Fig 1 The pathways of iodine metabolism in the thyroid
iodoprotein normally present in thyroid gland. The chemical characteristics of purified Tg have been extensively reviewed (Edelhoeh and Rall, 1964; Rall et al., 1964).

Thyroglobulin is a tetramer having molecular weight of about 660,000 (Edelhoeh, 1960). Its sedimentation coefficient ($S_{20,W}$) is approximately 19.45 (Derrien et al., 1949) and its isoelectric point is about 4.6 (Gottschalk and Ada, 1956). Thyroglobulin is a glycoprotein consisting of about 5650 aminoacid residues of which about 125 are tyrosyl units (Edelhoeh and Rall, 1964). Gottschalk and Ada (1956) reported that carbohydrate contents of Tg is about 9 per cent by weight, most of this carbohydrate is accounted for as glucosamine and mannose. Iodine content of Tg depends on the iodine intake of the subject. Density gradient centrifugation studies of Inoue and Taurog (1968) with $^{14}$C or $^{131}$I labelled thyroproteins revealed that the structure of Tg is influenced by the extent to which it is iodinated.

Thyroid Peroxidase and Organification of Iodine:

Halmi and Pitt-Rivers (1962) showed by radiiodine tracer techniques that iodide taken up by thyroid tissues is rapidly incorporated into Tg, so that free iodide itself normally constitutes less than 1 per cent of total intrathyroidal iodine. It is generally believed that for the iodination of Tg, iodide taken up by the thyroid must
first be converted to a more oxidized form, such as $I^0$, $I^+$, $I^-$, or $IOH_2^+$. $H_2O_2$ has been proved to be an obligatory biological oxidant for the oxidation of iodide to any one of these forms before its incorporation into organic molecule.

Studies over the past 30 years by various groups of workers indicate that $H_2O_2$ dependent oxidation of iodide might be enzymatic and the enzymes classed as peroxidase carry out this function. A substantial body of evidence establishes clearly that the peroxidase activity can be located and partially purified from homogenates of thyroid tissues (DeGroot and Davis, 1962; Yip, 1965; Mahoney and Igo, 1966; Hosoya and Morrison, 1967; Covel and Taurog, 1967; Taurog et al., 1970). These enzymes seem to be firmly associated with the insoluble elements of the cell, but they have been obtained in soluble forms by extracting the insoluble fractions with detergents or detergents and proteolytic enzyme(s) (DeGroot and Davis, 1962; Alexander, 1965; Yip, 1965; Mahoney and Igo, 1966; Hosoya and Morrison, 1967; Covel and Taurog, 1967). Extracted enzymes, after further purification by ammonium sulfate precipitation and column chromatography, seem like other peroxidases, to be heme-proteins (Yip, 1966; Hosoya and Morrison, 1967). Recent histochemical studies of Strum and Karnovsky (1970) indicate that the thyroid peroxidase...
is widespread within the cell and found to be distributed in the Golgi apparatus, the apical cell membrane and on the endoplasmic reticulum.

Tyrosine iodination can occur without the presence of a specific tyrosine iodinase, following peroxidation of iodide. In fact there is no firm evidence for the presence of tyrosine iodinase in the thyroid. The relative specificity of iodination for $T_G$ may be occasioned more by the presence of high concentrations of non-iodinated thyroglobulins as acceptor protein, rather than the presence of a specific enzyme mediating iodination or carrying reactive iodine to thyroglobulin.

PEROXIDASES

Peroxidases and catalases are collectively known as "hydroperoxidases", a group of hemoproteins. As early as 1855, Schönbien observed that the oxidation of certain organic compounds by dilute solutions of $H_2O_2$ could be catalyzed by 'substances' occurring in plants and animals, but he regarded both the 'activation' and the decomposition of the $H_2O_2$ as being due to the same 'substance'. It was not until the end of the last century that the name 'peroxidase' was coined by Linossier who in 1898 detected an oxidase-free preparation of peroxidase from pus. For many years, the distinction between the
peroxidases (enzymes that normally catalyze oxidations involving H$_2$O$_2$) and the catalases (enzymes which, under normal biological conditions, can cause the decomposition of H$_2$O$_2$ to oxygen and water) was not realized. Keilin and Hartree (1936, 1945) observed that catalase can act as a peroxidase in certain special circumstances and so they suggested that this peroxidative activity of catalase may perhaps represent a major biological function of this enzyme. Conversely, when high concentrations of H$_2$O$_2$ are present, peroxidase loses its peroxidative activity, but still can act as catalase (Keilin and Mann, 1937).

Pathways of Peroxidase Catalyzed Reactions:

A number of attempts beginning from 1940 had been taken to elucidate the mechanisms of peroxidase catalyzed reactions (Chance, 1943; 1949; 1949a, 1949b; 1950; 1951; George, 1952; 1953; 1953a; 1953b; Yamazaki et al., 1960, Bjorksten, 1970). Most of the studies to investigate the mechanisms of peroxidatic reactions are carried out with horseradish peroxidase (HRP) and cytochrome C peroxidase. All of them are believed to be capable of forming one or more of a series of intermediate complexes - identifiable by their optical and magnetic properties - in the course of their reaction with peroxide and donor.
Peroxidases catalyze the overall peroxidative reaction:

\[ \text{H}_2\text{O}_2 + 2e + 2\text{H}^+ \rightarrow 2\text{H}_2\text{O} \]

where \(2e\) represents two reducing equivalents. A wide variety of substances such as phenols, aromatic amines, enediols like ascorbic acid are capable of supplying reducing equivalents. As mentioned earlier peroxidase in resting state has an oxidation state of +3. Upon addition of one molecule of \(\text{H}_2\text{O}_2\), a green product, compound I, is formed. This substance has an oxidation state which is two equivalents higher than that of ferriperoxidase, that is +5. The electronic location of the five reducing equivalents in compound I is not known. Compound I is very unstable and readily interacts with the hydrogen donor forming the compound II which is pink in colour and have the oxidation state +4. The entire cycle may be summarized as follows:

\[ \text{Fe}^{3+} \rightarrow \text{Compound I} \quad \text{(oxidation state: +5)} \]

\[ \text{Compound I} \rightarrow \text{Compound II} \quad \text{(oxidation state: +4)} \]

\[ \text{Sum:} \quad 2\text{AH}_2 + \text{H}_2\text{O}_2 \rightarrow 2\text{AH}^\prime + 2\text{H}_2\text{O} \]
Hence, peroxidase catalyzes one-equivalent transfers from substrate and produces free radicals as primary product. Electronic resonance spectroscopy of the oxidation of substrates by peroxidase and H₂O₂ has established this mechanism conclusively. The free radical products then react according to their individual chemistries by dimerizing, disproportionating or undergoing further oxidation.

\[
dimerization: \quad AH + AH \xrightarrow{\text{dimerization}} AH - AH
\]

\[
disproportionation: \quad 2AH \xrightarrow{\text{disproportionation}} A + AH_2
\]

\[
further oxidation: \quad AH \xrightarrow{\text{further oxidation}} A + e^- + H^+
\]

Inhibitors of Peroxidase:

Inactivation of peroxidase may be brought about by various ways among the inhibitors, substances which can become more or less firmly (and sometimes reversibly) attached at the sixth coordination position of the iron atom in the prosthetic group of the enzyme are widely studied. This is the case for the inactivation of peroxidases by an excess of H₂O₂ (formation of enzymatically inactive 'Compound III'), and by cyanide, sulphide and fluoride, where spectroscopically distinct compounds are formed. In general, other ions have a much lower inhibitory power on peroxidase. However, bromide (Keilin, 1936, Mitsui, 1954), azide (Keilin, 1936), ferrocyanide, hydroxylamine,
phenylhydrazine (Ebihara, 1939) and thiocyanate (Morita, 1954) reduce the activities of various peroxidases, probably, as a result of coordination at the iron atom of the prosthetic group. The inhibitors of peroxidase have been reviewed in details by Saunders et al. (1964).

Sources of Peroxidase:

Peroxidases are evenly distributed throughout the nature. The richest sources of plant peroxidases are the roots of horseradish (Sumner and Howell, 1936) and the sap of the fig tree (Michlin, 1956). In vertebrates peroxidase has been identified in thyroid gland (Dempsey, 1944; DeRobertis and Grasso, 1946; Ljunggren, 1957; Alexander, 1960; Bhattacharya and Datta, 1970; Haldar and Datta, 1972) salivary glands (Branchet and Gener, 1944; Arvy et al., 1957; Alexander, 1959; Thomson and Morell, 1967; Hati et al., 1968; Bal et al., 1972; Mahajani et al., 1973), milk (Elliott, 1932), leucocyte (Agner, 1958), thymus and ovary (Branchet and Gener, 1944), and uterus (Martin et al., 1958) etc. Abrams, Altschul and Hogness (1942) characterized cytochrome C peroxidase in yeast and, Shaw and Hager (1961) isolated chloroperoxidase from Caldariomyces fumago. Among these a few have been purified to homogeneity and their physico-chemical properties extensively investigated. Table 1 shows
the properties of various peroxidases.

Table 1

Properties of Various Peroxidases

<table>
<thead>
<tr>
<th>Peroxidase</th>
<th>Mol.Wt.</th>
<th>Heme/mole</th>
<th>Maximum Soret peak</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactoperoxidase</td>
<td>78,000</td>
<td>1</td>
<td>412, 438</td>
<td>Morrison et al., 1957; Rombauts et al., 1967; Dolman et al., 1968</td>
</tr>
<tr>
<td>Thyroid peroxidase</td>
<td>160,000</td>
<td>3</td>
<td>412, 425</td>
<td>Hosoya and Morrison, 1967</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>149,000</td>
<td>2</td>
<td>430, 475</td>
<td>Agner, 1958; Shultz and Kaminker, 1962</td>
</tr>
<tr>
<td>Horseradish peroxidase</td>
<td>40,000</td>
<td>1</td>
<td>403, 440</td>
<td>Keilin and Hartree, 1951</td>
</tr>
<tr>
<td>Chloroperoxidase</td>
<td>42,000</td>
<td>1</td>
<td>400, 409</td>
<td>Morris and Hager, 1966</td>
</tr>
<tr>
<td>Cytochrome C peroxidase</td>
<td>49,000</td>
<td>1</td>
<td>408, 438</td>
<td>Yonetani and Ray, 1965</td>
</tr>
<tr>
<td>Submaxillary peroxidase</td>
<td>40,000</td>
<td>2</td>
<td>406, 422</td>
<td>Bal et al, 1972</td>
</tr>
</tbody>
</table>
Peroxidase Catalyzed Iodination Reactions:

The ability of peroxidases to catalyze the oxidation of iodide is well known and seems to be ubiquitous to hemiprotein peroxidases. Most studies of peroxidase catalyzed - iodination, consider this class of enzymes as a redox catalyst for iodide oxidation, and little attention has been given to the possible mechanism of non-specificity in regard to the substrates to be iodinated by the enzyme.

There are two schools of thoughts regarding the enzymatic iodination. According to DeGroot and Davis (1961) two separate enzymes namely, peroxidase and iodinase are involved in enzymatic iodination reactions while the other school (Taurog and Howells, 1966; Hosoya et al. 1962) believes that peroxidases catalyze not only the peroxidation of iodide to "active iodine", but it can also catalyze the incorporation of active iodine into tyrosine or other substrates.

Morrison et al. (1970) proposed that iodination involves what might be thought of as two sites: the heme prosthetic group, and a second site. This second site may be a functional group on the enzyme, or it may simply be a binding site for the compound which is iodinated. In the case of thyroid peroxidase (Yip, 1965), it is possible to separate the ability to catalyze the iodination reaction while the guaiacol oxidation activity is unaffected. Morrison et al. (1970) found that when the enzyme is treated with trypsin for 1 hour digest at a concentration of trypsin/peroxidase (1:10) at 25°, there is 57 per cent loss of the ability
to catalyze iodination reaction but no loss in the ability to catalyze oxidation of guaicol.

The concept of the participation of a single enzyme to carry out both peroxidative and iodinating functions, put forward by Alexander and Corcoran (1962), has gradually gained ground. Recent studies with the purified peroxidases like lactoperoxidase (LP), horseradish peroxidase (HRP), myeloperoxidase (MP), thyroid peroxidase and submaxillary peroxidase (Björksten et al., 1963; Hosoya and Morrison, 1967; Taurog, 1970; Morrison and Bayse, 1970; Bayse and Morrison, 1971; Critchlow and Dunford, 1972; Mahajani et al., 1973) clearly indicate that hemoprotein peroxidases have both peroxidative as well as iodinase activities. Using LP as a model iodinating enzyme Morrison and Bayse (1970) concluded that the enzyme has two active sites, one is halogenation site and the other is peroxidative site. The halogenation site may be equivalent to a trans-iodination site in the enzyme. This would be comparable with the enzyme activity labeled "iodinase". As in the absence of peroxide LP does not iodinate tyrosine even in the presence of $I_2$ or $I^-$, Morrison and Bayse (1970) concluded that the enzyme does not have "iodinase" activity.

Mechanism of Iodination:

Peroxidase can participate in iodination reactions, and equations (a - c) have been proposed.
a. \[ \text{H}_2\text{O}_2 + \text{I}^- \rightarrow \text{"oxidized I"} \]
\[ \text{I}^- \rightarrow \text{I}^+ + e \]
\[ \text{I}^- \rightarrow \text{I}^+ + 2e \]

b. \[ 2\text{I}^+ + \text{I}^- \rightarrow \text{I}_3^- \]
\[ \text{I}^+ + 2\text{I}^- \rightarrow \text{I}_3^- \]

c. \[ \text{"oxidized I"} + \text{HO-} \rightarrow \text{HO-} \]

The peroxidase is involved in the oxidation of iodide as illustrated in reaction a, although the mechanism of oxidation is unknown. Iodide may be oxidized by a one or a two electron transfer process, and in either case, \( \text{I}_3^- \) would be produced (equation b). The role of peroxidase in iodination, according to the above scheme, is simply to oxidize iodide and the phenolic compound. Thus the overall iodination reaction, involving at least three substrates, is unquestionably a multistep process. Hence, the kinetics of the peroxidase-catalyzed iodination are complex. A number of investigators (Frieden, 1959; Celand, 1963; Fromm, 1967) worked on the problem of multisubstrate reactions and developed equations which made it possible to derive information concerning the mechanism of catalysis from initial rate studies. Kinetic studies of Morrison and Bayse (1970) with \( \text{LP}^+ \) indicate that a mechanism is operative which does not require that substrates, peroxide, iodine and tyrosine, be present on the enzyme simultaneously. This observation is
consistent with a mechanism in which the enzyme is first oxidized to the relatively stable forms of peroxidases produced by the interaction of peroxide and the enzyme. Maloof and Soodak (1963) presented evidence that iodination proceeds through intermediate formation of sulphenyl iodide. DeGroot (1965) pictured this view as follows:

\[
\begin{align*}
\text{Protein} & \quad \text{I}^- + \text{H}^+ \\
S & \quad \text{S} \\
\rightarrow & \\
\text{Protein} & \quad \text{SH} \quad \text{SH} \\
\text{Tyrosine} & \\
\text{Peroxidase} & \quad \text{Protein} \\
\rightarrow & \\
\text{Monoiodotyrosine} & \\
\end{align*}
\]

Fawcett (1966; 1968) also found the evidence for the involvement of sulphenyl iodide while studying the iodination of tyrosine. Studies with soluble preparation of thyroid peroxidase and myeloperoxidase (MP) (Gross and Sizer, 1959; Yamazaki et al., 1960, Yamazaki and Piette, 1961; Yip and Hadley, 1966) indicate that the enzyme makes direct peroxidatic attack to generate free radical which is then secondarily iodinated. On the basis of this idea iodination of tyrosine is believed to occur via free radical mechanism according to the scheme:
IODINE METABOLISM IN SUBMAXILLARY GLAND

The knowledge of the structure and function of salivary glands is essential in understanding the nature of saliva. Much of the information about salivary function has been obtained from investigations in animals. Two types of cells, i.e., serous and mucous, have been detected in the three pairs of salivary glands. Submaxillary and sublingual glands are called mixed glands because they contain both the types of cells. But mucous type of cells are seldom present in the parotid gland. Both sympathetic and parasympathetic nerves innervated the salivary glands (Garret, 1966; 1966a; Fritz, 1971).
Endocrine Function of Salivary Glands:

Godolowski and Calandra (1960) showed that submaxillary and possibly sublingual elaborate a factor which inhibits the action of insulin. Katagiri and Higashijo (1940) observed the atrophy of testis and hypertrophy of uterus and adrenal cortex on ligation of rat parotid gland ducts and extirpation of the submaxillary gland. A detailed report on biochemical aspects of a purified salivary glands' hormone called 'parotin' has been published by Ito (1954; 1960). Fleming (1960) studied the effect of parotin in mice.

Influence of Endocrine Glands on Salivary Glands:

Lacassagne (1940; 1940a) observed that salivary glands of female mouse assumed the histological characteristics of that of male when the male sex hormone was administered to a female mouse. Buillard and Delsuc (1941) reported the atrophy of salivary glands on castration. Testosterone injection in mice resulted hypertrophy of tubules (Reynaud, 1950) while gonadectomy caused decrease in the weight of submaxillary gland (Atkinson et al., 1959). Influence of other endocrine glands on salivary glands has been published by Lacassagne and Chamorrow (1940).

Thyroidectomy (Arvy and Gave, 1950), administration of thiourea (Arvy et al., 1950) and hypophysectomy (Lacassagne and Chamorrow, 1940) induced atrophy of the
submaxillary gland. Eartly and Leblond (1954) found that administration of thyroxine alone to hypophysectomized animals do not restore the submaxillary gland to its normal state, although thyroxine alone in thyroidectomized state would bring the submaxillary gland in its normal state. They further suggested that the granular tubules of submaxillary gland might be under the control of a hypophyseal factor. Grad and Leblond (1949) had reported previously that the simultaneous injection of thyroxine and testosterone was more effective in restoring the gland to its normal condition in thyroidectomized animals than either of hormones alone would do so. Fawcett and Kirkwood (1954) suggested that the salivary glands control the blood thyroxine level and iodine pool of thyroid gland by deiodinating thyroxine and its analogs via saliva and gastrointestinal tract. But Ruegamer (1955) found no deiodinating activity in dog's salivary glands. Taurog and his co-workers (1959) have suggested that the submaxillary iodide pump unlike that of thyroid is not affected by thyroid stimulating hormone (TSH). Fluorescent dye technique studies by Leonara and Steinman (1968) suggest that there might exist a hypothalamic - parotid gland endocrine axis.

Perioxidase and Iodinase Activities Of Submaxillary Gland:

The presence of perioxidase and tyrosine iodinase activities in rat submaxillary gland has been demonstrated by Fawcett and Kirkwood (1954a). They also found that the
enzyme is that it is strongly stimulated in the presence of iodide. Another peroxidase having no iodinating activity has been purified to homogeneity from goat submaxillary gland by Bal et al. (1972).

Effect of Cold Stress on Submaxillary Peroxidase:

Cold stress is known to cause a profound alteration of cell metabolism. Chauhan et al. (1969; 1971) and Ghosal et al. (1972) demonstrated that protein synthesis in brain, heart and liver of rat increased significantly when the animals were exposed to cold. Bhattacharyya et al. (1972) studied the effect of cold stress on submaxillary peroxidase activity in both normal and thyroidectomized rats. They observed that when normal rats were exposed to $3^\circ + 1^\circ$C for 5 hours, peroxidase activity of submaxillary gland increased up to about 125 per cent. The stimulation of the enzyme activity was independent of sex. However, no change in peroxidase activity was observed when thyroidectomized rats were subjected to cold stress. This group of workers also observed that the stimulation of peroxidase activity under cold stress was abolished when inhibitors of RNA synthesis i.e. actinomycin D and rifampicin were administered, indicating that the increased enzyme activity was probably mediated through increased synthesis of the enzyme itself. The foregoing discussion clearly indicates that thyroid controls the submaxillary peroxidase activity through protein synthetic machinery. Hence a brief review on the effect of
thyroid hormones on generalized protein synthesis in different tissues is relevant and discussed in the following few pages.

THYROID HORMONES AND PROTEIN BIOSYNTHESIS

The high rate of protein turnover in animal cells makes regulation of protein synthesis a sensitive focal point of hormonal influence. The survival time for proteins may vary from the long-lived hemoglobins to the rapidly replenished inducible liver enzymes. Observations made some years ago revealed a good correlation between the circulatory level of many hormones and the total protein content of the target tissues. This was particularly obvious in the case of mammalian sex hormones acting on the accessory sexual tissues and certain muscles (Mueller et al., 1958; Kassenaar et al., 1962; Kochakian, 1965; Frieden, 1964) and growth hormone on liver and muscle (Geschwind et al., 1950; Gray and Young, 1954).

Thyroid hormones, thyroxine (T4) and triiodothyronine (T3) are classed as anabolic hormones and have shown to stimulate amino acid incorporations in liver, kidney, heart, and muscles (Tata et al., 1963; Michels et al., 1963; Sokoloff et al., 1964; Brown, 1966; Scow and Hagan, 1965; Buchanan and Tapley, 1966). T4 and T3 are also reported to increase the protein synthesis in vitro (Sokoloff and Kaufman, 1959; Sokoloff et al., 1964; Brown, 1966). Recently Baudry et al. (1971) observed that thyroidectomy decreases the biological half-life of mitochondrial proteins, especially those of their internal
membrane and plasma proteins and it has little influence on the other subcellular fractions (nuclei, microsomes, plasma membranes).

Tonoue and Yamamoto (1967; 1967a; 1967b; 1968) studied the effect of thyroidectomy and T₄ administration on the transport of α-aminoisobutyric acid -¹⁴C into pituitary cells, incorporation of ¹⁴C-leucine, alanine and glycine into the proteins of rat pituitary. The results of their experiments demonstrate that thyroidectomy causes stimulation of amino acid transport and incorporation into pituitary proteins. The stimulatory actions were suppressed by a single dose of thyroxine injection. Tonoue and Yamamoto (1968) also found that ¹⁴C-alanine incorporation into pituitary proteins of rat is inhibited even if thyroxine is added in vitro. Similar results were obtained by Matsuzaki and Shigeru (1971). These observations and earlier findings of Tata and Sokoloff suggest that the same hormone may have different action on different tissues. Tata (1964; 1966; 1968) and Yamamoto and Tonoue (1967) have described the probable mechanisms of action of thyroid hormones in terms of molecular genetics.

Selective Induction of Specific Proteins:

Over-all stimulation of amino acid incorporation does not always explain the biological specificity of different hormones even they were acting on the same tissue. Tipton and Nixon (1946) found succino oxidase and Cytochrome C oxidase
activities to be elevated in livers of hyperthyroid rats, and Drubkin (1950) obtained similar results for cytochrome C. Green (1959) has reported that thyroxine increases electron transport enzymes and those associated with mitochondrial membranes. Lee et al. (1959) and Lardy et al. (1960) observed the increased mitochondrial \( \alpha \)-glycerophosphate dehydrogenase activity in livers of thyroxine-treated rats. Thyroid hormones are also reported to increase the levels of carbamylphosphate synthetase (Paik and Cohen, 1960; Tatibana and Cohen, 1964; 1965), cathepsin (Weber, 1963; Tata, 1966a) collagenase (Gross, 1964) and \( \beta \)-glucoronidase (Kubler and Frieden, 1964) in tadpoles.

Roche et al. (1962) have reported the stimulation of amino acid uptake in mitochondria-free liver preparations by thyroxine but not by triiodothyronine. Bronk (1963) on the other hand presented evidence that thyroxine stimulates amino acid incorporation in isolated mitochondria. Carneiro and Sesso (1963) observed thyroxine injection leads to increase uptake of \( ^3H \)-phenylalanine and \( ^3H \)-tryptophan in liver but decreased uptake in villi, jejunum, tongue, stomach and cerebellum.

Alteration of cytoplasmic protein synthetic activity is preceded by changes in a variety of nuclear and cytoplasmic functions concerned with RNA synthesis and utilization. Stimulation of RNA synthesis in the hepatic nuclei was regarded as one of the primary events of \( T_3 \) action. Observations of Widnell and Tata (1963) focussed
the accelerated synthesis and turnover of nuclear and cytoplasmic RNA during the latent period of action of thyroid hormones. In reflection of these changes observed earlier, DNA dependent RNA polymerase activity in hepatic nuclei (Widnell and Tata, 1963) and mitochondria (Gadaleta et al. 1972; 1975) are also found to be stimulated by thyroid hormones. The mechanism of the stimulatory action of thyroid hormones on RNA polymerase is not known although suggestions based on indirect evidence have been made. Sokoloff et al. (1964), on the basis of their experimental observations, concluded that thyroid hormones have no effect on RNA synthesis. Recently Tonoue and Yamamoto (1968) observed that stable RNA : protein ratio or RNA : DNA ratio in pituitary of rat is increased on thyroidectomy but this increase is not suppressed by a single dose of thyroxine. The increased RNA synthesis in pituitary is in contrast to the hepatic RNA synthesis which is decreased by thyroidectomy.

EFFECTS OF INHIBITORS OF RNA AND PROTEIN SYNTHESIS ON HORMONAL ACTIVITY

The relatively early stimulation of RNA synthesis does not necessarily imply that the RNA newly formed under the influence of hormone is responsible for the biological activity of the hormone. However, such a view has received support from the numerous observations made on the suppression of hormonal activity by inhibitors of RNA and protein synthesis.
Actinomycin D and puromycin have been the two most commonly used inhibitors, the former because of its action on DNA-dependent RNA synthesis (Goldberg and Rabinowitz, 1962; Reich and Goldberg, 1964) and latter because of its action on polypeptide chain assembly. 5-Fluorouracil, an antimetabolite by virtue of being incorporated into RNA, and cycloheximide, an inhibitor of protein synthesis at the ribosomal level are also used.

Inhibitory effects do not indicate the site of action of the hormone. Inhibition of hormonal effect by actinomycin D has been interpreted as the proof for a direct hormonal involvement in the transcription process. For instance the antagonistic effect of this antibiotic in ecdysone-induced puffing of insect salivary glands (Clever, 1964) has been used as evidence to confirm the theory of direct action of the hormone on the gene (Karlson, 1963). Although actinomycin D inhibits the enhancement by cortisone of RNA synthesis and enzyme induction, the increased protein synthesis by rat-liver polyribosomes following adrenalectomy is not affected by actinomycin D. This led Breuer and Davis (1964) to conclude that cortisone and actinomycin D do not act at the same site. Conclusions drawn from inhibitions can lead to confusion especially when they relate to hormones with multiple actions, since it has not been established whether the multiplicity stems from a primary action at a single site or represents several independent actions. This
qualification is not valid for those cases where all the major physiological actions of hormones (i.e. estrogen, testosterone, thyroid hormones) are abolished by inhibitors of RNA and protein synthesis.

Inhibitors of RNA and protein synthesis themselves may have multiple sites of action or produce identical effects through different mechanisms. Actinomycin D, which was initially used as an inhibitor of mRNA synthesis (Reich and Goldberg, 1964) is now found to be an even more potent inhibitor of rRNA synthesis and ribosome formation in a variety of systems (Perry, 1963; Franklin, 1963; Lindeberg and Persson, 1972). According to Raacke (1971) and Fukuhara (1965) puromycin and cycloheximide, inhibitors of cytoplasmic protein synthesis also inhibit the synthesis or maturation of ribosomes, almost certainly through more than one mechanism.

Variations in the experimental conditions used for producing inhibition may account for many of the discrepancies. There are a few reports describing failure to inhibit hormonal effects on RNA and protein synthesis or the physiological activity of the hormones. It is sometimes observed that increasing the dose of the hormone could partially overcome the inhibition by actinomycin D but similar cases have not been reported for puromycin (Datta and Sen, 1965; Tata, 1966). It has been observed earlier that at high
doses (upto 5 mg per rat), actinomycin D has also been found to inhibit protein synthesis by causing the breakdown of mRNA in the cytoplasm. However, reports of Honig and Rabinovitz (1965) show that the antibiotic may not cause the breakdown of template RNA but may inactivate it by causing some disturbance in glucose metabolism. Puromycin has a strong glycogenolytic effect that is immediate and quite independent of its inhibitory action on protein synthesis at the ribosomal level (Tata, 1964). This antibiotic also causes the swelling of hepatic mitochondria (Grief and Chipkin, 1965) and this might explain the rather abrupt drop (within 1.5 hours) in the BMR of normal and thyrotoxic rats (Weiss and Sokoloff, 1963) Gustafsson et al. (1965). Other authors (Tata et al., 1963; Gustafsson et al. 1965) have reported that the decay of increased respiratory activity following a single administration of thyroid hormone is a very slow process, probably dependent on the long functional lifetime of newly synthesized mitochondria.

These considerations emphasize the need for caution in interpreting the abolition of hormonal activities by inhibitors of protein synthesis. Inhibitory effects in vivo per se do not tell us about the primary site or mechanism of action of the hormones. Nevertheless, if one considers these effects as complementary to other work on hormones and protein synthesis, the inhibitory effects strongly suggest that an undisturbed synthesis of proteins is essential for the expression of physiological activity of many hormones.