# The Effect of Thyroxine and Thiouracil on the Composition of Milk

## 3. THE CONCENTRATION OF THE MAJOR CONSTITUENTS AND OF SOME OF THE WATER-SOLUBLE VITAMINS IN COW'S MILK

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When lactating animals are treated with thyroxine, milk yield is almost invariably markedly stimulated (Graham, 1934*a*, *b*; Jack & Bechdel, 1935; Folley & White, 1936; Herman, Graham & Turner, 1938; Smith & Dastur, 1940; Owen, 1948*a*; Chanda & Owen, 1951). Usually the yield of fat is stimulated to a greater extent than that of the milk so that an increase in fat percentage results. This latter effect is, however, not always observed. In some experiments the solids-not-fat of the milk increase (Folley & White, 1936), but this again is not a constant finding (Archibald, 1945). The changes in the total nitrogen content of the milk caused by iodinated casein or thyroxine are also highly variable. Thus Ralston, Cowsert, Ragsdale, Herman & Turner (1940) reported a decline in the total nitrogen content of the milk. On the other hand, Van Landingham, Hyatt & Weakley (1946), Hibbs & Krauss (1947), Owen (1948*a*) and Chanda & Owen (1951) found no change in the total nitrogen content or in the partition of nitrogen in the milk. It came to be generally believed that, apart from its effect on fat, thyroxine caused only minor changes in the composition of the milk. Owen (1948*a*), however, found a significant increase in the phosphorus content of the milk without any concomitant change in either the nitrogen or the calcium contents. Chanda & Owen (1951) found that the goitrogenic substance thiouracil caused a decrease in the total phosphorus of milk without any change in its nitrogen content, and in the same experiments they showed that thyroxine increased the ester phosphorus and lipid phosphorus while decreasing the inorganic phosphorus in cow's milk. They also found that thiouracil produced changes in the partition of phosphorus which were the reverse of those produced by thyroxine. Changes in phosphorus partition, whether produced by thyroxine or thiouracil, were inversely correlated with changes in milk phosphatase. In the present paper the effects of thyroxine and thiouracil on the partition of aneurin in relation to changes in the phosphatase titre of the milk are described. The simultaneous changes produced in the concentrations of some of the major constituents of milk and in the concentrations of certain minerals and water-soluble vitamins were also followed.

## EXPERIMENTAL

There were five experiments, each divided into periods 1–3, the hormonal treatments being confined to period 2. Each period lasted 3 weeks, except for period 2 of Exp. 5 which was a fortnight. Three cows were used in Exp. 1, and six in each of the other four experiments. In Exp. 1, there was one control cow, one treated subcutaneously with 10 mg. DL-thyroxine per day, and a third which received, subcutaneously, 10 mg. thiouracil per day. In each of the other four experiments two cows received 10 mg. DL-thyroxine per day each, two received 20 mg. thiouracil per day each, and two were controls. For the purpose of calculating the responses to the drugs, milk yields of all the cows were recorded. In Exp. 2, however, the milk of one of the control cows was not chemically analysed. In Exp. 3, aneurin in the milk was partitioned and the milk was also analysed for riboflavin, creatine, creatinine, Ca, Mg, Na and K. Haemoglobin, and red and white cell counts were determined in the blood of cows in Exp. 3. Fat, solids-not-fat, ascorbic acid and phosphatase were determined in the milk in Exps. 1–3. In addition, in Exps. 1 and 2, lactose, chloride and freezing point were determined. The milk yields only are considered in Exps. 4 and 5. In Exp. 1, 10 mg. thiouracil were found to cause the milk phosphatase to increase at only half the rate at which 10 mg. thyroxine caused it to decrease (Chanda & Owen, 1951). The dose of thiouracil was therefore increased to 20 mg. in all subsequent experiments. 20 mg. thiouracil gave an increase of the same magnitude as the decrease caused by 10 mg. thyroxine (Chanda & Owen, 1951). Thyroxine was dissolved as described by Folley & White (1936) prior to injection. The solution contained 1 mg. DL-thyroxine sodium B.P. (British Drug Houses Ltd.) per ml. Since thiouracil, like thyroxine, is soluble in alkali, it was possible to prepare a solution of it in the same way. A weighed amount of thiouracil was dissolved in a minimal excess of 0·1 N-NaOH and neutralized to phenolphthalein with 0·1 N-HCl. The solution was then diluted with distilled water to 1 mg./ml. in Exp. 1, or 2 mg./ml. in the other experiments. The solutions of the drugs were preserved in the refrigerator. Samples for filling 10 ml. syringes for injection were 'flash pasteurized' in weighing bottles immediately before use by having their temperature momentarily raised to 80°.

### *Methods of analysis*

*Fat* was determined by the Gerber method (British Standards Institution, 1936). *Total solids* were determined by drying a weighed sample of the milk first on a water bath and then in an electric oven at 100°. *Solids-not-fat* were determined from the difference between total solids and fat, but were calculated to a fat-free basis. *Protein* was calcu-

lated by multiplying the total nitrogen by the factor 6·38. *Lactose* was determined polarimetrically by Vieth's method as described by Elsdon & Walker (1942), the necessary correction being made for the volume of the precipitate. *Chloride* was estimated by the method of Davies (1938). The *freezing point* was estimated by the improved Hortvet apparatus described by Temple (1937). *Calcium, magnesium* and *sodium* were estimated in milk samples which were evaporated in vitreosil basins on a water bath prior to being ashed in an electric muffle furnace which was thermostatically controlled at 600°. The ash was dissolved in warm 6N-HCl and made up to a known volume. The final stage in the estimation of calcium was the titration of the precipitated calcium oxalate with $KMnO_4$ as described by Hawk, Oser & Summerson (1947). Sodium was precipitated as sodium zinc uranyl acetate in sintered-glass filters, dried at 100° and weighed (Peters & Van Slyke, 1932). Magnesium was determined from the phosphate content (Fiske & Subbarow, 1925) of a magnesium ammonium phosphate precipitate. *Potassium* was precipitated as cobaltinitrite from a 2% trichloroacetic acid (TCA) filtrate of whole milk and the cobalt in the centrifuged and washed precipitate was determined by its colour reaction with nitroso-R-salt (Sideris, 1942). The potassium figures were corrected for the volume of the protein precipitated by TCA (Rowland, 1938) and for co-precipitated sodium. *Creatinine* was determined by treatment of the tungstic acid filtrate of milk with alkaline picrate solution, the resulting colour being read in a Spekker absorptiometer. *Creatine* was determined by subtracting the preformed creatinine from the total creatinine determined by the same method after autoclaving the protein-free filtrate with HCl (Hawk *et al.* 1947). *Phosphatase* was determined as already described by Chanda & Owen (1951). *Ascorbic* acid was estimated according to the method used by Mattick *et al.* (1945), precaution being taken that the samples of milk were made large enough to fill the preserving jars in which they were taken so as to prevent any aerobic oxidation. The milk was pipetted into the TCA-metaphosphoric acid mixture within 15 min. of the collection of the samples, to avoid formation of any dehydroascorbic acid. *Riboflavin* was determined in a Spekker fluorimeter by the method of Emmerie (1938) combined with Arnold's (1945) modification. *Aneurin* was determined fluorimetrically in *iso*butanol as thiochrome. The aneurin was partitioned as described by Houston, Kon & Thompson (1940). Free aneurin was determined in skimmed milk by Jansen's (1936) method, while cocarboxylase was determined from the difference between free aneurin and aneurin present in the takadiastase digest of the TCA extract of skim milk. Pepsin digestion of the TCA precipitate gave the protein-bound aneurin. Total aneurin (free plus cocarboxylase plus protein-bound) was independently determined by digestion of skim milk with takadiastase only, for it was found in confirmation of Houston *et al.* (1940) that pepsin digestion following takadiastase digestion did not materially increase the total.

## RESULTS AND DISCUSSION

*Milk yield.* Typical graphs showing the increases in milk yield brought about by thyroxine and the decreases brought about by thiouracil have already been given (Chanda & Owen, 1951). The quantitative responses in milk yield produced by the two drugs in all the cows used in the present series of experiments are now described. In calculating the positive or negative responses in milk yield it is obviously difficult to estimate what the yield would have been had no treatment been given, since after the first few weeks from calving, the yield of milk gradually declines. The increases caused by thyroxine and decreases caused by thiouracil were therefore complicated by this natural decline. To offset this difficulty, the average daily yield ($x$) of each cow during period 1 was analysed by co-variance with the average daily yield ($y$) in period 2, the cows being divided into three treatment groups—control, thyroxine and thiouracil. From the total sum of squares and products the variance due to treatment was eliminated. From the residual sum of squares and products, the following significant regression equation was established between $x$ and $y$

$$y = -0{\cdot}44 + 0{\cdot}94077x$$

($x$ = the actual milk yield in lb. in period 1; $y$ = the expected milk yield in period 2). Geometrically, this method of statistical analysis corresponds to using an extrapolation of a straight line fitted to the milk yield data of period 1 as the expected yield in period 2 (Owen, Smith & Wright, 1943). Since the effects of discontinuance of the hormone treatment are just as marked as those produced by its institution, period 3 cannot be regarded simply as a second control period, so that a line fitted to the data of periods 1 and 3 would overestimate the effect of thyroxine and underestimate that of thiouracil. The expected milk yields during period 2, calculated from this equation, compared favourably with the actual milk yields shown simultaneously by each of the nine control cows. The results for all the nine thyroxine-treated cows except one showed that the actual yields in period 2 were higher than the expected yields. For the sake of brevity the mean results of each group are recorded in Table 1 which shows that the average response was +13·2% in the thyroxine-treated cows during treatment, while

Table 1. *Effect of thyroxine and thiouracil on milk yield*

| Group of cows | No. of cows | Expected milk yield during period 2 calculated from the regression (lb./day)* | Actual milk yield (lb./day) | Response (%) |
|---|---|---|---|---|
| Control | 9 | 21·1 | 21·1 | — |
| Thyroxine (10 mg./day) | 9 | 17·1 | 19·3 | +13·2 |
| Thiouracil (20 mg./day) | 8 | 22·0 | 19·6 | −10·7 |

* 1 lb. = 454 g.

the average response was −10·7 % in the eight cows treated with 20 mg. thiouracil daily. The response by the cow in Exp. 1, which received only 10 mg. thiouracil, was only −5·8 %. The responses in the individual cows within groups varied very widely and the initial yields of cows did not seem to have any effect on the relative magnitude of the response caused by either thyroxine or thiouracil.

*Fat, solids-not-fat and protein content of milk.* The results for solids-not-fat have been calculated to a fat-free basis. In most of the animals, thyroxine caused an increase in the fat content of the milk. The effect of the hormone on the solids-not-fat content of milk was variable. In some of the cows there was a small increase, but in others there was no change. This contrast is particularly noticeable in cow no. 12 (Exp. 3) in which the greatest response in the fat content of milk was recorded without any change in the solids-not-fat. Thiouracil decreased the fat content of milk except in cow no. 8 (Exp. 2) for which the fat content of milk was considerably increased in successive periods. The effect of thiouracil on the solids-not-fat content of the milk was inconstant.

Table 2. *Effect of thyroxine and thiouracil on the contents of fat and solids-not-fat in milk*

| Exp. | Cow | Daily injection in period 2 | Fat (g./100 g. milk) Period 1 | Fat (g./100 g. milk) Period 2 | Fat (g./100 g. milk) Period 3 | Solids-not-fat (g./100 g. fat-free milk) Period 1 | Solids-not-fat (g./100 g. fat-free milk) Period 2 | Solids-not-fat (g./100 g. fat-free milk) Period 3 |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | None | 4·07 | 3·75 | 4·15 | 8·78 | 8·89 | 9·15 |
| | 2 | 10 mg. thyroxine | 3·83 | 4·20 | 5·00 | 8·84 | 9·11 | 9·92 |
| | 3 | 10 mg. thiouracil | 3·82 | 3·23 | 3·38 | 8·46 | 8·21 | 8·57 |
| 2 | 4 | None | 4·45 | 4·34 | 4·78 | 8·86 | 8·87 | 9·20 |
| | 5 | 10 mg. thyroxine | 4·51 | 5·08 | 4·83 | 9·45 | 9·96 | 9·61 |
| | 6 | 10 mg. thyroxine | 4·31 | 4·43 | 4·61 | 8·75 | 8·63 | 8·97 |
| | 7 | 20 mg. thiouracil | 4·06 | 3·62 | 3·46 | 8·48 | 8·53 | 8·52 |
| | 8 | 20 mg. thiouracil | 4·19 | 4·66 | 5·13 | 8·79 | 8·83 | 9·33 |
| 3 | 9 | None | 3·95 | 3·90 | 4·24 | 8·92 | 8·89 | 8·78 |
| | 10 | None | 4·28 | 3·95 | 3·98 | 8·88 | 8·81 | 8·54 |
| | 11 | 10 mg. thyroxine | 4·12 | 4·74 | 4·35 | 9·24 | 9·43 | 8·95 |
| | 12 | 10 mg. thyroxine | 4·14 | 5·24 | 4·74 | 9·01 | 8·97 | 8·72 |
| | 13 | 20 mg. thiouracil | 4·44 | 4·12 | 4·58 | 9·05 | 8·85 | 8·76 |
| | 14 | 20 mg. thiouracil | 3·91 | 3·47 | 4·32 | 9·02 | 9·01 | 8·84 |

The protein content of the fat-free milk in the cows used in Exps. 2 and 3 confirmed the findings in Exp. 1 already reported by Chanda & Owen (1951). Neither thyroxine nor thiouracil had any effect on the protein content of the fat-free milk.

*Lactose, chloride and freezing point.* These analyses, which were carried out in Exps. 1 and 2 only, show that, corresponding to a small increase in solids-not-fat (Table 2), there was a small increase in the lactose content of the milk, but the effect was not noticeable in all the treated cows. Thiouracil caused a small decrease in lactose content corresponding to a small decrease in solids-not-fat. Simultaneously with an increase in lactose content, there was a decrease in the chloride content of the milk in the thyroxine-treated cows. Thiouracil had the opposite effect. An increase in chloride content with a corresponding decrease in lactose was also noticeable in the control cows with the progress of lactation. There was thus a close negative correlation between the average lactose and chloride contents in Table 3 ($r = -0{\cdot}9933$). Davies (1936) pointed out that since lactose and chloride account for 80 % of the osmotic pressure of milk, there tends to be a constant relationship between these two constituents. He found from analysis of numerous milk samples that the relationship can be expressed as

$$\text{lactose content} = 6{\cdot}26 - 13{\cdot}5 \times \text{chloride content},$$

or in other words lactose + 13·5 times chloride should be constant and equal to 6·26. In the present investigation this constant has been calculated and recorded in Table 3 in which it is referred to as the lactose-chloride number. It was found that this number was fairly constant for all the cows in all the periods, but its actual value was somewhat lower than that of Davies (1936). The mean value in the present experiments was found to be 6·19. In conformity with this constancy of the relationship between lactose and chloride it was also found that the freezing point of the milk remained unchanged throughout the experiments (Table 3).

*Calcium, magnesium, sodium and potassium.* The calcium content of the milk showed no change attributable to hormonal effects, thus confirming the earlier observations of Owen (1948*b*). In conformity with this finding is the observation that calcium was not affected by thiouracil. In Exp. 3, the mean calcium contents, with their standard errors, of the two thyroxine-treated cows were 136·7 ± 3·1, 130·8 ± 3·1 and 121·2 ± 3·0 mg./100 g. milk in periods 1, 2 and 3 respectively. The corresponding figures for the two cows which were treated with thiouracil were 124·4 ± 2·8, 118·8 ± 3·0 and 118·3 ± 2·8, and for the two control cows 121·2 ± 3·3,

114·8 ± 3·4 and 110·3 ± 3·1. The failure of hormonal treatments to change the calcium content of milk is in sharp contrast to their effects on the phosphorus content which was increased by thyroxine (Owen, 1948*b*; Chanda & Owen, 1951) and decreased by thiouracil (Chanda & Owen, 1951). As pointed out earlier (Owen, 1948*b*), this can perhaps be related to the small amount of calcium required by the soft tissues. These observations, in conjunction with that of Owen (1948*b*), indicate that the metabolic pathways of calcium and phosphorus in adult animals are not so directly linked in the soft tissues as they are known to be in bone.

There was no indication that either drug caused any change in sodium, potassium or magnesium. The means and standard errors for all animals, without distinction of periods, were 64·3 ± 4·8, 149·6 ± 3·6 and 11·3 ± 0·4 mg./100 ml. milk for sodium, potassium and magnesium respectively.

experiment was, however, by injection, whereas, in the treatment of toxic goitre in humans, thiouracil is given by mouth and much larger doses of the drug per unit body weight are used. Since leucopaenia caused by thiouracil is an idiosyncrasy it is conceivable that treatment of a larger number might demonstrate its occurrence in cows. We have, however, never observed any untoward symptoms in cows treated with thiouracil. The average figures for blood constituents of all the cows in Exp. 3 were: haemoglobin 8·80 ± 0·17 g./100 ml., red blood cells 5·11 ± 0·14 millions/mm.$^3$, and white blood cells 9·31 ± 0·42 thousands/mm.$^3$.

*Creatine and creatinine content of milk.* To find whether the increase in ester phosphorus caused by thyroxine (Chanda & Owen, 1951) could be attributed to an increase in labile phosphate, such as creatine phosphate, the creatine content of the milk was estimated in Exp. 3. The results showed that

Table 3. *Effect of thyroxine and thiouracil on the lactose and chloride contents of milk*

| Exp. | Cow | Daily injection in period 2 | Period | Lactose (%) | Chloride (%) | Lactose chloride number* | Depression of freezing point (° C.) |
|---|---|---|---|---|---|---|---|
| 1 | 1 | None | 1 | 4·13 | 0·151 | 6·17 | 0·545 |
| | | | 2 | 4·09 | 0·155 | 6·18 | 0·541 |
| | | | 3 | 4·05 | 0·158 | 6·18 | 0·542 |
| | 2 | 10 mg. thyroxine | 1 | 4·22 | 0·145 | 6·18 | 0·546 |
| | | | 2 | 4·47 | 0·125 | 6·16 | 0·539 |
| | | | 3 | 4·16 | 0·152 | 6·21 | 0·544 |
| | 3 | 10 mg. thiouracil | 1 | 4·33 | 0·137 | 6·18 | 0·538 |
| | | | 2 | 4·06 | 0·156 | 6·17 | 0·542 |
| | | | 3 | 4·37 | 0·139 | 6·25 | 0·546 |
| 2 | 4 | None | 1 | 4·39 | 0·132 | 6·17 | 0·542 |
| | | | 2 | 4·21 | 0·148 | 6·21 | 0·539 |
| | | | 3 | 4·09 | 0·155 | 6·18 | 0·541 |
| | 5 | 10 mg. thyroxine | 1 | 4·67 | 0·112 | 6·18 | 0·545 |
| | | | 2 | 5·18 | 0·076 | 6·21 | 0·546 |
| | | | 3 | 4·76 | 0·105 | 6·18 | 0·542 |
| | 6 | 10 mg. thyroxine | 1 | 4·28 | 0·144 | 6·22 | 0·539 |
| | | | 2 | 4·32 | 0·138 | 6·18 | 0·542 |
| | | | 3 | 4·11 | 0·154 | 6·19 | 0·537 |
| | 7 | 20 mg. thiouracil | 1 | 4·25 | 0·142 | 6·17 | 0·547 |
| | | | 2 | 4·29 | 0·140 | 6·18 | 0·541 |
| | | | 3 | 4·35 | 0·139 | 6·23 | 0·545 |
| | 8 | 20 mg. thiouracil | 1 | 4·31 | 0·138 | 6·17 | 0·551 |
| | | | 2 | 4·01 | 0·159 | 6·16 | 0·547 |
| | | | 3 | 4·44 | 0·135 | 6·26 | 0·549 |

* Lactose content + 13·5 × chloride content = 6·26 (Davies, 1936).

*Blood analysis.* In view of reports that thiouracil treatment of toxic goitre in humans causes leucopaenia in a minority of patients (Grainger, Gregson & Pemberton, 1945; Himsworth, 1948), the blood of the cows in Exp. 3 was analysed for haemoglobin, red blood cells and white blood cells. The daily variations could not be related to the drug treatments, and there was no evidence of leucopaenia in the cows. The method of treatment in the present

neither thyroxine nor thiouracil caused any change in the content of either creatine or creatinine. An analysis of variance showed that there was no significant variation in the creatine content of the milk between animals. There was, however, a significant decrease from period to period. This variation was attributable to advancing lactation since there was a regular trend of decrease during successive periods in all the cows. The averages

in periods 1–3 of creatine + creatinine (measured as creatinine) were $13{\cdot}7 \pm 0{\cdot}50$, $13{\cdot}1 \pm 0{\cdot}52$ and $11{\cdot}5 \pm 0{\cdot}62$ mg./100 g. in the thyroxine-treated cows, respectively. The corresponding figures for the cows which received thiouracil were $14{\cdot}0 \pm 0{\cdot}49$, $13{\cdot}2 \pm 0{\cdot}54$ and $11{\cdot}4 \pm 0{\cdot}62$, and for the control cows $12{\cdot}8 \pm 0{\cdot}50$, $13{\cdot}4 \pm 0{\cdot}50$ and $11{\cdot}8 \pm 0{\cdot}54$ mg./100 g. Creatine contributed a relatively constant percentage to these totals ($71{\cdot}2 \pm 4{\cdot}1$ %).

The lack of change in the creatine content of milk in thyroxine-treated cows is in sharp contrast to that of urinary creatine which was found to be markedly increased in cows treated with thyroxine (Owen, 1948*a*). The statement of Basu & Mukherjee (1943) that ester phosphorus in milk is exclusively the labile creatine phosphate is not supported by these results. In spite of the large increase in the ester phosphorus caused by thyroxine (Chanda & Owen, 1951), no changes occurred in the creatine content of the milk. Moreover, the amount of creatine found in the milk could have accounted for only 1·4 mg. ester phosphorus, which is lower than the smallest ester phosphorus figure observed by Chanda & Owen (1951) during thiouracil treatment.

### *Water-soluble vitamins*

*Ascorbic acid.* The results for the five cows in Exp. 2 are recorded in Fig. 1 which shows that, in the thyroxine-treated cows, the ascorbic acid content of the milk decreased during treatment, while in the cows which were treated with thiouracil it increased. The control cow showed that the stage of lactation had little effect on the ascorbic acid content of the milk. In Exps. 1–3 the mean decrease caused by 10 mg. thyroxine in five cows was 25 % (range 14–34 %) while the mean increase caused in four cows by 20 mg. thiouracil was 24 %. In the cow dosed with 10 mg. thiouracil the decrease was 10 %.

Decreases in ascorbic acid content of milk after treatment of cows with iodinated casein have been recorded by Van Landingham, Henderson & Weakley (1944). Similar decreases were observed by Bartlett, Rowland & Thompson (1949) who attributed them to the iodide in the iodinated casein. Since, however, iodinated casein was not used in the present experiments, it is clear that it is the thyroxine itself which produces these effects. The minute dose of iodine given in 10 mg. thyroxine per day could not be expected to influence the ascorbic acid content of the milk. Furthermore, there is other evidence, albeit indirect, that the thyroid probably governs the ascorbic acid content of milk.

*Riboflavin.* The mean riboflavin contents of the milk of six cows (Exp. 3) in individual periods are recorded in Table 4 with their standard errors. The mean results for the two thyroxine-treated cows showed a decrease in the treatment period (period 2) compared with the initial control period (period 1), but this also occurred in one of the control cows and in the two thiouracil cows. The results were therefore analysed statistically. The analysis of variance, also recorded in Table 4, showed that the differences between cows were significant, but that the differences between periods within cows were not. The mean values in period 1 showed a range of 83–127 μg./100 ml. fat-free milk. It can, therefore, be concluded that neither the drugs nor the stage of lactation caused any change in the riboflavin content.

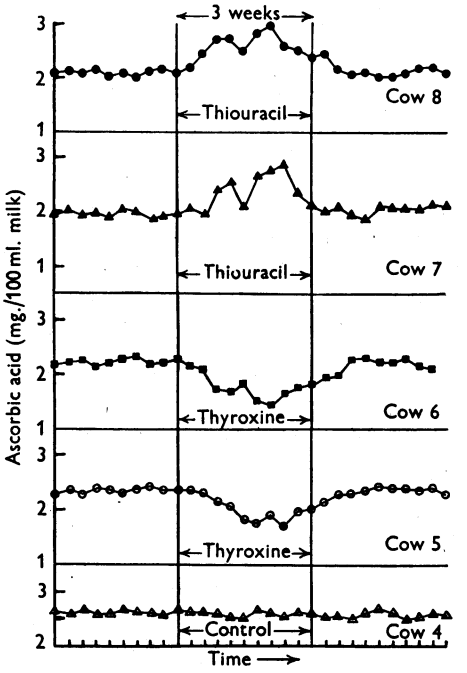


Fig. 1. Effect of thyroxine and thiouracil on the ascorbic acid content of cow's milk.

Bartlett *et al.* (1949) reported a 17 % decrease in riboflavin in the milk of cows treated with thyroxine. The Arizona workers (Kemmerer, Bolomey, Vavich & Davis, 1946) also reported a decrease, the concentration of riboflavin during the treatment period dropping almost to nil. These values, as Kon & Henry (1949) pointed out, are inconceivably low. In view of the finding of Bartlett *et al.* (1949) the present results of 2-day values were further analysed statistically to compare the rates of decrease in groups of cows during period 2. The differences between the three regressions were found not to be statistically significant. Kon & Henry (1949), quoting Thompson (1945), stated that neither thyroxine nor iodinated protein had any effect on the riboflavin content of milk.

Table 4. *Effect of thyroxine and thiouracil on the riboflavin content of cow's milk*

| Cow | Daily injection in period 2 | Riboflavin (μg./100 ml. fat-free milk ± S.E.) Period 1 | Period 2 | Period 3 |
|---|---|---|---|---|
| 9 | None | 103·2±1·6 | 105·4±2·2 | 102·2±4·2 |
| 10 | None | 82·5±1·7 | 77·2±2·9 | 77·0±2·4 |
| 11 | 10 mg. thyroxine | 111·5±2·8 | 97·5±3·1 | 108·1±3·6 |
| 12 | 10 mg. thyroxine | 103·0±1·5 | 95·1±2·9 | 100·2±3·4 |
| 13 | 20 mg. thiouracil | 104·6±1·6 | 103·5±2·6 | 99·5±4·1 |
| 14 | 20 mg. thiouracil | 127·2±2·2 | 115·7±2·5 | 107·0±4·2 |

*Analysis of variance of the average results for riboflavin*

(N.S., not significant.)

| Source of variation | Degrees of freedom | Sum of squares | Mean square | Variance ratio ($e^{2s}$) |
|---|---|---|---|---|
| Total | 17 | 2687·58 | — | — |
| Cows | 5 | 2302·18 | 460·44 | 20·39* |
| Periods | 2 | 159·61 | 79·81 | 3·53 (N.S.) |
| Error | 10 | 225·79 | 22·58 | — |

* $P<0·001$.

*Total aneurin and the partition of aneurin.* The cocarboxylase and protein-bound aneurin in the milk studied in Exp. 3 are shown graphically in Fig. 2. In this diagram the individual 2-day data throughout the experiment are shown. The average values for total aneurin in each period are recorded in Table 5, where the phosphatase and the partition of aneurin between the free, the cocarboxylase and the protein-bound forms are also recorded. It can be seen from Table 5 that the total aneurin content of the milk gradually decreased in all the cows in successive periods. There were large variations in the total aneurin values between cows. An analysis of variance of the total aneurin showed that the differences between cows and between periods were statistically significant. Due to the significant difference between cows, comparisons cannot be made between the controls and the treated cows in the same period; and from a comparison of the same cow from period to period, the effect of the hormone treatment is difficult to establish, but it would appear that the normal rate of the decrease was slightly retarded by thyroxine and somewhat enhanced by thiouracil. In spite of the smallness of the change in the total aneurin, the partition of aneurin showed considerable change. Table 5 shows that under the influence of thyroxine the cocarboxylase in the milk increased markedly at the expense of the free aneurin. There was also a small increase in the protein-bound aneurin. These changes are well shown in Fig. 2. Decreases in the free form of aneurin in the milk of cows fed iodinated protein were reported earlier by Bartlett *et al.* (1949). The present experiments have shown that these decreases are accompanied by an increase in the cocarboxylase and in the protein-bound forms of aneurin when thyroxine is administered subcutaneously. At the peak of hormonal response, the cocarboxylase in the thyroxine cow no. 12, accounted for 38 % of the total aneurin compared with only 21 % in the pretreatment period. The present results showed that thiouracil increased the free aneurin content and decreased the contents of both

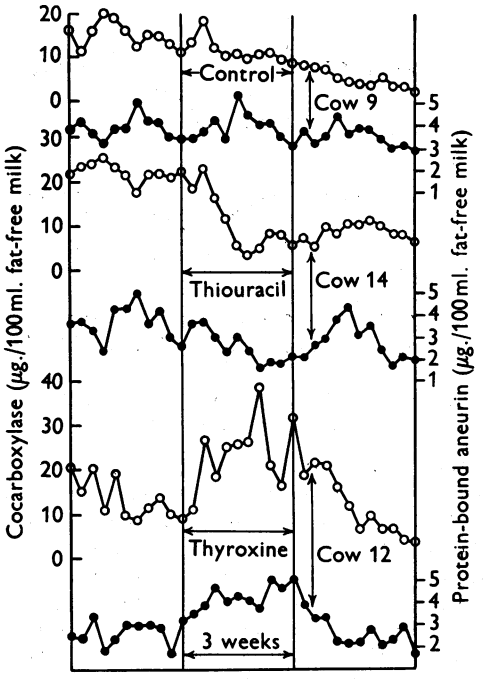


Fig. 2. Effect of thyroxine and thiouracil on the cocarboxylase and protein-bound aneurin content of cow's milk. ○—○, cocarboxylase; ●—●, protein-bound aneurin.

Table 5. *Partition of aneurin in fat-free milk*

| Cow | Daily injection in period 2 | Period | No. of analyses | Total aneurin (μg./100 ml.) | Percentage of total aneurin as: Free vitamin | Cocarboxylase | Protein-bound | Phosphatase (phenol units)* |
|---|---|---|---|---|---|---|---|---|
| 9 | None | 1 | 11 | 39·4 | 50·4 | 38·0 | 7·3 | 59 |
| | | 2 | 10 | 37·0 | 55·6 | 31·0 | 7·9 | 58 |
| | | 3 | 11 | 37·1 | 72·7 | 14·9 | 6·9 | 109 |
| 10 | None | 1 | 11 | 46·5 | 48·0 | 39·9 | 7·6 | 47 |
| | | 2 | 10 | 41·6 | 64·3 | 24·8 | 6·5 | 67 |
| | | 3 | 11 | 40·5 | 70·6 | 18·5 | 6·6 | 78 |
| 11 | 10 mg. thyroxine | 1 | 11 | 46·5 | 64·2 | 26·2 | 5·9 | 70 |
| | | 2 | 10 | 46·0 | 30·7 | 58·2 | 7·3 | 35 |
| | | 3 | 11 | 41·1 | 62·2 | 26·2 | 6·9 | 84 |
| 12 | 10 mg. thyroxine | 1 | 11 | 42·0 | 58·1 | 31·5 | 6·7 | 67 |
| | | 2 | 10 | 41·0 | 28·4 | 58·1 | 10·8 | 21 |
| | | 3 | 11 | 39·2 | 56·9 | 30·9 | 7·0 | 73 |
| 13 | 20 mg. thiouracil | 1 | 11 | 45·8 | 45·0 | 42·5 | 7·6 | 39 |
| | | 2 | 10 | 39·1 | 68·4 | 21·1 | 7·3 | 70 |
| | | 3 | 11 | 37·1 | 67·2 | 21·6 | 7·4 | 73 |
| 14 | 20 mg. thiouracil | 1 | 11 | 50·3 | 42·7 | 44·6 | 7·4 | 37 |
| | | 2 | 10 | 45·0 | 66·8 | 23·4 | 5·7 | 64 |
| | | 3 | 11 | 44·3 | 68·3 | 20·5 | 6·4 | 59 |

* See Chanda & Owen (1951).

cocarboxylase and protein-bound aneurin (Fig. 2). The same sort of effect was noticed in the control cows (Table 5) as in the thiouracil cows, but it is evident from Fig. 2 and Table 5 that these natural effects were intensified by thiouracil. Thus, the minimum percentage of total aneurin in the form of cocarboxylase was decreased from 10 % in period 1, to 8 % in period 2 in the control cow (no. 10) while the corresponding decrease was from 18 to 4 % in the thiouracil-treated cow (no. 14).

During the last period of the experiment, the combined effect of advancing lactation and the cessation of thyroxine treatment produced some dramatic changes. The phosphatase titre increased rapidly and there was a big increase in free aneurin at the expense of cocarboxylase (Table 5). Protein-bound aneurin was also reduced. When thiouracil was discontinued, advancing lactation offset the expected sharp decrease in the phosphatase titre (Table 5) but protein-bound aneurin and cocarboxylase increased (Fig. 2). The effect of advancing lactation can be seen in the control animal (no. 9), in which, during period 3, a marked increase in phosphatase was accompanied by an increase in free aneurin and by corresponding decreases in cocarboxylase and protein-bound aneurin (Table 5). These effects were as unmistakable as those produced artificially by thiouracil in cows 13 and 14.

*Correlation between phosphatase and phosphorylated aneurin.* A positive correlation between phosphatase and free aneurin was observed to be a normal occurrence in the milk of cows and goats (Houston *et al.* 1940) and of sows (Braude *et al.* 1947) and also in human milk (Chanda, Owen & Cramond, 1951). This was confirmed in the present work.

There was a close positive correlation between phosphatase and free aneurin in the milk of all the cows (Table 6), and these correlations held whether the results for free aneurin were expressed in units per 100 ml. milk or as percentages of the total

Table 6. *Correlations of the percentages of aneurin in the free and cocarboxylase forms with phosphatase in the cow's milk*

(Number of pairs of observations was 28 in each case.)

| Cow | Treatment | Coefficient* of correlation of phosphatase with: Free aneurin × 100 / Total aneurin | Cocarboxylase × 100 / Total aneurin |
|---|---|---|---|
| 9 | None | +0·9490 | −0·8960 |
| 10 | None | +0·8932 | −0·8473 |
| 11 | Thyroxine | +0·8794 | −0·8898 |
| 12 | Thyroxine | +0·9514 | −0·9185 |
| 13 | Thiouracil | +0·8419 | −0·8257 |
| 14 | Thiouracil | +0·8328 | −0·7869 |

* Every coefficient shown in the table is significant at $P < 0{\cdot}001$.

aneurin. There were also (Figs. 3 and 4) large negative correlations (Table 6) between phosphatase and cocarboxylase. The curves in Figs. 3 and 4 fitted by the method of least squares, show for a thyroxine and a thiouracil cow a curvilinear relationship between phosphatase and cocarboxylase similar to that found in human milk by Chanda *et al.* (1951).

In Fig. 5, in which mean values for phosphatase and for protein-bound aneurin are plotted, the regression line, fitted by the method of the least squares indicates a significant negative but non-linear correlation between the two variables.

*Biochemical significance of the relation of phosphatase to phosphorylated aneurin.* Like ester phosphorus, which was increased dramatically by thyroxine (Chanda & Owen, 1951), cocarboxylase and protein-bound aneurin were also markedly increased by the hormone. Thiouracil caused a significant decrease in cocarboxylase. Normally a higher proportion of phosphorylated aneurin is a characteristic of early lactation (Houston *et al.* 1940; Chanda, 1951). In late lactation the amount of phosphorylated aneurin is only 10% of the total aneurin so that the ratio of total to free aneurin approaches unity. The higher proportion of phosphorylated aneurin in early lactation and during thyroxine treatment may be related to the greater activity of the mammary gland. The phosphatase titre in milk becomes small, perhaps due to its retention in the gland at the peak of lactation or when thyroxine is administered to the cow. As the free aneurin in the milk of thyroxine-treated cows was observed to decrease with a concomitant decrease in the phosphatase, the contents of cocarboxylase and protein-bound aneurin were found to increase. The larger amount of phosphorylated aneurin appeared to occur at the expense of free aneurin because the total aneurin remained approximately constant. With thiouracil the proportion of free aneurin was increased at the expense of the phosphorylated fractions. When the mammary gland worked faster under the influence of thyroxine more of the phosphoric esters were synthesized (Chanda & Owen, 1951). This in turn was accompanied by an increase in the cocarboxylase. During hypothyroidism there is a lowering in the metabolic rate and consequently less of the milk aneurin is in the phosphorylated form. The lesser amount of phosphatase secreted in the milk during thyroxine treatment (Folley & White, 1936) may therefore denote a retention of the enzyme for synthetic activity in the gland. This hypothesis is supported by the close negative correlations observed between phosphatase and phosphorylated aneurin. The function of the milk phosphatase may thus be anabolic in the mammary tissue even though in milk after secretion it appears to be mainly hydrolytic (Graham & Kay, 1934). The interdependence of phosphorylated compounds and phosphatase may be related to the power of thyroxine to increase the concentration of enzymes in the body (Williams-Ashman, 1948). The reverse changes caused by thiouracil are also explicable on this basis. The negative correlations between phosphorylated compounds and phosphatase observed in this and in

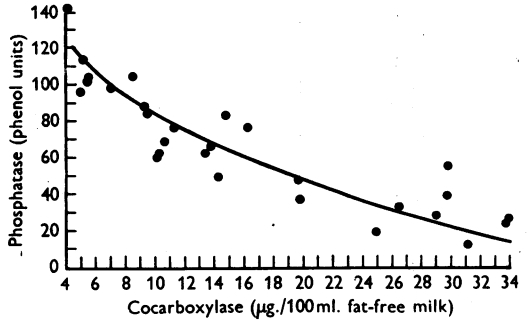


Fig. 3. Correlation between cocarboxylase and phosphatase in the milk of thyroxine-treated cow no. 11.

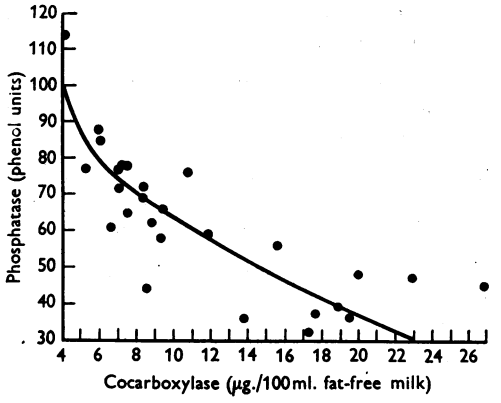


Fig. 4. Correlation between cocarboxylase and phosphatase in the milk of a thiouracil-treated cow.

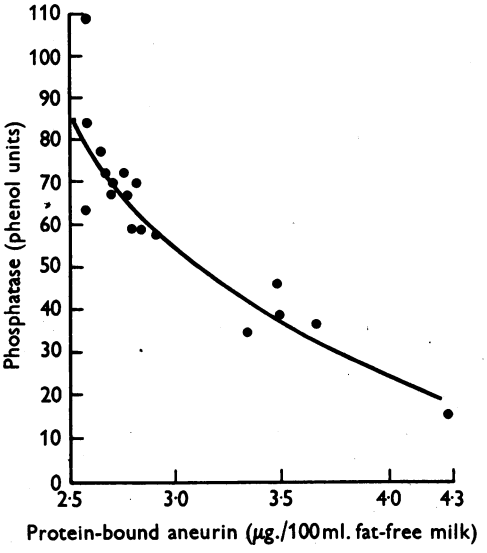


Fig. 5. Correlation between protein-bound aneurin and phosphatase in cow's milk. The ordinate of each point represents the mean value of phosphatase for a given cow in a given period, and its abscissa represents the corresponding mean value of protein-bound aneurin.

previous experiments (Chanda *et al.* 1951; Chanda & Owen, 1951) may imply a synthetic role for phosphatase in syntheses by the mammary gland.

## SUMMARY

1. The effect of thyroxine and thiouracil on the composition of cow's milk has been investigated, and the relation of phosphatase to the partition of aneurin has been specially studied. The following are the main results:

2. Thiouracil (20 mg. per cow per day subcutaneously) decreased the milk yield of cows by 10·7 %, while DL-thyroxine (10 mg. per cow per day subcutaneously) increased it by 13·2 %.

3. Thyroxine caused an increase in the fat of milk in some of the cows and thiouracil caused a decrease.

4. There was a small increase in the lactose content of milk in the thyroxine-treated cows corresponding to a simultaneous decrease in the chloride content. Thiouracil caused the reverse effects. There was an inverse relationship between lactose and chloride, such that

lactose content = 6·19 − 13·5 × chloride content.

The freezing point of the milk was not changed by either drug.

5. The calcium, sodium, potassium, magnesium, protein, creatine and riboflavin contents of the milk were not demonstrably affected by either thyroxine or thiouracil. The creatine content of milk decreased with advancing lactation. Creatine phosphate was not found in the milk.

6. Thyroxine decreased the ascorbic acid content of the milk by 25 %, while thiouracil caused an increase of 24 %. When the dosage of thiouracil was halved, the increase was only 10 %.

7. Thyroxine did not affect the total aneurin content of the milk, but thiouracil caused a small but statistically significant decrease. Thyroxine increased markedly the cocarboxylase content of the milk, while a small but statistically significant increase also occurred in the protein-bound aneurin fraction. These increases were mostly at the expense of free aneurin which was decreased by thyroxine injection. Thiouracil changed the partition of aneurin in the opposite way. There were close negative correlations between phosphatase and cocarboxylase. Likewise there were close negative correlations between phosphatase and protein-bound aneurin. The correlations between phosphatase and free aneurin were positive. These correlations were found in all cows, irrespective of treatment with thyroxine or thiouracil. Implications of these correlations in the biochemistry of milk secretion have been discussed.

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# Uptake of Potassium Tellurite by a Sensitive Strain of *Escherichia coli*

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As early as 1913 Schurmann & Hajos added potassium tellurite to a differential medium for the isolation of the diphtheria bacillus, and this use of potassium tellurite has now become familiar. Cavazutti (1921) and Joachimoglu (1920, 1922) found that in general Gram-positive organisms were less affected by tellurite than Gram-negative, and that the activity of related compounds decreased in the order $TeO_3^{2-} > SeO_3^{2-} > TeO_4^{2-} > SeO_4^{2-}$. Fleming (1932, 1942) demonstrated the value of potassium tellurite when used with penicillin and gentian violet for the isolation of certain bacteria from badly contaminated sources. Penicillin and potassium tellurite were both active at high dilution on entirely different bacterial species so that, with a few exceptions, bacteria which were penicillin-sensitive were tellurite-resistant and vice versa. Strains of *Escherichia coli* sensitive to tellurite could rapidly acquire resistance by serial subcultivation in increasing concentrations of the salt (Fleming & Young, 1940) and the antigenic and fermentation characteristics of both sensitive and naturally resistant strains of *Esch. coli* appeared to be identical. There was also some evidence of a very high absorption of tellurite by actively growing bacteria. Growth on tellurite agar yields black colonies, and granules can be seen inside the cells which are presumably composed of elementary tellurium (Hewitt, 1951).

In the present communication, as an approach to the means by which potassium tellurite ($K_2TeO_3$) inhibits bacterial growth, the mechanism of assimilation of potassium tellurite by bacterial suspensions is studied by the quantitative determination of the uptake using $^{127}Te$ incorporated into potassium tellurite as radioactive tracer.

Although the tellurium was added to the bacterial suspensions as the alkaline salt $K_2TeO_3$, the true substrate of the assimilation process at pH 5·5 may be $TeO_2$, $H_2TeO_3$ or $HTeO_3^-$. The $pK_a$ values of the two dissociation stages of tellurous acid were found to be 8–9 and > 10, so that only about 0·1 % of $H_2TeO_3$ is ionized at pH 5·5. Whatever the nature of this substrate, however, or of the final chemical form in which Te accumulates in the cells, uptakes and concentrations have been expressed throughout in terms of $K_2TeO_3$.

## METHODS

### *Preparation of radioactive potassium tellurite*

Acidic residues, containing radioactive Te obtained in the preparation of $^{131}I_2$, were supplied by the Atomic Energy Research Establishment, Harwell, with an activity of approximately 5 mc. in 100 ml. Oxalate, sulphate and chromic ions were also present, and elementary Te was slowly precipitated from the acid solution by the addition of 10 g. of $Na_2S_2O_5$ to 50 ml. of residues. After 48 hr. the precipitation appeared to be complete and the Te was separated and washed five times with distilled water. The moist Te was dissolved in a slight excess of 50 % (v/v) $HNO_3$, and the solution was cleared by centrifuging and diluted with distilled water to 6 ml. On addition of 20 ml. of ethanol a flocculent white precipitate of $TeO_2$ appeared. After standing overnight this was washed three times with distilled water in the centrifuge and dried for 24 hr. *in vacuo* over $P_2O_5$. The product was weighed, and the theoretical amount of 1 % (w/v) KOH solution added to form $K_2TeO_3$. The solution was finally diluted to a concentration of 1000 $\mu$g. $K_2TeO_3$/ml.

The overall yields of $K_2TeO_3$ were 100–150 mg., with an initial specific activity of about 0·5 $\mu$c./mg. and a radioactive recovery of 2 %. The half life of this solution corresponded exactly with that of $^{127}Te$ (90 days).