Iodinated compounds in milk after radioiodide administration

Radioiodide administered to lactating animals appears in high concentration in the milk[1]. Inorganic iodide is the major constituent but protein-bound radioiodine is also found[1,2]. In order to study the nature of this protein-bound fraction, rats and rabbits at about the tenth day of lactation were injected subcutaneously with 500–750 μc of carrier-free ^{131}I. Milk and plasma samples were obtained 3 to 4 h later as previously described[1]. Small amounts of thiouracil or thiosulphate were added to all samples immediately after collection to prevent oxidation of iodide and hence the possibility of iodination *in vitro*. Ascending paper chromatograms (Whatman No. 1) of both milk samples and acid–butanol extracts[3] of milk before and after hydrolysis with trypsin at pH 8.5 were prepared in various solvents:

Butanol–dioxane saturated with 2 *N* NH_4OH (4:1:5).
Butanol saturated with 2 *N* acetic acid.
tert.-Amyl alcohol saturated with 2 *N* NH_4OH.

Chromatograms were autoradiographed or scanned with an automatic recording strip-counter. The positions of carriers were determined by spraying with $PdCl_2$ for iodide and diazotised sulphanilic acid for iodinated amino acids[4].

The only substances identified in chromatograms of unhydrolysed skim milk were iodide and an iodine-containing compound which remained at the origin and was therefore thought to be protein. In rabbits, about 15% of the activity was in this fraction and in rats about 40%, which corresponded to the proportion of the total activity precipitated by 10% trichloroacetic acid. In a rabbit treated with thiouracil before injection of ^{131}I only iodide was present in the milk and the thyroid gland. Chromatograms of hydrolysed milk did not show significant amounts of radioactivity at the origin but two peaks other than iodide were observed. The larger of these was found in the position of carrier monoiodotyrosine (MIT). The second peak corresponded approximately with the position of the added carrier thyroxine (T_4). No consistent evidence of radioactivity in the positions of carriers diiodotyrosine (DIT) or 3,5,3′-triiodothyronine (T_3) was obtained. Preparatory chromatograms were run of hydrolysed milk extracts and the areas corresponding to MIT + DIT and T_4 + T_3, eluted and re-chromatographed both in one and two dimensions.

The identification of radioactive MIT was confirmed but DIT was not found. Eluates from the T_4/T_3 area did not yield thyroxine but two other radioactive spots were observed. The nature of these substances is unknown. Compounds with similar mobilities in the three solvents, namely 3,3′,5-triiodothyronine, 3,3′-diiodothyronine, tetraiodothyroacetic acid, and tetraiodothyropropionic acid, did not correspond with either spot.

These compounds are thought to be formed in the mammary-gland tissue or in the milk *in vivo* for the following reasons. Firstly, except in the case of one rat which received 750 mc of ^{131}I and in which a small amount of a material (? thyroglobulin) remaining at the origin of the chromatograms was observed, only iodide was found on chromatograms prepared from plasma of the experimental animals. Moreover, even after destruction of the rat thyroid by ^{131}I, free MIT does not appear in the blood[5]. Secondly, the most likely source of these compounds would be the thyroid gland, yet in one rabbit which was surgically thyroidectomized 3 days before the administration of ^{131}I, the usual compounds were found in the milk. Thirdly, MIT is known to have a relatively short half-life in the blood and to be rapidly deiodinated after injection[6]. Fourthly, ROCHE *et al.* have described very similar findings after incubation of milk with ^{131}I *in vitro*[7].

The possibility of the transfer of MIT or DIT from blood to milk assuming that they might perhaps be present in blood in such low concentration as to be undetectable was not examined directly but that of another compound (T_4) was. A lactating rabbit received 20 μc (approximately 1 μg) of ^{131}I-labelled T_4 intravenously and serial samples of milk and plasma were obtained over the next 6 h as described[1]. Slowly increasing amounts of ^{131}I were found in successive milk samples. At 3 h after injection the level was equal to that in plasma and at 6 h was 1.5 times the plasma concentration as compared with the 20–30 times concentration gradient observed as early as 45 min after injection of radioiodide[1]. The radioactivity in the milk in the present experiment was thought to be due to the contamination with radioiodide (8%) of the injected labelled T_4 and to the progressive deiodination of T_4 which is known to occur in the rabbit[8]. Chromatograms indicated that although in the plasma 5–15% of the activity was iodide and the rest thyroxine, only iodide and a small amount of MIT could be detected in milk.

The conclusions drawn from these experiments are that the trichloracetic acid precipitable, non-dialysable fraction of the radioactivity found in rat and rabbit milk after radioiodide administration represents an iodine-containing protein (S) which is formed in the glands or in the milk. On enzymic hydrolysis it yields MIT and at least two other labelled compounds which do not appear to be any of the known naturally occurring iodinated amino acids or their derivatives.

Similar findings have been reported previously for the rat[2] and the dog[9] but in ruminants (goat[10] and cow[11]) only iodide has been observed in the milk after ^{131}I administration. No metabolically active compounds have been chromatographically detected in milk. This is in agreement with previous results based on biological testing[12].

The relative scarcity or absence of DIT in the presence of large amounts of MIT in milk is puzzling; enzymic deiodination of DIT in the body does not appear to stop at the MIT stage[6]. Failure to iodinate beyond the MIT stage appears to be a more likely explanation and may be simply the consequence of an excess of tyrosine with respect to the available iodine. However, it is of interest that an excess of MIT over DIT appears to be characteristic of the iodinated proteins formed by several "primitive" or "abnormal" biological iodinating systems such as the lactating breast *in vivo*, cell-free preparations of functioning mammary tissue or thyroid *in vitro*[13,14], the developing chick thyroid[15], carcinomas of the thyroid[16,17] and certain adenomas of the thyroid[18].

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1 K. BROWN-GRANT, *J. Physiol.*, 135 (1957) 644.
2 G. D. POTTER AND I. L. CHAIKOFF, *Biochim. Biophys. Acta*, 21 (1956) 400.
3 J. GROSS, *Brit. Med. Bull.*, 10 (1954) 218.
4 J. GROSS AND C. P. LEBLOND, *Endocrinology*, 48 (1951) 714.
5 W. TONG, A. TAUROG AND I. L. CHAIKOFF, *J. Biol. Chem.*, 195 (1952) 407.
6 J. B. STANBURY, A. A. H. KASSENAAR, J. W. A. MEIGER AND J. TERPSTRA, *J. Clin. Endocrinol. and Metabolism*, 16 (1956) 735.
7 J. ROCHE, R. MICHEL AND E. VOLPERT, *Compt. rend. soc. biol.*, 148 (1954) 21.
8 K. BROWN-GRANT AND J. G. GIBSON, *J. Physiol.*, 127 (1955) 341.
9 L. VAN MIDDLESWORTH, *J. Clin. Endocrinol. and Metabolism*, 16 (1956) 989.
10 W. E. WRIGHT, J. E. CHRISTIAN AND F. N. ANDREWS, *J. Dairy Sci.*, 38 (1955) 31.
11 R. F. GLASCOCK, *J. Dairy Research*, 21 (1954) 318.
12 K. L. BLAXTER, *Vitamins and Hormones*, 10 (1952) 217.
13 A. TAUROG, G. D. POTTER, W. TONG AND I. L. CHAIKOFF, *Endocrinology*, 58 (1956) 132.
14 A. TAUROG, G. D. POTTER AND I. L. CHAIKOFF, *J. Biol. Chem.*, 213 (1955) 119.
15 J. B. TRUNNELL AND P. WADE, *J. Clin. Endocrinol. and Metabolism*, 15 (1955) 107.
16 J. R. TATA, J. E. RALL AND R. W. RAWSON, *J. Clin. Endocrinol. and Metabolism*, 16 (1956) 1554.
17 P. G. STANLEY, *Biochem. J.*, 63 (1956) 581.
18 R. PITT-RIVERS, D. HUBBLE AND W. H. HOATHER, *J. Clin. Endocrinol. and Metabolism*, in the press.

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Isolation of acetyl peptides from acetylchymotrypsin

During the last few years several papers have been published on the reaction of α-chymotrypsin with diisopropylphosphorofluoridate (DFP). This reaction resulted in a complete inhibition of the enzymic activity and was accompanied by the introduction of the diisopropylphosphoryl group in the enzyme molecule (chymotrypsin-DP). SCHAFFER *et al.*[1] have demonstrated that upon acid hydrolysis of chymotrypsin-DP, the phosphorus moiety of DFP was present in the peptide chain Gly·Asp·phospho Ser·Gly. This sequence could be extended to Gly·Asp·phosphoSer·Gly·Glu. These results were partially confirmed[2]. We found that upon proteolytic digestion of chymotrypsin-DP a peptide could be isolated which contained a diisopropylphosphoryl group substituted to the hydroxyl group of a serine residue[3–5]. The amino-acid sequence in this peptide was shown to be Gly·Asp·Ser·Gly·Gly·Pro·Leu. These results indicate that the attack of DFP on the enzyme is directed against the hydroxyl of a serine residue which is occurring in the peptide sequence Gly·Asp·Ser·Gly.

There is good reason to believe that the site on the enzyme which combines with DFP is identical to that part of the enzyme where substrate hydrolysis is performed[6]. Moreover, it is attractive to consider related mechanisms to govern substrate hydrolysis and inhibition by DFP. In the former case a labile acetyl–enzyme complex is assumed to be formed which breaks down rapidly in water; in the latter case an analogous but stable enzyme–DP complex is formed. These