Mechanism of Iodide Action on Cholesterol Metabolism

By HELEN B. BROWN, Ph.D., AND IRVINE H. PAGE, M.D.

Iodide retards or prevents hypercholesterolemia and the development of experimental arteriosclerosis in rabbits. "Protection" is estimated from reduction of plasma and/or hepatic cholesterol. Small doses of iodide do not "protect." Iodide in large doses prevents the hypercholesterolemia resulting from exogenous cholesterol and reduces hepatic cholesterol. This effect is independent of the thyroid gland and is not related to "thyroxin-like" plasma iodine. The influence of iodide on cholesterol metabolism seems to be related to the presence of a butanol-insoluble protein-bound iodine compound in the plasma.

IODIDE is almost traditional in treatment of arteriosclerosis. Its use probably began from a confusion of this condition with tertiary syphilis, and continues here and there, although without convincing clinical demonstration. Its modern use depends in part on the fact that iodide inhibits experimental hypercholesterolemia and atherosclerosis in rabbits. The purpose of this study is to explore the mechanism of this action.

A major possibility is that iodide alters thyroid function. Clinically and experimentally, hypothyroidism is associated with hypercholesterolemia and increased incidence of arteriosclerosis, while the reverse seems to be true in spontaneous or induced hyperthyroidism. Consequently, one explanation of iodide protection is that it depends on increased formation of thyroxin-like substances. Less obvious is the possibility that the effect of iodide may be independent of thyroid or thyroid-like functions. Thus, Ungar found that iodide protected thyroidectomized cholesterol-fed rabbits. However, such extrathyroidal protection could not be demonstrated by Turner and Khyatt.

In this study we propose (1) to investigate the role of the thyroid gland in iodide protection, (2) to determine minimum protective iodide dosages and plasma iodine levels consistent with protection, and (3) to correlate the several fractions of circulating iodine with concentrations of serum and hepatic cholesterol, by studies in normal and thyroidectomized cholesterol-fed rabbits.

METHOD

1. Animal Care

New Zealand white rabbits of uniform stock, 15 to 18 weeks of age (2 to 3 Kg. body weight) were selected, kept in individual cages and fed unrestricted amounts of Pratt's Rabbit Pellets. Their drinking water contained no iodine while the pellets yielded intake of 0.2 to 0.3 mg. of iodide daily.

The experiments were grouped in two series and appropriately subgrouped (table 1). Distribution within groups of five or six (experimental) or three or four (control) as to weight and sex were approximately uniform. Supplementary feedings were given six days a week in gelatin capsules.

Series 1 consisted of eight groups of normal rabbits. The experimental groups were fed 0, 1, 10, 20 or 40 mg. of iodide as potassium iodide and, in the first eight weeks, 200 mg. of cholesterol, increased to 400 mg. in the subsequent 15 weeks. Control groups received 0, 1 or 20 mg. of iodide and no cholesterol.

Series 2 consisted of thyroidectomized rabbits (experimental) fed 0, 1 or 40 mg. of iodide with 400 mg. of cholesterol for 10 weeks; control groups received iodide without cholesterol or (one group of normal rabbits) cholesterol without iodide.

The animals were weighed weekly. Blood was taken from ear veins, biweekly at first and triweekly.

* Cholesterol used in these experiments was very kindly furnished by Dr. Augustus Gibson of Merck and Co.
in the later phases of the study. At the end of the experiment the animals were killed by bleeding and autopsies were performed. Liver, spleen, kidneys, heart and aorta were weighed and sampled for Procedure. Six 2 ml. samples of heparinized plasma, pooled by groups, were transferred into 40 ml. centrifuge tubes for protein precipitation (Somogyi\textsuperscript{20}). The supernatant fluid was decanted after centrifugation and used for direct determination of inorganic iodide.\textsuperscript{21}

The separation of fractions was then carried out by a modification of the method of Taurog and Chaikoff.\textsuperscript{22} The protein precipitates were thrice washed with 20 ml. redistilled water until the wash was chloride-free. Two precipitates were used for determination of total protein-bound iodine. The four remaining were used for butanol extraction. Butanol extraction of the acidified precipitate was substituted for direct extraction of plasma, since thyroxin- and diiodotyrosine-like iodine fractions are extractable for acidified hydrolysates of thyroid tissue.\textsuperscript{23} Precipitates were brought to pH 3 to 4 by addition of 10 per cent sulfuric acid (0.5 ml.) and extracted, first with 25 ml. and twice with 15 ml. portions of butanol, by vigorous hand shaking for one and one-half minutes. Successive butanol extracts of each precipitate were decanted and combined.

Two extracts were made alkaline by addition of 0.5 ml. of a 5 per cent sodium carbonate–4 normal sodium hydroxide mixture and butanol removed by distillation under reduced pressure. The last traces of butanol were removed by addition of 90 per cent ethyl alcohol and re-evaporation. These residues were used for determination of total butanol-soluble iodine. The remaining two butanol extracts were treated with the sodium carbonate–sodium hydroxide mixture (50 ml. and 30 ml. in succeeding ex-

### Table 1.—Summary of Experiments

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Sex</th>
<th>Daily Intakes (6 X week)</th>
<th>Mean body wt. Kg. weeks</th>
<th>Group</th>
<th>No. of Sex</th>
<th>Daily Intakes (6 X week)</th>
<th>Mean body wt. Kg. weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chol</td>
<td>Iodine</td>
<td>0</td>
<td>8</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>F</td>
<td>mg.</td>
<td>mg.</td>
<td>2.5</td>
<td>3.2</td>
<td>3.8</td>
<td>M</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2.5</td>
<td>3.2</td>
<td>3.8</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2.7</td>
<td>3.2</td>
<td>3.9</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1</td>
<td>20</td>
<td>2.6</td>
<td>3.2</td>
<td>3.9</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>3</td>
<td>400*</td>
<td>2.7</td>
<td>3.3</td>
<td>4.1</td>
<td>4†</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>2</td>
<td>400</td>
<td>2.5</td>
<td>3.2</td>
<td>4.0</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>2</td>
<td>400</td>
<td>2.5</td>
<td>3.3</td>
<td>4.0</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>3</td>
<td>400</td>
<td>2.6</td>
<td>3.2</td>
<td>3.9</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2</td>
<td>400</td>
<td>2.6</td>
<td>3.2</td>
<td>3.9</td>
<td>8</td>
</tr>
</tbody>
</table>

Age at start, 15-18 weeks

* For eight weeks, cholesterol-fed rabbits received 200 mg. cholesterol daily, then 400 mg. for the following 15 weeks

2. Chemical Methods

Cholesterol determinations were done by the Schoenheimer-Sperry method.\textsuperscript{19} The scheme of analysis of plasma iodine fractions is as follows:

Pooled Heparinized Plasma

\[
\text{ZnSO}_4 \downarrow \text{NaOH} \quad \text{Supernatant Fluid} \leftarrow \rightarrow \text{Protein Precipitate}
\]

INORGANIC IODIDE

\[
\text{Butanol} \quad \text{pH} \quad \text{Extraction} \quad \text{Butanol extract} \quad \text{Residue} \downarrow \rightarrow \text{Aqueous layer}
\]

BUTANOL-SOLUBLE–ALKALI INSOLUBLE IODINE FRACTION (THYROXIN-LIKE)

\[
\text{Butanol-Soluble Iodine Fraction} \quad 5\% \text{Na}_2\text{CO}_3 \quad 4\% \text{NaOH} \quad \text{Butanol-Insoluble Iodine Fraction}
\]

Age at start, 15-18 weeks

Duration of experiment 10 weeks

† Unoperated control rabbits
tractions) and used for determination of butanol-soluble alkali-insoluble iodine. The residue remaining after the butanol extraction (freed from butanol traces by evaporation with 90 per cent ethyl alcohol under vacuum, or by the simpler method of washing twice with ethyl ether) was used for analysis of butanol-insoluble iodine. Iodine-free whole wheat flour was used as organic material in ashing the butanol extracts.

Iodine determinations were done by the method of Barker. The time of ceric sulfate reduction was prolonged from 15 to 30 minutes which gave the same range of color change for 0.01 to 0.05 μg. iodine as for 0.02 to 0.10 μg.

A sample of normal dog plasma was fractionated in parallel with each group of samples as a low iodine (2 μg. per 100 ml.) protein-rich reference.

The entire method was tested by recoveries of iodine compounds added to plasma. Ninety-five to 100 per cent potassium iodide and 92 to 94 per cent thyroxin and diiodotyrosine were recovered when any one or a combination of the three were added to the same plasma. Eighty-five to 90 per cent of added thyroxin was obtained by fractionation with no interference from added potassium iodide or diiodotyrosine. These recoveries are similar to those reported by others.

Contamination of the protein precipitate by plasma inorganic iodide after three washings amounted to 0.5 per cent of the inorganic iodide fraction. This occlusion of inorganic iodide leads to an appreciable error only in the determination of protein-bound and butanol-soluble iodine in the thyroidecomized rabbits fed 40 mg. iodine. Results have been corrected for this error (table 3). Fractionation carried out on three-day dialyzed and undialyzed portions of the same plasma containing 3, 18 and 50 μg. per 100 ml. protein-bound iodine and up to 250 μg. per 100 ml. inorganic iodide showed no differences. Further extraction of the residue with three butanol washings removed no more iodine.

**Results**

1. Tissue Cholesterol Changes and Iodide

Under the conditions of these experiments cholesterol feeding did not produce aortic atherosclerosis, gross or microscopic. Plasma and hepatic cholesterol increased; the cholesterol contents of aorta, heart, spleen and kidney remained normal. The protective function of iodide was therefore estimated from its effect on plasma (table 2) and hepatic cholesterol (table 3).

The results in normal rabbits (series 1) were as follows. Iodide alone did not alter the plasma cholesterol level. It did reduce hepatic total and ester cholesterol. Cholesterol feeding alone increased plasma and hepatic cholesterol. Iodide given with cholesterol reduced this hypercholesterolemia at a dosage of 20 mg. and prevented it at 40 mg.; 1 and 10 mg. had no effect during cholesterol feeding. Total and esterified hepatic cholesterol were not changed by 20 and 40 mg. iodide dosages, but 1 and 10 mg. fed with cholesterol increased the proportion of hepatic ester cholesterol.

In the rabbits of series 2, thyroidecomy alone elicited hypercholesterolemia without changing hepatic cholesterol. Iodide did not influence this hypercholesterolemia but did reduce hepatic cholesterol. Cholesterol feeding raised both plasma and hepatic cholesterol; the exogenous hypercholesterolemia was much greater than in normal animals on the same diet. Iodide in amounts of 1 and 40 mg. given with cholesterol reduced exogenous hypercholesterolemia. In contrast to its effect in normal rabbits, iodide reduced hepatic total and esterified cholesterol. Data from series 1 and 2 are summarized in table 4.

The outstanding difference between normal and thyroidecomized rabbits was that 20 or 40 mg. iodide dosages were required to produce significant reduction in plasma or hepatic cholesterol in normal cholesterol-fed animals while 1 mg. was effective after thyroidecomy.

2. Plasma Iodine Fractions and Iodide

Iodide administration increased plasma inorganic and total protein-bound iodine in both normal and thyroidecomized rabbits in proportion to dosage (table 5). In normal animals inorganic iodide rose from 2.3 μg. per 100 ml. on the basal diet to 285 on 40 mg., and the protein-bound iodine from 7.1 to 42.3 μg. per 100 ml. The mean increments were greater in thyroidecomized rabbits. One mg. of iodide raised plasma protein-bound iodine to 26 μg. per 100 ml. in thyroidecomized animals as compared with the 20 mg. required to produce the same level in normal animals.

The fractions of the protein-bound iodine are also given in table 5. The butanol-soluble fraction was between 5 and 10 μg. per 100 ml. in all animals on iodide dosages less than 40 mg. The butanol-soluble alkali-insoluble iodine ("thy-
roxin-like) was even more constant, ranging from 4.6 to 7.3 μg. per 100 ml. in normal and thyroidectomized animals independent of iodide dosage. An exception occurred in thyroidectomized animals fed 40 mg. for 10 weeks.

**Table 2.—Mean Values of Total Plasma Cholesterol**

<table>
<thead>
<tr>
<th>Group</th>
<th>Daily Intake</th>
<th>Pre-exper. period</th>
<th>Period on 200 mg. chol., 8 weeks</th>
<th>Period on 400 mg. chol., 15 weeks</th>
<th>Comparison between Groups. p values compared with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chol.</td>
<td>Iodide</td>
<td>mg.</td>
<td>mg.</td>
<td>mg./100 ml.</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>36 ± 1.7 (35)</td>
<td>33 ± 3.5 (16)</td>
<td>42 ± 3.5 (24)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
<td>31 ± 2.9 (12)</td>
<td>36 ± 3.2 (18)</td>
<td>47 ± 6.4 (18)</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>20</td>
<td>36 ± 3.8 (12)</td>
<td>37 ± 3.5 (20)</td>
<td>92 ± 11.4 (30)</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>0</td>
<td>40 ± 3.4 (21)</td>
<td>110 ± 10.9 (30)</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>1</td>
<td>44 ± 4.5 (18)</td>
<td>69 ± 10.3 (30)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>10</td>
<td>35 ± 1.8 (15)</td>
<td>57 ± 4.5 (24)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>20</td>
<td>35 ± 3.8 (20)</td>
<td>47 ± 4.3 (30)</td>
<td>&gt;0.3</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>40</td>
<td>33 ± 3.5 (12)</td>
<td>84 ± 13.8 (23)</td>
<td>0.01</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>0</td>
<td>95 ± 11.4 (16)</td>
<td>95 ± 11.5 (18)</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>40</td>
<td>33 ± 46.1 (31)</td>
<td>107 ± 11.8 (34)</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>0</td>
<td>217 ± 13.2 (24)</td>
<td>353 ± 46.1 (31)</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>40</td>
<td>209 ± 13.5 (32)</td>
<td>209 ± 13.5 (32)</td>
<td>—</td>
</tr>
</tbody>
</table>

The figures preceded by ± denote the standard error of the mean; those in parentheses, the number of values in the series. The p values are taken from Fisher's table of t. Values obtained on one rabbit are not included in average of group 7, series 1.

* Normal rabbits.

Butanol-insoluble protein-bound iodine accounted for most of the increased protein iodine. Normally none is present. More appeared in thyroidectomized than in normal animals on the same iodide intake; the amount in plasma of thyroidectomized animals on 1 mg. iodide daily was the same as in normals on 20 mg. Thyroidectomized controls had a small amount of this fraction in the plasma which can be attributed to the iodide content of the basal diet (0.2 to 0.3 mg. iodide daily).

Reduction in plasma and/or hepatic cholesterol as described above was accompanied by a rise in inorganic and protein-bound iodine in both normal and thyroidectomized rabbits. The butanol-soluble fractions, which include the alkali-insoluble iodine, were not altered. Significant decreases in plasma and/or hepatic cho-

**Discussion**

1. **Iodide and Cholesterol Metabolism**

Evaluation of the experimental conditions under which iodide induces changes in cholesterol-fed animals clarifies some of the conflicting reports in the literature. Inorganic and organic iodine compounds fed with cholesterol tend to maintain blood cholesterol at normal levels, reduce hepatic cholesterol and retard or prevent
development of plaques in the aorta of normal rabbits. Deposition may be prevented under cholesterolemia and atherosclerosis develop before iodide is administered, no fall in cholesterol

<table>
<thead>
<tr>
<th>Normal Series 1 Group No.</th>
<th>Thyroid-ectomized Series 2 Group No.</th>
<th>Diet</th>
<th>Total Cholesterol % fresh tissue</th>
<th>Comparison between Groups. p values compared with Normal Chol. fed.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 &amp; 2</td>
<td>1</td>
<td>Normal (and noneffective iodide)</td>
<td>.247 ± .010 (11)</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>2 &amp; 3</td>
<td>Iodide (effective)</td>
<td>.195 ± .010 (8)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>4 &amp; 5</td>
<td>Cholesterol</td>
<td>.359 ± .026 (14)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5 &amp; 6</td>
<td>1 &amp; 10 mg. iodide</td>
<td>.347 ± .040 (10)</td>
<td>&lt;0.01</td>
<td>0.7</td>
</tr>
<tr>
<td>7 &amp; 8</td>
<td>20 &amp; 40 mg. iodide</td>
<td>.432 ± .051 (10)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>6 &amp; 7</td>
<td>1 &amp; 40 mg. iodide</td>
<td>.287 ± .190 (10)</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Because thyroidectomy did not influence hepatic cholesterol, values of both series are grouped for statistical treatment. The figures preceded by ± denote the standard error of the mean; those in parentheses the number of values in the series. The p values are taken from Fisher's table of t. Livers from one rabbit in group 2 and one rabbit in group 6, series 2, were not analyzed.

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Normal Rabbits, Cholesterol in</th>
<th>Thyroidectomized Rabbits, Cholesterol in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Liver</td>
</tr>
<tr>
<td>Basal Diet</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Iodide 1-10 mg.</td>
<td>normal</td>
<td>raised below normal</td>
</tr>
<tr>
<td>Iodide 20-40 mg.</td>
<td>normal</td>
<td>raised above normal</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>raised above normal</td>
<td>raised above normal</td>
</tr>
<tr>
<td>Cholesterol and Iodide 1-10 mg.</td>
<td>same as cholesterol level</td>
<td>normal</td>
</tr>
<tr>
<td>Cholesterol and Iodide 20-40 mg. I</td>
<td>normal</td>
<td>same as cholesterol level</td>
</tr>
</tbody>
</table>

some circumstances without significant reduction of hyperlipemia.

This action of iodide on cholesterol metabolism depends upon the time it is given. If hyper-

or regression of lesions occur either in thyroidectomized or normal animals. The amount of cholesterol in the diet is important. Iodide can only give protection when
the amounts of cholesterol are not excessive. Usually no protection has been reported when rabbits were fed over 500 mg. a day.\textsuperscript{9, 14, 28} Turner and Bidwell\textsuperscript{29} found that, on a diet containing 500 mg., the protective effect of iodide was lost within four months. Our rabbits were of iodide to become manifest on blood and hepatic cholesterol. The evidence of these experiments is in favor of the concept that atherosclerosis is reduced in cholesterol and iodide-fed thyroidectomized rabbits\textsuperscript{6} and in disagreement with the view\textsuperscript{17} that hypercholesterolemia

<table>
<thead>
<tr>
<th>Iodide Intake</th>
<th>Inorganic Iodide</th>
<th>Protein-Bound Iodine</th>
<th>Fractions of Protein-Bound Iodine</th>
<th>Plasma and/or Hepatic Cholesterol Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Butanol-Soluble</td>
<td>Butanol-Soluble Alkali-Insoluble</td>
</tr>
<tr>
<td>Normal</td>
<td>2.3</td>
<td>7.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal</td>
<td>—</td>
<td>8.0</td>
<td>8.0</td>
<td>2.8</td>
</tr>
<tr>
<td>1 mg.</td>
<td>3.3</td>
<td>15.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1 mg.</td>
<td>—</td>
<td>13.1</td>
<td>7.0</td>
<td>4.6</td>
</tr>
<tr>
<td>10 mg.</td>
<td>38</td>
<td>22.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10 mg.</td>
<td>—</td>
<td>23.8</td>
<td>10.9</td>
<td>4.7</td>
</tr>
<tr>
<td>20 mg.</td>
<td>87</td>
<td>28.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>20 mg.</td>
<td>—</td>
<td>29.4</td>
<td>8.8</td>
<td>5.5</td>
</tr>
<tr>
<td>40 mg.</td>
<td>285</td>
<td>42.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>40 mg.</td>
<td></td>
<td>46.0</td>
<td>24.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

**Normal Rabbits**

**Thyroidectomized Rabbits**

<table>
<thead>
<tr>
<th>Iodide Intake</th>
<th>Inorganic Iodide</th>
<th>Protein-Bound Iodine</th>
<th>Fractions of Protein-Bound Iodine</th>
<th>Plasma and/or Hepatic Cholesterol Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Butanol-Soluble</td>
<td>Butanol-Soluble Alkali-Insoluble</td>
</tr>
<tr>
<td>Normal</td>
<td>4.2</td>
<td>9.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal</td>
<td>—</td>
<td>9.2</td>
<td>6.0</td>
<td>5.6</td>
</tr>
<tr>
<td>1 mg.</td>
<td>63</td>
<td>25.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1 mg.</td>
<td>—</td>
<td>25.6</td>
<td>7.7</td>
<td>5.3</td>
</tr>
<tr>
<td>40 mg.</td>
<td>2000</td>
<td>65.0*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>40 mg. 8 wks.</td>
<td>—</td>
<td>58.9*</td>
<td>14.2*</td>
<td>7.3</td>
</tr>
<tr>
<td>40 mg. 10 wks.</td>
<td>3000</td>
<td>94.0*</td>
<td>26.5*</td>
<td>16.2</td>
</tr>
</tbody>
</table>

Inorganic and protein-bound iodine values are averages of 20 to 30 individual determinations. Fraction averages are of 2 to 4 pools of 10 to 12 individual plasmas taken at various times from rabbits in the same group.

* Corrected for inorganic iodide remaining after three washings of protein precipitate.

more resistant to the effects of cholesterol feeding in that 400 mg. daily did not elicit atherosclerosis after 15 weeks.

The species of experimental animal is also important in the action of iodide on cholesterol metabolism. Chickens are more sensitive to cholesterol feeding than rabbits and develop atherosclerosis on minimum amounts.\textsuperscript{30} They show no reduction of plasma cholesterol and only slight reduction in the size of aortic plaques on very large amounts of iodide fed along with cholesterol.\textsuperscript{31}

Our experiments show that the presence of the thyroid gland is not necessary for the effect and atherosclerosis are unaffected in such animals.

The increased sensitivity of thyroidectomized rabbits to iodide feeding remains unexplained except as it represents impaired ability to remove iodine from circulation in absence of the thyroid gland.

Differences in iodide requirement of normal and thyroidectomized rabbits emphasize the importance of iodide dosage. In normal animals fairly large amounts of iodide are required to reduce plasma and/or hepatic cholesterol. Minimum reduction was obtained on 20 to 25 mg. iodide daily. A maximum effect may be ob-
tained on less than 100 mg. iodide a day. Forty (reported here) to 80 mg. were about as effective as 382.5

These results with large amounts of iodide contrast with the increased plasma and hepatic cholesterol and accelerated aortic deposition produced by 1 to 10 mg. in normal animals. In our own experiments, 1 and 10 mg. increased only hepatic esterified cholesterol significantly. The nature of this paradoxical response to small iodide dosage is not apparent.

Specific aspects of the action of iodide on cholesterol metabolism can be noted. First, iodide in large doses depresses or prevents hypercholesterolemia of exogenous origin while it does not prevent endogenous hypercholesterolemia elicited by thyroidectomy.

Second, iodide reduces hepatic cholesterol. Although large doses of iodide did not change hepatic total cholesterol in normal cholesterol-fed rabbits, decreases in hepatic cholesterol were observed in all other iodide-treated groups. The difference between normal and thyroidectomized rabbits fed cholesterol and iodide may be attributable to the greater sensitivity of thyroidectomized animals to iodide. But this explanation does not account for the decreased hepatic cholesterol in normal rabbits fed iodide without cholesterol. The lack of hepatic response of cholesterol-fed normal rabbits to iodide is more likely the result of an equilibrium between inverse effects on hepatic cholesterol of large and small amounts of iodide.

Third, iodide reduces hepatic esterified cholesterol to such an extent that only free cholesterol was present in the livers of over half the iodide-fed rabbits showing reduced total hepatic cholesterol.

Lastly, plasma cholesterol rise is controlled only with simultaneous iodide and cholesterol feeding. Iodide does not alter plasma cholesterol in normal animals, in thyroidectomized hypercholesterolemic rabbits, or in rabbits with hypercholesterolemia present before iodide feeding.

These changes in cholesterol metabolism due to iodide are distinct from those due to thyroidal hormone. The thyroid depresses hypercholesterolemia, either exogenous or endogenous, without a significant effect on the cholesterol content of the whole body or the liver.33-37

2. Iodide and Plasma Iodine

Plasma inorganic and protein-bound iodine increased in both thyroidectomized and intact animals receiving iodide (table 5). Normally, iodine in the blood of animals and human beings on a low iodine diet consists of a few μg. per 100 ml. in inorganic and protein-bound form.10c

The protein-bound iodine can be extracted from plasma with butanol and separated into a diiodotyrosine-like fraction, soluble in strong alkali, and a thyroxin-like portion, insoluble in alkali. These fractions have been characterized chemically23 and chromatographically.38-40

When no excess iodine has been ingested, the protein-bound iodine is almost entirely thyroxin-like and varies with thyroid activity.10d

But when iodides are administered, the thyroxin-like portion remains low even though the inorganic and total protein-bound iodine greatly increase. This phenomenon has been observed in patients.23, 41, 42

The same is true in rabbits. The “thyroxin-like” iodine fraction amounted to 4 to 7 μg. per 100 ml. in the plasma of our iodide-fed rabbits, both normal and thyroidectomized. It fell within the normal range of plasma protein-bound iodine found in human beings,43 85 per cent or more of which consists of “thyroxin-like” iodine.10d However, this fraction is very high in our thyroidectomized rabbits when compared with 1 μg. per 100 ml. plasma protein-bound iodine found by others in thyroidectomized animals on a normal diet.44

The nature of this butanol soluble-alkali insoluble iodine in the plasma of our iodide-fed rabbits is unknown and its thyroxin content is not yet evaluated. A major portion is probably inactive as judged by the large amount (5.6 μg. per 100 ml.) present in the thyroidectomized animals on the basal diet. Certainly changes in cholesterol metabolism do not appear to be related to this fraction, because the same amount was present in plasma of all iodide-fed animals, whether plasma and/or hepatic cholesterol was significantly reduced or not.

In contrast, the butanol-insoluble fraction of the protein-bound iodine occurs in large...
amounts in plasma of rabbits treated with iodide and increases directly with increasing iodide dosage. It is distinct from the “hormonal” iodine. The thyroid gland contains a butanol-insoluble iodinated protein which yields butanol soluble–alkali insoluble iodine on hydrolysis. However, we were unable to recover this latter substance from plasma. We are conducting further studies on its chemical nature.

Butanol-insoluble iodine is present in animal tissues, other than the thyroid and plasma, as a result of normal metabolism of iodine compounds. Gross and Leblond found radioactive butanol-insoluble iodine metabolites in all tissues, notably in the liver, of rats given radioactive thyroxin. These metabolites disappeared more slowly from the tissues than the butanol-soluble ones. Possibly they are produced in sufficient quantity with prolonged iodine intake to accumulate in the plasma as well as in the tissues. A decrease in plasma and/or hepatic cholesterol with a rise in butanol-insoluble iodine suggests a relationship between the two. This would explain the difference in effective iodide dosage between normal and thyroidectomized rabbits described above. The relationship between this fraction and cholesterol metabolism in cholesterol-fed rabbits is such as to suggest that the protective action of iodide in arteriosclerosis may be related to establishment of adequate plasma and tissue concentrations of butanol-insoluble protein-bound iodine.

**Summary and Conclusions**

1. The effects of graded dosages of potassium iodide on blood and tissue cholesterol and the plasma iodine fractions in normal and thyroidectomized cholesterol-fed rabbits have been evaluated.

2. A “protective” effect of iodide against cholesterol deposition was estimated from reduction of plasma and/or hepatic cholesterol. In both normal and thyroidectomized rabbits: (a) iodide reduced total hepatic cholesterol without affecting the plasma content; (b) cholesterol feeding increased plasma and hepatic cholesterol; (c) iodide fed with cholesterol counteracted these increases; (d) esterified hepatic cholesterol varied as the total cholesterol.

3. Thyroidectomized rabbits were more sensitive than normal to the effect of iodide on endogenous cholesterol.

4. The endogenous hypercholesterolemia resulting from thyroidectomy was not affected by iodide.

5. Plasma inorganic and protein-bound iodine increased in proportion to the iodide dosage, more so in thyroidectomized than in normal rabbits. Fractionation of the protein-bound iodine showed that the butanol-soluble, alkali-insoluble iodine remained the same in all iodide-fed rabbits. The butanol-insoluble fraction accounted for the progressive increase in protein bound iodine.

6. In animals in which iodide decreased plasma and/or hepatic cholesterol, the butanol-insoluble fraction was present in amounts of 20 µg per 100 ml or more.

7. The effect of iodide on cholesterol metabolism is independent of the thyroid gland; it is manifest after thyroidectomy; it is not related to the “thyroxin-like” plasma iodine; the iodide effect is expressed primarily on hepatic cholesterol while a thyroidal action is not.

8. The protective action of iodide against arteriosclerosis seems to be related to the presence of a butanol-insoluble iodine compound in the plasma which may act by altering hepatic cholesterol metabolism.

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HELEN B. BROWN and IRVINE H. PAGE

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