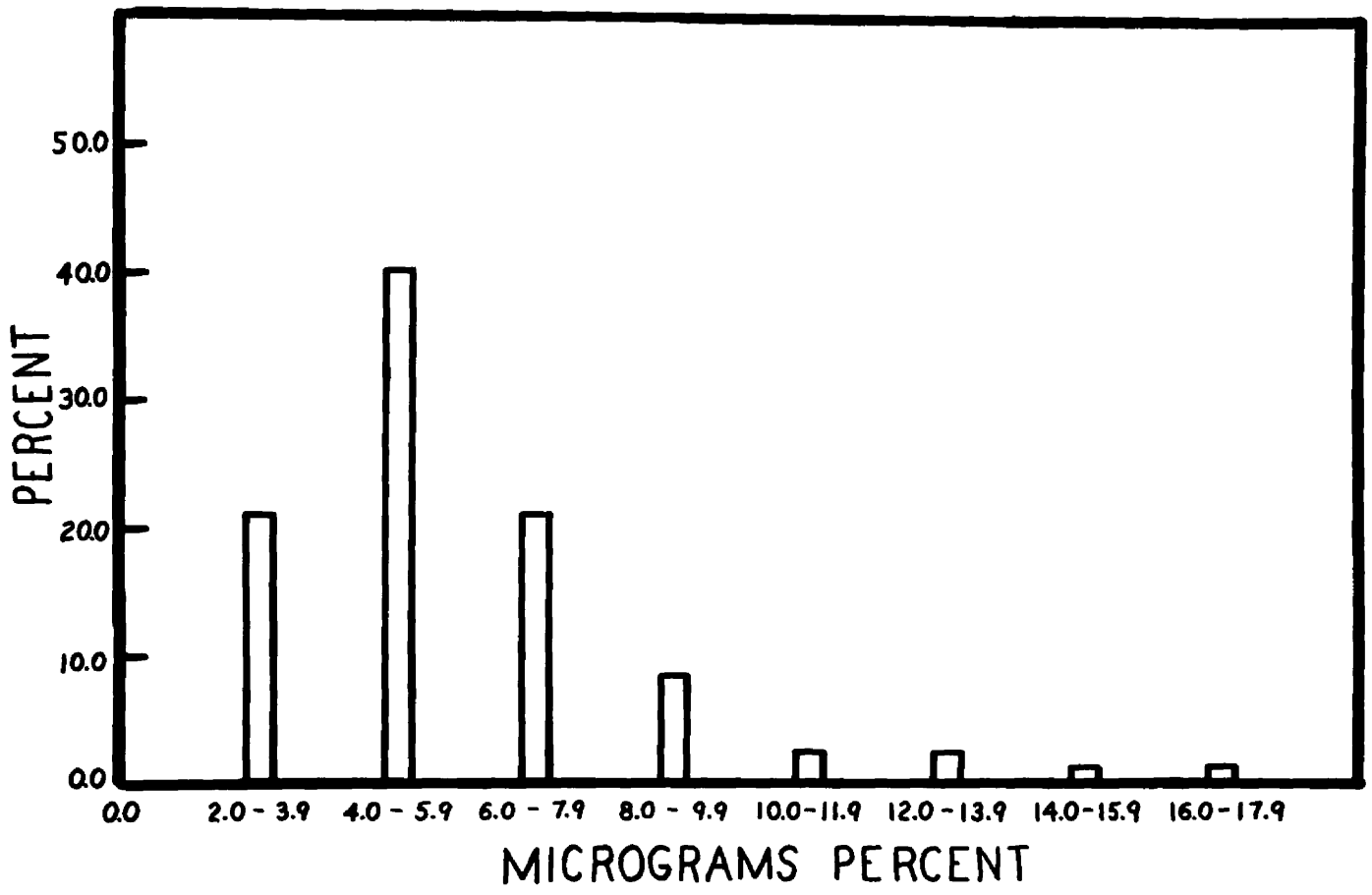

50.0
40.0
30.0
20.0
10.0
0.0
PERCENT
0.0
2.0 - 3.9
4.0 - 5.9
6.0 - 7.9
8.0 - 9.9
10.0-11.9
12.0-13.9
14.0-15.9
16.0-17.9
MICROGRAMS PERCENT

Figure 2. The distribution of thirty plasma protein-bound iodine determinations on thirty calves under 48 hours old. The concentrations varied from 6.0 to 29.7 micrograms percent and averaged 12.8 micrograms percent.

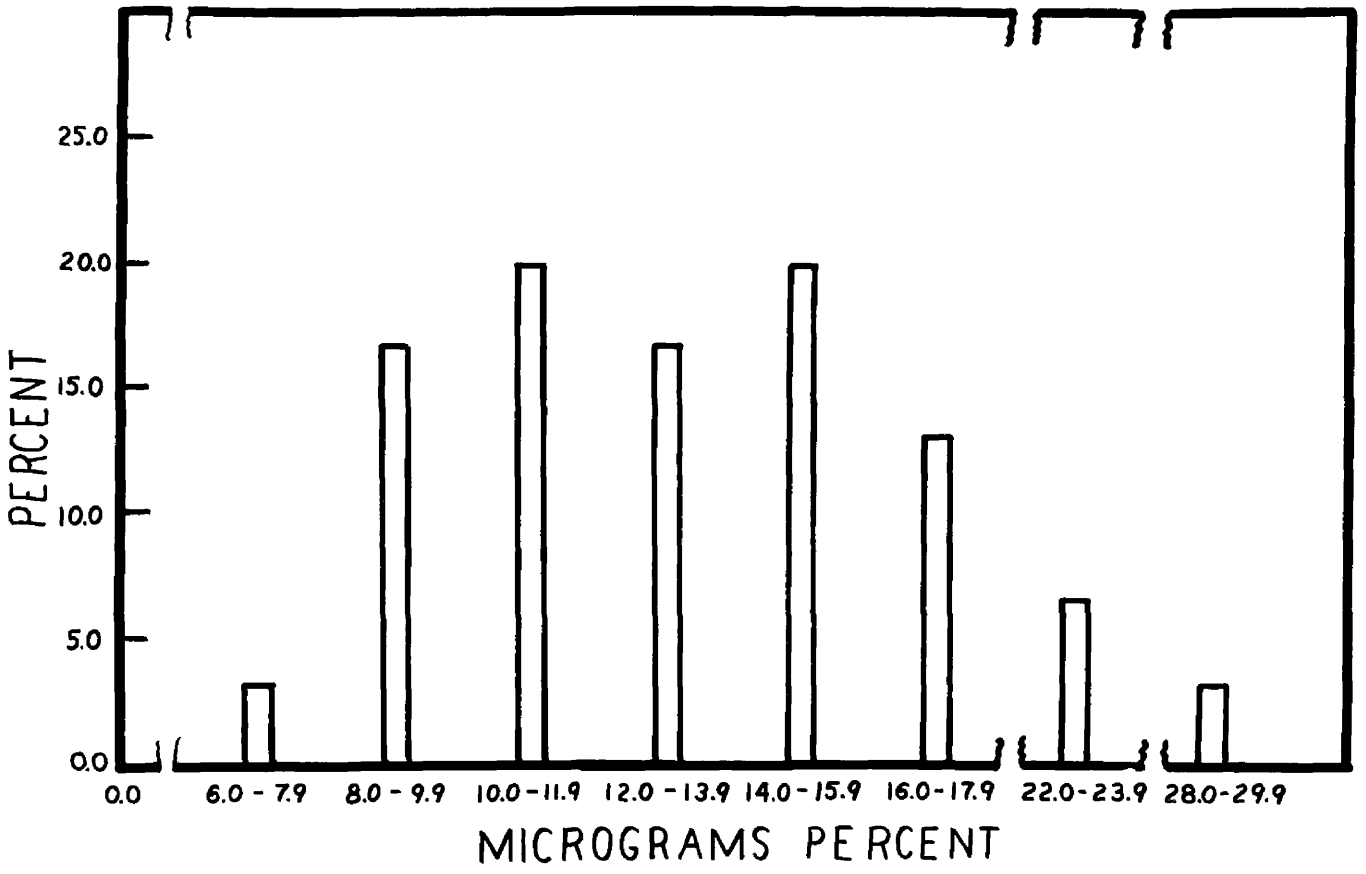

PERCENT
25.0
20.0
15.0
10.0
5.0
0.0
0.0
6.0 - 7.9
8.0 - 9.9
10.0 - 11.9
12.0 - 13.9
14.0 - 15.9
16.0 - 17.9
22.0 - 23.9
28.0 - 29.9
MICROGRAMS PERCENT

Figure 3. The distribution of 114 plasma protein-bound iodine determinations on ninety-one calves between 48 hours and 12 months old. The values ranged from 2.7 to 18.0 micrograms percent and averaged 7.3 micrograms percent.

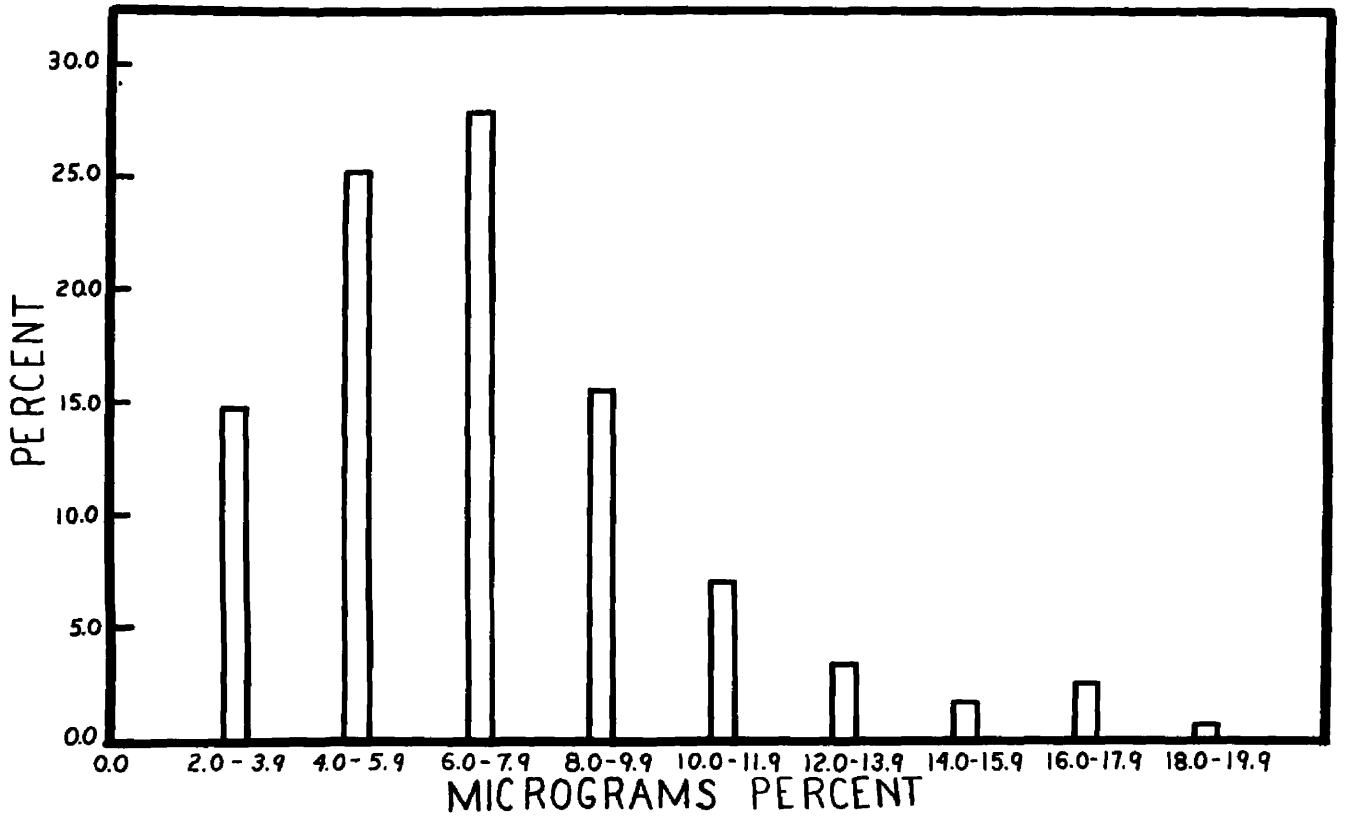

30.0
25.0
20.0
15.0
10.0
5.0
0.0
PERCENT
0.0
2.0 - 3.9
4.0 - 5.9
6.0 - 7.9
8.0 - 9.9
10.0 - 11.9
12.0 - 13.9
14.0 - 15.9
16.0 - 17.9
18.0 - 19.9
MICROGRAMS PERCENT

Figure 4. The distribution of sixty plasma protein-bound iodine determinations on twenty-six heifers 13 to 24 months old. The range of values was from 3.0 to 15.3 micrograms percent with an average of 6.2 micrograms percent.

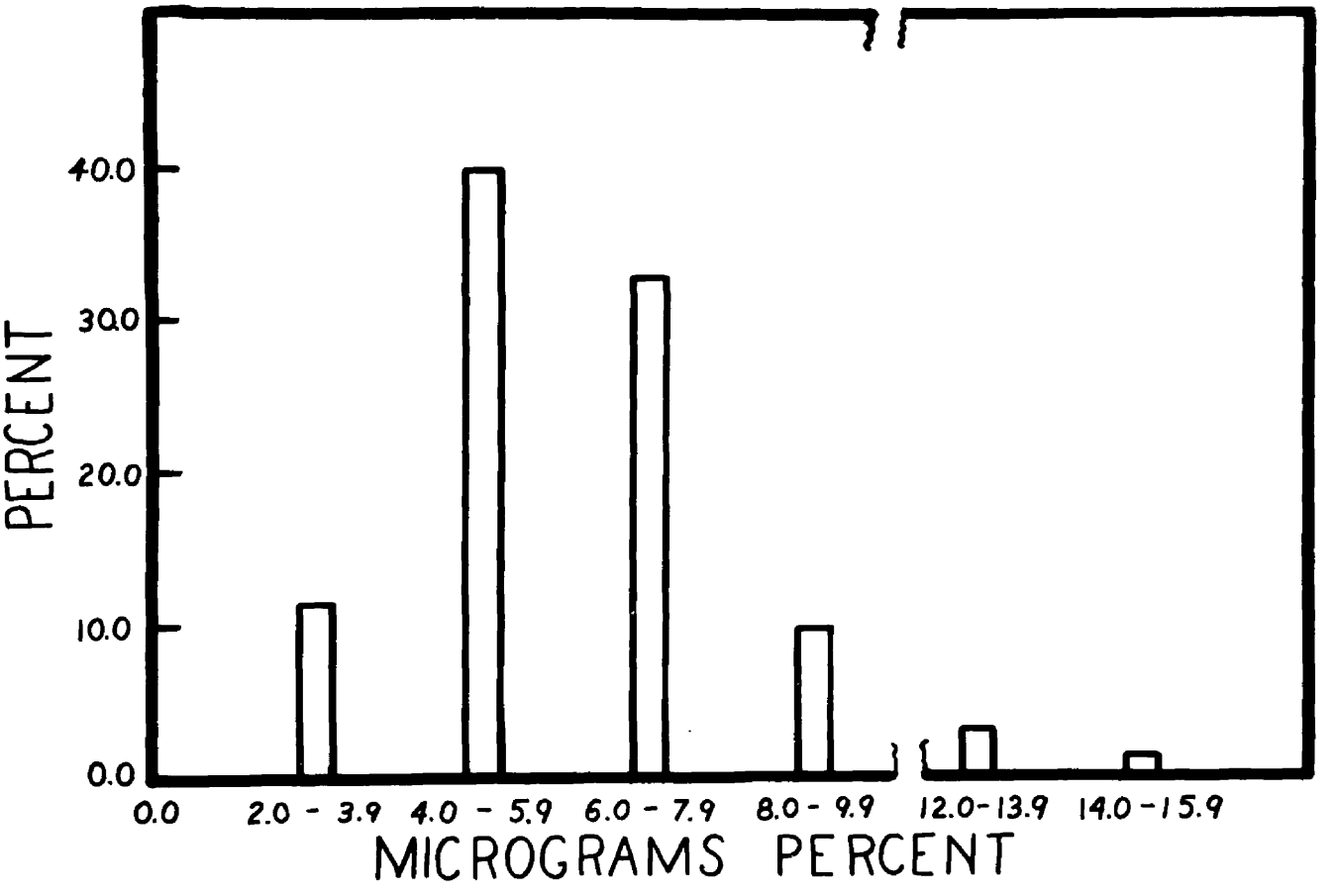
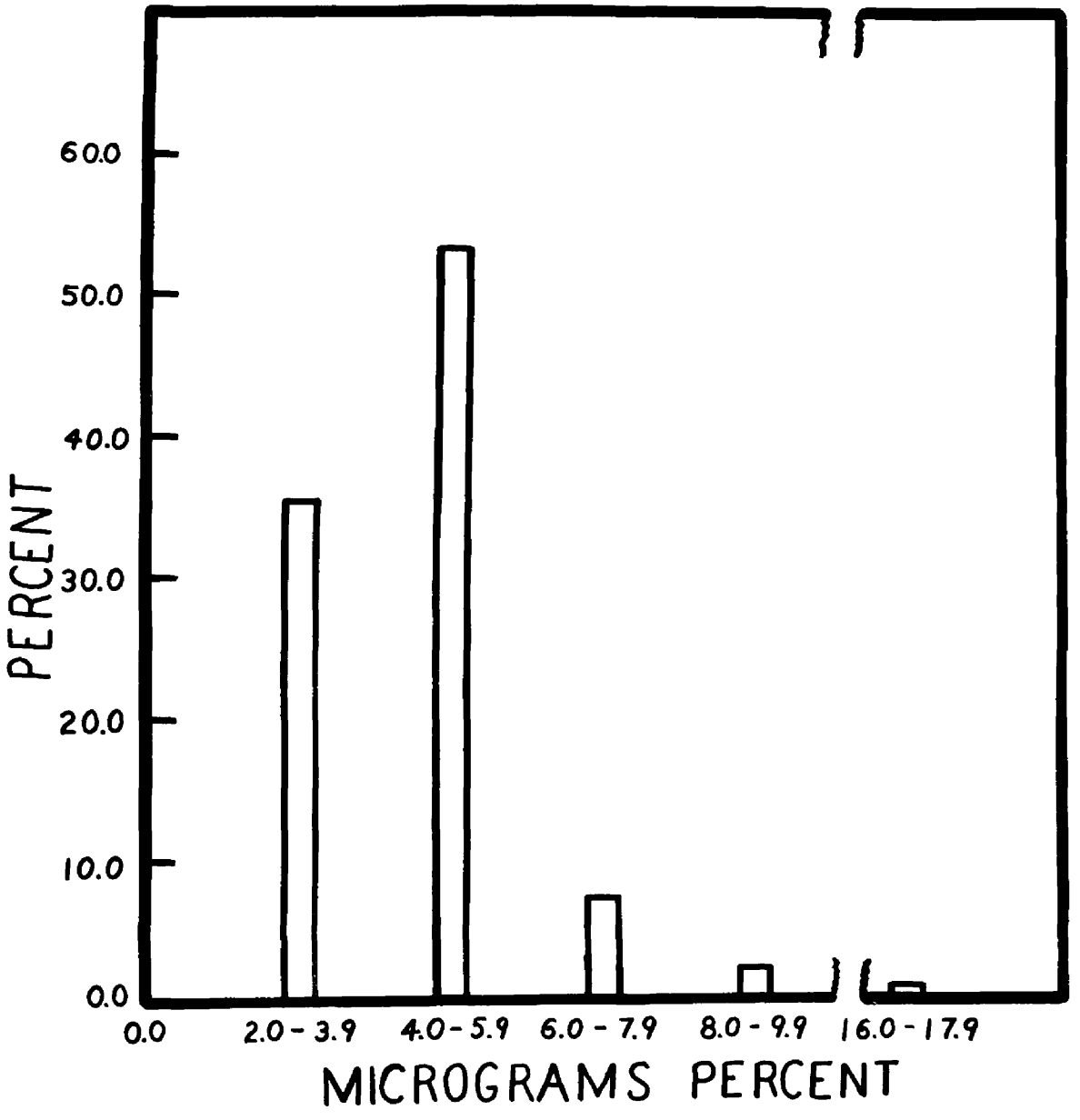

PERCENT
40.0
30.0
20.0
10.0
0.0
0.0
2.0 - 3.9
4.0 - 5.9
6.0 - 7.9
8.0 - 9.9
12.0-13.9
14.0-15.9
MICROGRAMS PERCENT

Figure 5. The distribution of ninety-two plasma protein-bound iodine determinations on thirty cows over 24 months of age. The values averaged 4.6 micrograms percent with a range of 2.1 to 16.5 micrograms percent.


60.0
50.0
40.0
30.0
20.0
10.0
0.0
PERCENT
0.0
2.0 - 3.9
4.0 - 5.9
6.0 - 7.9
8.0 - 9.9
16.0 - 17.9
MICROGRAMS PERCENT

percent of the determinations on calves between 48 hours and 12 months old were less than 10.0 micrograms percent. In addition, the values fell as low as 2.7 micrograms percent. The downward trend continued in heifers between 12 and 24 months of age (Figure 4) with 85.0 percent of the values below 8.0 micrograms percent. The concentration of cows over 24 months of age was generally still lower (Figure 5). In this group, nearly 90 percent of the values were less than 6.0 micrograms percent.

In view of these data, it is suggested that a certain range of plasma protein-bound iodine concentrations be considered normal for animals of each of the different age groups. These suggested ranges are shown in Table V, and were selected so as to include 85 to 90 percent of the determinations in each case.

Calves Before and After First Nursing

Since the protein-bound iodine concentration in the plasma of young calves was considerably higher than in older animals, a more intensive study was made of this age group.

TABLE V

NORMAL RANGE OF PLASMA PROTEIN-BOUND IODINE CONCENTRATIONS OF DAIRY ANIMALS AT DIFFERENT AGES

Age	No. of Animals	No. of Determinations	Avg. γ %	Suggested Normal Range γ %	% of Determinations Included
Under 48 hours	30	30	12.8	8.0 - 18.0	86.7
48 hours - 12 months	91	114	7.3	3.5 - 12.0	87.7
13 - 24 months	26	60	6.2	3.5 - 10.0	85.0
Over 24 months	30	92	4.6	3.0 - 8.0	87.0

As shown in Table VI, the level of organic iodine in the newborn calf before it has nursed is approximately the same as is found in older calves. Once the calf has had an opportunity to nurse, however, the organic iodine of the plasma increases markedly. Two other phenomena associated with calving which the writer feels are of significance are that colostrum contains a much larger amount of iodine, particularly organic, than milk (see Table VII) and that the plasma protein-bound iodine of the dam is significantly lower on the day of calving than either before or after parturition (Table VIII). Furthermore, colostrum is known to contain large amounts of globulin and albumin, while milk contains only traces of these proteins (61).

In view of this, it is believed that the high postnursing protein-bound iodine concentration in the plasma of dairy calves can be explained by the following: Shortly before calving, the mammary gland is unusually permeable to globulin and albumin. This sudden drain depletes the circulating protein of the cow and also her circulating thyroid hormone, which is attached to the albumin and globulin fractions of the blood. As the calf nurses, it injests enough iodine to raise its own level of organic

TABLE VI

PLASMA PROTEIN-BOUND IODINE LEVELS IN CALVES BEFORE AND AFTER FIRST NURSING

Calf No.	Micrograms Percent P.B.I.		
	Before Nursing	After Nursing	Percent Increase
A	7.1	8.2	15.5
B	11.1	12.5	12.6
C	5.9	13.9	101.7
D	9.3	14.9	60.7
E	14.1	29.7	110.6
F	6.9	15.6	126.1
G	7.7	10.5	36.4
Average	8.9	15.0	66.2

TABLE VII

THE IODINE CONCENTRATION OF MILK AND COLOSTRUM

Sample No.	Milk		Colostrum	
	PBI γ %	Total Iodine γ %	PBI γ %	Total Iodine γ %
1	4.5	12.2	-	20.2
2	6.1	13.6	-	28.8
3	-	8.0	25.0	30.9
4	-	8.1	21.9	-
5	5.4	7.2	*	*
6	-	9.0	*	*
7	-	9.9	*	*
8	7.2	9.3		
9	6.1	-		
10	-	6.7		

* Iodine concentrations above 35.0 micrograms per-cent.

TABLE VIII

CHANGES IN PLASMA PROTEIN-BOUND IODINE LEVELS OF COWS AT CALVING TIME

Cow No.	PBI Level in Micrograms Percent		
	Before Calving	At Calving	After Calving
K 5	3.8	2.6	4.1
15	4.1	1.4	5.0
17	3.8	2.5	3.7
101	4.5	2.0	4.8
115	4.1	3.0	6.7
128	4.1	3.5	3.7
132	6.3	2.3	5.4
134	5.1	2.9	3.9
Average	4.5	2.5*	4.6

* Highly significantly lower than levels before or after calving (T = 5.30).

iodine to a relatively high level. It is well known that the colostrum quickly changes to normal milk and, as that occurs, an equally marked fall in the organic iodide in the calf's plasma takes place due to excretion or thyroidal storage of the iodine.

While the above is conjecture, it is supported by the histological appearance of the thyroid gland during the first few days of extrauterine life. Plate 1 shows the histology of the gland at 12 hours, and 3, 4, and 6 days of age (see also Table IX). At 12 hours after birth there was little evidence of colloid storage or secretory activity of the gland. The follicles were small and relatively free of colloid. The secretory cells were of the columnar type associated with a relatively inactive gland.

These results do not agree with those of Koneff and his associates (111), who found a marked increase in colloid storage as fetal calves neared term. Since only one calf was sacrificed in the present study, it is quite possible that its thyroid gland was unusually inactive or deficient in iodine. On the other hand, there may be a reduction in stored hormone at birth.

At three days, a marked shortening of the secretory cells and filling of the follicles with colloid had taken place.

Plate 1. Sections of thyroid glands of calves at various times after birth.

A. The thyroid gland of a calf 12 hours old. The follicles were few and nearly devoid of colloid. The secretory cells were columnar and poorly defined. The general appearance of the gland was one of inactivity.

B. A section from a calf 3 days old. The follicles were somewhat more numerous and turgid than those in the younger calf. The amount of colloid in the follicles had increased. The secretory cells were more cuboidal.

C. A section from a 4-day-old calf. The numerous follicles were well filled with colloid. Little undifferentiated tissue remained in the gland.

D. A section from the thyroid from a 6-day-old calf. The follicles were filled with deep-staining colloid. The secretory cells were well defined and no undifferentiated tissue remained in the gland.

All glands presented a normal gross appearance.

Magnification: Each division on the scale equals 10 microns.

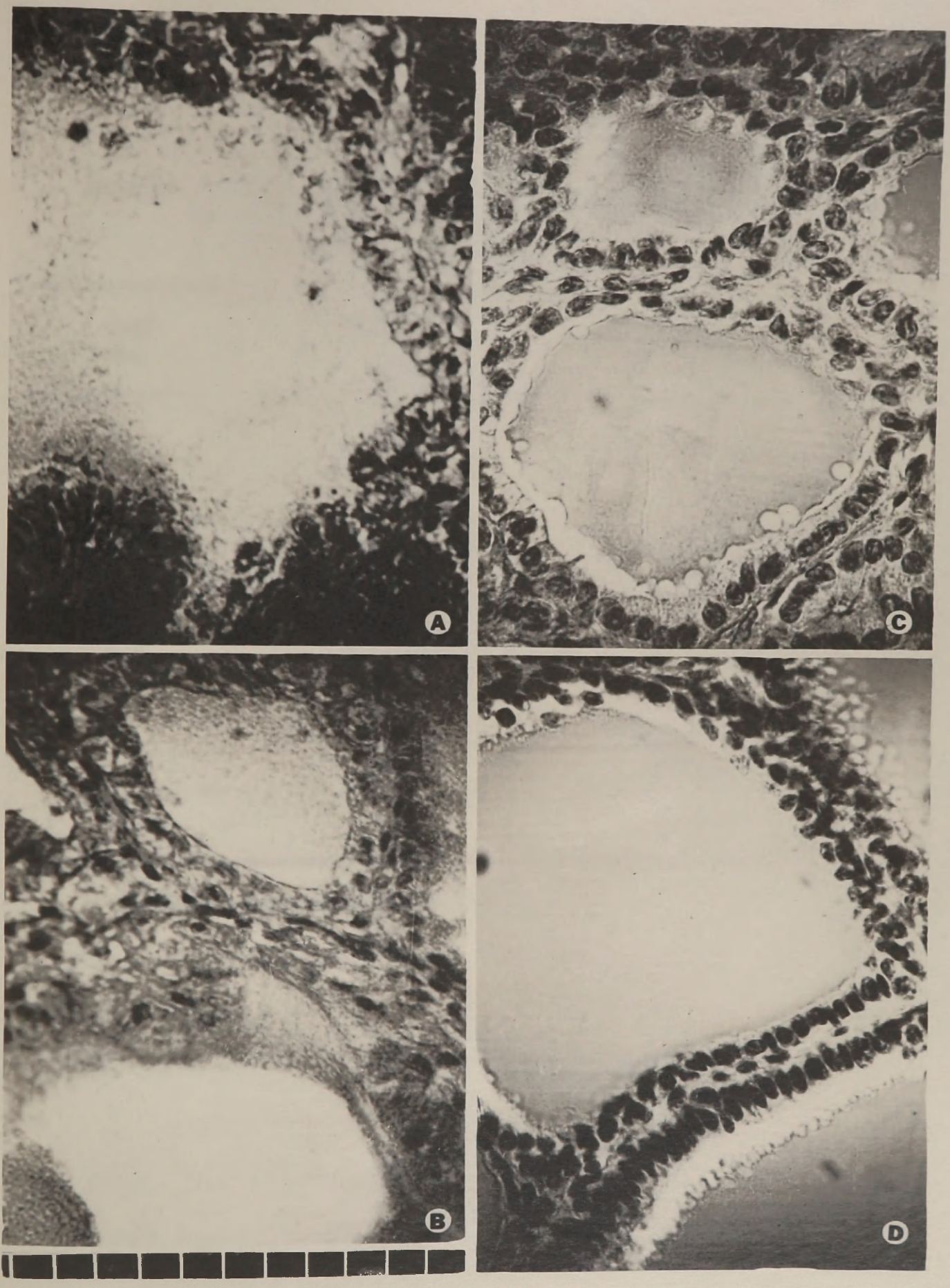

A
B
C
D

TABLE IX

PLASMA PROTEIN-BOUND IODINE CONCENTRATION OF CALVES WHOSE THYROIDS WERE EXAMINED HISTOLOGICALLY

Calf No.	Concentration of Iodine γ %	Age
191	23.8	12 hours*
193	9.8	6 hours
	15.5	30 hours
	3.1	80 hours*
149	22.7	6 hours
	8.3	3 days
	11.7	4 days*
192	3.7	6 days*

* Time of thyroidectomy.

Presumably this indicated a greater secretory and storage activity on the part of the gland.

By the fourth and sixth days the follicles were numerous and distended with colloid. The glands gave every indication of being in a normal, active condition.

Whether the organic iodine obtained from the colostrum is actually thyroxine is not presently known. Thyroxine has been the only major form of organic iodine found in the circulation of rats, dogs, and other laboratory animals, but no information is available on dairy cattle. It is known that iodine can be organically combined in the blood without passing through the thyroid and, since cattle rations are often supplemented with iodized salt, they may represent an exception. Normally, the mammary gland is impervious to thyroxine, but it is unreasonable to expect this to be true at a time when albumin and globulin appear to penetrate it in large amounts (61). At any rate, the colostrum appears to supply massive amounts of precipitable iodine to the young calf, which stores it in its own thyroid gland.

The physiological significance of these data is obscure. At present, one can only suggest that they represent one of the

protective mechanisms whereby the calf is prepared to meet the stresses imposed upon it during its adjustment to the new environment.

Jersey and Holstein Cows

Plasma samples were obtained on four different occasions from seven Jersey and nine Holstein cows from one of the college herds. The collection dates (June, August, November, and March) correspond roughly to the four seasons, although they were not selected with this in mind. All of the animals were over 18 months old and, with two exceptions, were in milk on one or more of the sampling dates. Cow K 104 had milked previously, but was dry and open during this time, and K 131 calved for the first time after the last bleeding date.

The results of the protein-bound iodine determinations are shown in Table X. The statistical analysis summarized in Table XI showed no significant difference between cows or breeds. No significant seasonal differences were found for the Jersey cows, although the November level was lower than in other months. The concentration of the Holstein cows was also lower at the November and March samplings than at the

TABLE X

PLASMA PROTEIN-BOUND IODINE LEVELS IN JERSEY AND HOLSTEIN COWS OVER EIGHTEEN MONTHS OLD

Herd No.	Breed	Age in Mos.	Sampling Dates				Average γ %
			6-12 1951 γ %	8-14 1951 γ %	11-12 1951 γ %	3-18 1952 γ %	
K 5	J	72	5.1	4.8	3.8	4.1	4.45±0.28
K 10	J	72	4.8	4.2	4.9	4.4	4.58±0.13
K101	J	54	6.9	6.0	4.5	4.8	5.55±0.88
K104	J	52	3.6	5.7	3.6	5.4	4.65±0.27
K105	J	47	5.1	5.1	5.0	4.9	5.02±0.06
K115	J	34	4.8	4.5	4.1	6.7	5.02±0.05
K132	J	22	6.0	6.6	4.4	5.4	5.60±0.66
Average		50	5.19 ± 0.87	5.27 ± 0.66	4.33 ± 0.23	5.10 ± 0.62	4.97±0.76
K 15	H	72	5.7	4.8	3.3	4.1	4.50±0.56
K 17	H	66	3.0	5.4	3.8	3.7	3.98±0.73
K 18	H	68	5.1	8.1	3.8	4.7	5.42±2.66
K 19	H	48	3.0	3.0	4.4	4.6	3.75±0.57
K 20	H	55	3.9	5.1	3.0	6.0	4.50±0.31
K109	H	44	4.2	5.7	4.2	5.0	4.78±0.34
K128	H	23	5.7	5.4	4.1	3.1	4.58±1.04
K131	H	19	7.5	7.2	5.2	4.8	6.18±1.35
K134	H	19	8.4	6.3	5.1	3.9	5.92±2.82
Average		46	5.17 ± 3.12	5.67 ± 1.86	4.10 ± 0.48	4.44 ± 0.58	4.84±1.93

TABLE XI

SIGNIFICANCE OF VARIATIONS IN PLASMA PROTEIN-BOUND IODINE LEVELS OF JERSEY AND HOLSTEIN COWS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Jerseys				
Cows	6	5.29	.88	1.35
Season	3	3.96	1.32	2.03
Holsteins				
Cows	8	22.24	2.78	2.01
Season	3	13.53	4.51	3.27*
Combined				
Breed	1	.26	.26	.13
Season	3	15.18	5.06	2.49

* Significant at the 5-percent level.

June and August bleedings. The seasonal variation was statistically significant in this breed. The considerable variation in iodine concentration of the same cow on different dates would lead one to wonder if the variability was due to a real difference, or if it was merely a reflection of the inadequacy of a single sample for expressing a cow's protein-bound iodine level. In order to test this, samples were drawn from a cow on 6 consecutive days, and the iodine concentration determined (Table XII). From these data it may be observed that a single plasma sample is sufficient to determine the approximate level for a cow, but two or more samples should be taken for exact information.

The iodine values reported here are somewhat higher than those reported by Long et al. (120) and Reece and Man (156). The former workers reported that Jersey and Holsteins averaged 4.11 and 2.73 micrograms percent, respectively, while the latter found Jersey cows averaged 4.6 micrograms percent. In the present study the average levels for Jersey and Holstein cows were 4.97 and 4.84 micrograms percent, respectively. The Ohio workers reported their breed differences to be statistically significant.

TABLE XII

DAY-TO-DAY CHANGES IN PLASMA PROTEIN-BOUND IODINE CONCENTRATION IN A JERSEY COW

Sampling Date	Colorimeter Reading	Micrograms Percent PBI
May 8	16.2	3.7
9	16.3	3.7
10	16.0	3.6
11	15.3	3.3
12	16.9	4.2
13	16.0	3.6
Average		3.65 ± .32

The fallacy of comparing animals of different ages has previously been pointed out, and insufficient data is available for determining the ages of the animals used in these studies. In spite of this, it is evident that the concentrations reported by the Ohio workers are decidedly below those obtained in this laboratory. Whether these differences were due to the herds studied or were inherent in the techniques used is not known. However, one plasma sample was exchanged between the two laboratories for comparative analysis. Our value was approximately 10 percent higher than the one reported by the Ohio workers.

The possibility of a difference in the organic iodine concentration in the plasma of cows of the same breed in different herds is illustrated by the results obtained when plasma iodine determinations were made on a group of Jersey cows recently moved from California to the Michigan State College campus.

These cows were brought to the college farm in August, 1951, and were sampled on September 24 and November 6. The plasma levels of the cows over 18 months of age in this herd are shown in Table XIII. The concentration of organic iodine

TABLE XIII

PLASMA PROTEIN-BOUND IODINE LEVELS OF A HERD OF JERSEY COWS BROUGHT FROM CALIFORNIA

Herd No.	Age in Months	Sampling Dates		Average γ %
		9-24-51 γ %	11-6-51 γ %	
332	77	2.6	4.3	3.4
C30	71	3.6	4.3	4.0
C47	59	1.5	2.4	2.0
C51	55	2.7	3.9	3.3
C62L	44	4.8	2.6	3.7
C62R	43	5.4	3.3	4.4
C79	31	3.3	4.0	3.6
C84	24	3.3	3.2	3.2
C85	24	1.5	4.2	2.8
C89	22	3.0	4.7	3.8
C98	20	3.0	4.4	3.7
327	78	2.6	3.3	3.0
349	64	2.0	3.4	2.7
358	54	2.0	3.3	2.6
Average	49.0	3.0 ± .90	3.7 ± .19[a]	3.3 ± .38[b]

[a] Differences between dates were not significant.

[b] Significantly different from cows in Table IV. (t for November 6 and 12 = 2.07. t of over-all averages = 2.93.)

in the plasma was unexpectedly low at the first sampling, and was only a little higher in November: The differences were not significant. The average value from both samplings was highly significantly less ($t = 3.92$) than the over-all average of the Jersey and Holstein cows previously discussed. When the samples of November 6 were compared with the values obtained on November 12 (Table IX), the difference was less, but was still significant at the 5-percent level ($t = 2.07$).

Whether the difference between the two herds was due to genetic or environmental factors is not known. It might be postulated that cows from areas bordering the seacoast (where the iodine content of the feed is entirely adequate to meet the animals' needs) have thyroid glands which are not immediately capable of secreting normal amounts of hormone when the cow is moved to an iodine-deficient area and placed on a ration containing no supplemental iodized salt, as these were. After a time hypertrophy of the gland occurs and the amount of thyroxine secreted increases, thus causing a rise in plasma protein-bound iodine.

On the other hand, the possibility exists that the stress of shipment for a long distance produced a temporary depression

of thyroid activity which, after a period of adjustment to the new environment, will return to normal. It is hoped that another series of determinations can be run to see if, after several months of adjustment, the plasma iodine levels of the California Jerseys have reached those of comparable cows from the main college herd. Until that is done, the possibility of true genetic differences between these herds is not ruled out. The California Jerseys are relatively highly inbred, and it is not unlikely that the endocrine system has been affected in the process.

With regard to the effects of pregnancy and lactation on the organic iodine fraction of plasma, no relationship could be established with the limited data of this study.

Cases of Sterility

During the course of this work, a group of cows being studied as sterility cases by members of the college's pathology and physiology departments were sampled. All of these cows had histories of many breedings without conceiving, but none of them exhibited organic or pathological indications of inability to be impregnated. Some of the cows had been brought to the college and placed on a good ration as a preliminary to further

treatment; others were sampled on the farm. Of nineteen cows, four had been pronounced pregnant by a veterinarian when sampled.

The average values obtained are shown in Table XIV. The values obtained are markedly lower than those of the Holstein and Jersey cows in Table IX ($t = 3.23$, which is highly significant). The values are very similar to those obtained on the Jersey cows from California.

No one has yet shown any close connection between thyroid activity and reproduction of dairy cattle. Even thyroidectomized cattle have been capable of reproduction, even though estrus was not observed.

It has been mentioned previously, however, that the ovaries appear to exert a marked influence on plasma protein-bound iodine. In particular, the plasma level is maintained in thyroidectomized females (143), and limited estrogen administration increases thyroxine secretion (233). In view of these relationships, it is possible that, in some cases of sterility due to hypoovarian function, the usually low plasma protein-bound iodine concentration is due to hypogonadism rather than to hypothyroidism per se, although it is not known if alterations

TABLE XIV

MICROGRAMS PERCENT OF PROTEIN-BOUND IODINE IN THE PLASMA OF "STERILE" COWS

Animal No.	Breed	Age in Years	No. of Determinations	Avg. γ %	Notes
API	G	4	1	3.9	Open
S2	J	3	4	3.9	Open
S5	G	3	2	4.3	Pregnant
S6	H	4	1	2.7	Pregnant
S7	H	5	1	3.9	Pregnant
S9	H	2	1	4.2	Pregnant
S10	J	3	3	4.0	Open
S11	J	3	1	5.4	Open
S12	H	4	3	3.5	Open
S13	H	2	4	4.4	Open
S14	J	2	2	3.4	Open
S15	H	2	5	4.8	Open
S16	J	3	1	3.6	Open
871X	J	2	1	3.3	Open
908X	H	4	1	4.5	Open
914X	H	2	1	3.0	Open
915X-1	H	2	1	3.0	Open
915X-2	H	2	1	3.3	Open
915X-3	H	2	1	2.1	Open
Average				3.7 ± .93*	

* Highly significantly different from cows in Table IV ($t = 3.23$).

in ovarian activity, short of oophorectomy, are capable of influencing the plasma iodine concentration.

Identical Twins

Five pairs of twin heifers believed to be identical on the basis of markings, hair sworls, and blood type were sampled on several occasions. It was thought that the plasma protein-bound iodine levels of the identical twins might be quite similar. However, the results, summarized in Table XV, show that the values obtained on these animals were no more alike than those from unrelated animals.

Each pair of twins, with the exception of Pair 4, were on identical rations and maintained under the same environmental conditions. The writer knows of no data on identical twins of any species in which their rates of thyroxine secretion are compared. It appears that their identical inheritance does not necessarily extend to the functioning of the endocrine system which is subject to modification by environmental influences.

TABLE XV

PLASMA PROTEIN-BOUND IODINE LEVELS OF IDENTICAL TWIN HEIFERS

Animal No.	Twin Pair	Breed	Age in Mos.	Sampling Dates 6-12 1951 γ %	6-26 1951 γ %	7-10 1951 γ %	7-24 1951 γ %	8-22 1951 γ %	Avg. γ %
T 1	1	H[1]	22	6.9	5.7	6.6	3.0	3.6	5.2
T 2	1	H	22	4.2	12.6	7.2	5.1	4.5	6.6
T 3	2	H J[2]	16	15.3	8.1	9.9	6.0	7.5	9.4
T 4	2	H J	16	12.9	8.1	7.2	5.7	3.0	7.4
T 5	3	H J	17	4.8	-	6.6	3.6	5.1	5.0
T 6	3	H J	17	7.5	6.6	7.8	4.5	4.2	6.1
T 7	4	G H[3]	32	4.5	9.0	5.4	2.1	2.7	4.7
T 8	4	G H	32	4.2	16.5	5.4	2.7	3.6	6.5
T 9	5	H	22	-	-	-	-	4.8	4.8
T10	5	H	22	-	-	-	-	3.3	3.3

[1] Holstein.

[2] Holstein-Jersey cross.

[3] Guernsey-Holstein cross.

DISCUSSION

Reece and Man (156) have recently cast doubt upon the validity of the plasma level of protein-bound iodine as a measure of thyroid activity in dairy cattle by showing that two breeds of cows with different protein precipitable iodine levels had the same concentration of butanol extractable iodine in the plasma.

The organic iodine level in the plasma has been thoroughly demonstrated to be a reliable indicator of thyroid activity in humans, dogs, rats, rabbits, and other laboratory animals, and it is difficult to understand why cattle should be an exception. Even though cattle rations are frequently supplemented with iodized salt, it seems unlikely that the amount usually supplied would cause any significant rise in the nonthyroidal plasma protein-bound iodine. In this connection, no effect could be observed when cows in this study which had not previously received iodized salt were supplemented with it. This is, of course, considered to be an iodine-deficient region, and the amount of iodine in the supplemented diet may have been no

more than adequate. Perhaps the results would have been different if the supplemental iodine had been in excess of the animals' needs. There is little doubt but what, if sufficiently large amounts of iodine are injested, it is possible to build a concentration of nonthyroactive organic iodine in the plasma sufficient to mask any malfunctioning of the thyroid gland. Obviously, the relation of the protein-bound iodine in dairy cattle plasma to the circulating thyroid hormone must be thoroughly investigated.

Nevertheless, it has been clearly shown that the plasma protein-bound iodine concentration of cattle in the herds studied generally fell within certain well-defined ranges. There would seem to be no good reason why these ranges should not apply to cows in other herds as well. Until evidence to the contrary is forthcoming, it is suggested that cows on similar levels of iodine intake who deviate markedly from these tentative ranges do so because of thyroid hypo- or hyperfunctioning.

On the basis of the data reported here, it is proposed that the following ranges of plasma protein-bound iodine concentration be considered normal for dairy cattle: calves under 48 hours of age, 8.0 to 18.0 micrograms percent; animals

between 2 days and 12 months old, 3.5 to 12.0 micrograms percent; animals 13 to 24 months of age, 3.5 to 10.0 micrograms percent; and cows over 24 months old, 3.0 to 8.0 micrograms percent.

It is nearly impossible to determine a normal range for young calves because of the variable effect of colostrum. Their iodine levels may be even less than 8.0 micrograms percent if the blood sample is taken before the calf has nursed, and after nursing it may exceed 20.0 micrograms percent if the colostrum is rich in iodine. The iodine concentration in the plasma above 8.0 to 12.0 micrograms percent appears to have little or no relation to thyroid activity at this age.

Since no studies of the effect of hypo- or hyperthyroidism on the plasma level of protein-bound iodine in cattle have been conducted, it is impossible to relate these pathological conditions to the suggested normal ranges. It may be found that these ranges can be considerably enlarged before thyroid dysfunction is encountered. It is known that cows receiving thyroprotein (protamone) at the rate of 1 gram daily per 100 pounds of body weight show protein-bound iodine concentrations of more than 20.0 micrograms percent. Undoubtedly, as the

relationship of the protein-bound iodine in the plasma to thyroid activity is better understood, some revision of these ranges will be advisable.

In spite of the well-known effect of thyroxine or thyroprotein administration on milk and butterfat production, no relationship between the organic iodine level of the plasma and production of the cows studied was found. Neither did there appear to be any close relationship to the stage of pregnancy. This is not to say that there is no correlation, since the numbers were too limited and the range of production too small for the data to be in any way conclusive.

The question of genetic differences in thyroid activity of cattle is intriguing. If the differences in the plasma protein-bound iodine between cows are related to their productive ability, it may at some future date be possible to select potentially high-producing cows from their protein-bound iodine levels as calves. Or, perhaps one of the causes of the phenomenon of "nicking" is the fortunate mating of a bull and cow whose relatively high degree of thyroid functioning is transmitted to their offspring.

The little effect of season on the iodine values found in this work is not surprising, in view of the small amount of hot summer weather which occurs in this area. There is little reason to doubt that the hot summer months of more southerly states would cause a depression of thyroid activity, and in those areas seasonal changes in the plasma protein-bound iodine may be expected.

The recent use of thyroprotein (protamone) as a stimulant to milk production has been a cause of concern to some because of the possibility of certain unscrupulous dairymen using it on test cows. Such practices may easily be detected through determination of the plasma or milk iodine. The concentration of iodine in both milk and plasma is far greater than would be expected in untreated animals. Furthermore, the iodine level does not fall to normal for a period of days or weeks after administration of the drug ceases.

The areas of future work in this field seem well defined. First, it is essential that the protein-bound iodine compounds in dairy-cattle plasma be identified and the relation of the plasma organic iodine to thyroid activity established. If the relationship is close, then the factors affecting the plasma

protein-bound iodine concentration and the relation of this iodine level to performance of the animal must be thoroughly examined. Until this is done, the value of the plasma concentration of protein-bound iodine as a measure of thyroid activity in dairy cattle will be open to question.

However, regardless of the final conclusion regarding the value of the plasma protein-bound iodine as a measure of thyroid function in dairy cattle, it will be interesting to pursue this work further and investigate the relation of the organic-iodine level to the performance of the animal.

The relation of nutrition and many other environmental factors to the protein-bound iodine concentration of cattle must be studied. The correlation between this iodine level in the plasma and the rate of growth or fattening, breeding efficiency, milk production, and even longevity would be valuable. The effects of estrus, gestation, production, temperature, light, sex, and breed on the plasma protein-bound iodine should be known in order to properly evaluate the data obtained from these studies.

It is one of the functions of the researcher in dairy production to devise means whereby the dairy animal can produce

food with greater efficiency. The plasma level of protein-bound iodine may become a useful indicator of the probable performance of an individual animal. Until this is resolved, these investigations should be given every encouragement.

SUMMARY AND CONCLUSIONS

The plasma concentration of protein-bound iodine of dairy cattle has been shown to vary with the age of the animal. Soon after birth, the level rises as a result of the injestion of colostrum, which initially contains a large amount of iodine, most of which is in organic combination. This is accompanied by a corresponding drop in the protein-bound iodine level of the dam's plasma.

After the second day, the iodine level of the calf's plasma falls to one which is maintained for the first year to 18 months. A small decrease is observed between 18 months and 2 years, and a further fall occurs during the productive life of the animal.

It is suggested that normal ranges of protein-bound iodine be set as follows: Calves under 48 hours old, 8.0 to 18.0 micrograms percent; animals up to 12 months of age, 3.5 to 12.0 micrograms percent, those 13 to 24 months old, 3.5 to 10.0 micrograms percent; and cows over 24 months old, 3.0 to 8.0 micrograms percent. These ranges will probably require adjustment as more data become available.

Considerable variation in the organic iodine levels of cows sampled at different times of the year were found, although the seasonal changes were significant only for the Holstein breed.

The plasma protein-bound iodine levels of the Jersey and Holstein cows in the same herd were not significantly different. The iodine levels of cows from other herds were different.

Several cows with histories of poor conception rates were found to have low plasma protein-bound iodine concentrations. The significance of this is not known.

Identical twin heifers were no more alike in their plasma organic iodine levels than nonrelated animals.

No relationship of the plasma protein-bound iodine concentration to gestation or lactation could be found from the limited data of this study.

Initially colostrum is high in protein-precipitable iodine, due probably to the infiltration of plasma proteins into the udder. This provides the newborn calf with a relatively rich source of iodine, which photomicrographs indicate is stored in the thyroid gland. The physiological importance of this iodine

is not known. It is suggested that this is one of the means whereby the calf is prepared for adjustment to its new environment.

Normal milk is much lower in total iodine than colostrum, although the inorganic fraction remains relatively constant.

The need for further work in identifying the organic iodine compounds in the plasma and establishing the relationship of the plasma protein-bound iodine to thyroid function in dairy cattle is emphasized.

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