SUMMARY

1. The method of Kekwick & Mackay (1954) for the fractionation of human plasma proteins was used successfully for the separation of the serum proteins of guinea pig, rat, rabbit, cat, dog, monkey, ox, horse and sheep.

2. By variations in pH, ionic strength, temperature and solvent concentration, three fractions consisting essentially of a mixture of α- and β-globulins (G2); γ-globulin (G3) and albumins (AP) were isolated and concentrated. From rat, rabbit, cat, dog, ox and horse serum electrophoretically pure γ-globulin could be prepared from the fraction G3. From the fraction AP electrophoretically pure albumin was prepared from guinea pig, rabbit and sheep; and 98% pure albumin from dog and cat.

3. Tests on the fractions showed that cholesterol was largely concentrated in the fraction G2. The natural antibodies (Salmonella agglutinin) were concentrated in G2 in rat, ox, sheep and dog serum; and in G2 and G3 in horse, guinea pig and rabbit serum. It is suggested that the crude albumin might find a use as a vehicle for radioactive isotopes.

The author wishes to thank Dr R. A. Kekwick for his continuous helpful criticism, Dr B. A. D. Stocker for Salmonella strains, Miss Jean Sharrard for her technical assistance and the Animal Physiology Laboratory, Babraham, for supplies of sheep and ox serum.

REFERENCES


Studies on Ali-esterases and Other Lipid-hydrolyzing Enzymes

2. THE EFFECT OF HEPARIN INJECTION ON ALIMENTARY LIPAEMIA AND ON THE PLASMA ESTERASES*

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(Received 2 December 1954)

Although heparin does not affect the turbidity of lipaemic plasma when added in vitro, Hahn (1943) and Weld (1944) showed that a visible alimentary lipaemia in dogs disappears within a few minutes after the intravenous injection of small amounts of heparin. The post-heparin plasma contains a factor which is capable of clearing lipaemic plasma in vitro (Anderson & Fawcett, 1950); this factor has the properties of a protein and could therefore be an enzyme (Anfinsen, Boyle & Brown, 1952; Spitzer, 1952; Levy & Swank, 1954).

The clearing factor causes a decrease in the size of the large lipoprotein globules, apparently by hydrolysing the triglycerides to produce free fatty acids and glycerol (Graham et al. 1951; Swank & Wilmot, 1951; Anfinsen et al. 1952; Shore, Nichols & Freeman, 1953; Korn, 1954). It appears to be inhibited by small amounts of protamine (Brown, 1952; Spitzer, 1953) and by large amounts of sodium chloride (Brown, Boyle & Anfinsen, 1953), to be activated by heparin and to act specifically on lipoprotein (Korn, 1954). Pancreatic lipase also exhibits clearing activity in vitro and in vivo (Spitzer, 1952, 1953), but the clearing factor can be found in the post-heparin plasma of pancreatectomized dogs (Fasoli, Glassman, Magid & Foa, 1954).

It is reported that the clearing factor can be separated from plasma 'esterase' by fractional precipitation with ethanol (Brown et al. 1953) and that it does not hydrolyse β-naphthyl laurate (Brown, Baker & Kaufman, 1954). However, Levy & Swank (1954) found that the rate of hydrolysis of tributyrin by dog plasma increased 50–100% within a few minutes after intravenous injection of heparin, while the esterase activity of normal dog plasma was not affected by incubation with heparin in vitro. On injecting small amounts of protamine after injecting the heparin, the activity

of the clearing factor in the post-heparin plasma is inhibited and the esterase activity returns to normal.

However, it is known that the hydrolysis of tributyrin by normal dog plasma is due almost entirely to the presence of pseudocholinesterase (cf. Richter & Croft, 1942). In the present investigation we have found that the 50–100 % increase in the rate of hydrolysis of tributyrin by the plasma after injection of heparin is due to the appearance of another enzyme capable of hydrolysing tributyrin, the activity of the pseudocholinesterase remaining unaltered. It seemed of interest, therefore, to investigate a possible relationship between this tributyrinase and the clearing factor in post-heparin plasma.

METHODS

Esterase activity was measured manometrically by the Warburg method at 37-5 ° and pH 7.4 in a medium containing 0.025 M NaHCO₃ and saturated with 5% CO₂ and 95% N₂ (v/v). The esterase inhibitors used were physostigmine salicylate (eserine), diethyl p-nitrophenyl phosphate (E 600, paraoxon), diisopropyl p-nitrophenyl phosphate (DINP) and tri-o-cresyl phosphate (TOCP). The inhibitors were incubated with the esterase preparation at 37-5 ° for 30 min. before adding the substrate to measure the residual esterase activity (cf. Myers & Mendel, 1933).

Pseudocholinesterase activity was determined with 0.03 M butyrylcholine as substrate (Cohen, Kalabek & Warrins, 1949) and true cholinesterase with 0.03 M acetyl-β-methylcholine (Mendel, Mundell & Rudney, 1943); both enzymes are inhibited almost completely when incubated with 5 x 10⁻⁴ M eserine for 30 min. The ali-esterase activity can usually be determined with tributyrin as substrate; the ali-esterases (B-type esterases) of serum are not strongly inhibited by low concentrations of eserine but can be completely inhibited by low concentrations of E 600 and related compounds (cf. Aldridge, 1953a). The activity of the A-esterase (E 600-esterase) can be determined with phenyl acetate as substrate in the presence of 5 x 10⁻⁴ M E 600 or DINP, the diisopropyl analogue of E 600 (cf. Aldridge, 1953b; Mounter & Whitaker, 1953). Tributyrin and phenyl acetate were prepared as aqueous emulsions with 5 % (w/v) gum acacia so that the final reaction mixture in the Warburg vessel contained 0.2 % (v/v) ester and 0.1 % gum acacia. The lipase activity of the plasma was determined titrimetrically with an aqueous emulsion of olive oil and gum acacia as described by Cherry & Crandall (1932). In all experiments the esterase activity was calculated from the initial rate of hydrolysis after appropriate corrections had been made for the slow non-enzymatic hydrolysis of the substrate in question.

To determine the activity of the clearing factor, 0.3 ml. of lipaemic dog plasma was mixed with 0.1 ml. of the plasma to be tested, and the extinction measured at 680 mλ in a Beckman spectrophotometer (cf. Anderson & Fawcett, 1950; Spitzer, 1952; Brown et al. 1953; Levy & Swank, 1954). The lipaemic blood samples were drawn from a normal dog 3–4 hr. after feeding 5 g. of margarine or butter/kg. body weight; all blood samples were mixed with 0.1 vol. of 5% (w/v) sodium citrate as described by previous investigators (cf. Levy & Swank, 1954). The initial extinction of the plasma mixture was usually 0.35–0.45 and decreased rapidly in the presence of clearing factor; the initial rate of clearing, which is almost linear, was used as a measure of the clearing-factor activity. In experiments with inhibitors, the post-heparin plasma was mixed with an equal volume of water or inhibitor solution and allowed to stand for 30 min. before samples were removed to test for tributyrinase and clearing-factor activities. Under these conditions, E 600 is destroyed very rapidly (Aldridge, 1953b, c) while DINP is relatively stable (Aldridge, 1953c). Control experiments in which E 600 was incubated with the lipaemic dog plasma or in which an equivalent amount of p-nitrophenol was added to the post-heparin plasma showed no significant inhibition of the clearing factor.

Small amounts of heparin were injected intravenously into man, dog and rabbit and the post-heparin blood samples were drawn 5–7 min. later. Larger doses were injected intraperitoneally into rats, mice and guinea-pigs, and the animals were killed by exsanguination 15 min. later. In several experiments, guinea pigs under Nembutal anaesthesia were injected with heparin in the jugular vein. In these experiments it was found that small doses of heparin injected intravenously into animals after Nembutal anaesthesia produced approximately the same amount of clearing factor as is obtained when massive doses of heparin are administered intraperitoneally (see Table 3). Apparently the heparin is slowly absorbed from the peritoneal cavity. This fact is responsible for the large differences in the amounts of heparin which were administered intravenously and intraperitoneally to various species of animals in the experiments described below.

Table 1. Effect of intravenous heparin injections on the esterases of dog blood

<table>
<thead>
<tr>
<th>Source of esterases</th>
<th>Substrate</th>
<th>0</th>
<th>20</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Tributyrin</td>
<td>93</td>
<td>166</td>
<td>150</td>
<td>212</td>
</tr>
<tr>
<td></td>
<td>Butyrylcholine</td>
<td>1560</td>
<td>1480</td>
<td>1410</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Acetyl-β-methylcholine</td>
<td>126</td>
<td>114</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Phenyl acetate</td>
<td>87600</td>
<td>92500</td>
<td>73300</td>
<td>—</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>Tributyrin</td>
<td>313</td>
<td>297</td>
<td>320</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Acetyl-β-methylcholine</td>
<td>240</td>
<td>260</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

A male dog, weighing approx. 16 kg., was injected intravenously with various amounts of heparin (135 units/mg.). Samples of blood were removed before the injection and 5–7 min. later, and mixed with 0.1 vol. of 5% (w/v) sodium citrate. The average activities of the various esterases in the normal and post-heparin citrated blood samples were determined.
RESULTS

In confirmation of the results obtained by Richter & Croft (1942), it was found that the hydrolysis of tributyrin by normal dog plasma can be largely inhibited by $5 \times 10^{-4} \text{M} \text{ eserine}$, a concentration which inhibits the activity of the pseudocholinesterase almost completely. Similar results were obtained with $5 \times 10^{-4} \text{M} \text{ DINP}$; this compound causes complete and irreversible inhibition of the pseudocholinesterase. The small portion of the esterase activity of normal plasma towards tributyrin which cannot be inhibited by high concentrations of DINP or E600 (see Table 2) is probably due to the large amount of A-esterase present (see Table 1).

Intravenous injection of heparin had no significant influence on the activity of the pseudocholinesterase, true cholinesterase and A-esterase of plasma nor on the true cholinesterase and aliesterase of the erythrocytes of the dog (Table 1). However, in confirmation of the results obtained by Levy & Swank (1954), it was found that the total activity towards tributyrin increases by 60–130% shortly after the injection of heparin. The increase in activity is due to the appearance of an eserine-resistant tributyrinase in the plasma (see Table 3). This enzyme is also resistant to inhibition by $5 \times 10^{-7}$ and $5 \times 10^{-4} \text{M} \text{ DINP}$ (Tables 2 and 3), but is strongly inhibited by $5 \times 10^{-4} \text{M} \text{ DINP}$ or $5 \times 10^{-4} \text{M} \text{ E600}$ (Table 2).

Similar results were obtained with human plasma. The hydrolysis of tributyrin by normal human plasma is due almost entirely to the pseudocholinesterase present (Esson & Stedman, 1937; Richter & Croft, 1942; Adams & Whittaker, 1949) and can be inhibited by $5 \times 10^{-4} \text{M} \text{ eserine}$ or DINP (Tables 2 and 3). After injection of heparin, the plasma is found to contain another esterase which is capable of hydrolysing tributyrin and which is not inhibited by $5 \times 10^{-4} \text{M} \text{ eserine}$ or DINP (Table 3); the properties of this enzyme resemble those of the tributyrinase found in post-heparin dog plasma (Table 2).

The hydrolysis of tributyrin by the normal plasma of rats, rabbits, mice and guinea pigs, on the other hand, is due mainly to the presence of an aliesterase which cannot be inhibited by low concentrations of eserine (Esson & Stedman, 1937; Richter & Croft, 1942) but which can be inhibited by $5 \times 10^{-7}$ or $5 \times 10^{-4} \text{M} \text{ DINP}$ (Table 2). The hydrolysis of tributyrin by rat serum in the presence of $5 \times 10^{-4} \text{M} \text{ DINP}$ followed the pattern observed with dog and human plasma; normal plasma exhibits a slight activity whereas the post-heparin plasma exhibits a high DINP-resistant tributyrinase activity (Tables 2 and 3). Analogous results were obtained with rats which had been injected with

<p>| Table 2. Inhibitions by E600 and DINP of the hydrolysis of tributyrin by plasma from different species |
|-------------------------------------------------|----------------------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Species</th>
<th>Post-heparin</th>
<th>Post-heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Dog</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Rat</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

<p>| Table 3. Rate of hydrolysis of tributyrin (μl. CO₂/ml. plasma/30 min.) |
|-------------------------------------------------|----------------------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Species</th>
<th>Post-heparin</th>
<th>Post-heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Dog</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Rat</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*Note: The table includes data for different concentrations of E600 and DINP, with rates measured in microliters of CO₂ per milliliter of plasma per 30 minutes.*
previously with TOCP in order to inhibit the ali-esterases (Mendel & Myers, 1953); in this case the plasma contains only the DINP-resistant esterases (Table 3). It might be noted that the injection of TOCP did not affect the liberation of clearing factor into the plasma after heparin injection (Table 3).

A different type of result was obtained with rabbits. The injection of heparin did not cause the liberation of an appreciable DINP-resistant tributyrinase activity into the plasma, although the post-heparin plasma exhibited a high clearing activity (Table 3).

The results fail to support the suggestion of Levy & Swank (1954) that the tributyrinase in post-heparin dog plasma might be concerned with its clearing activity. Further experiments were carried out in order to substantiate this conclusion. Post-heparin plasma from rat and dog was heated at 48–50° for various periods. Both the DINP-resistant tributyrinase and the clearing factor were partially inactivated; however, the clearing factor was more sensitive to destruction than the tributyrinase (Table 4). Post-heparin plasma was also

### Table 3. Effect of heparin injections on the clearing activity and on the hydrolysis of tributyrin by plasma from different species

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of measurements</th>
<th>Heparin injection</th>
<th>Route of injection</th>
<th>Rate of hydrolysis of tributyrin by the plasma in the presence of the following inhibitors (μl CO₂/ml plasma/30 min.)</th>
<th>Clearing activity of the plasma (E₆₈₀ μM/100 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dose (units/kg.)</td>
<td></td>
<td>No inhibitor</td>
<td>5 × 10⁻⁴M DINP</td>
</tr>
<tr>
<td>Dog</td>
<td>13</td>
<td>0</td>
<td>Intravenous</td>
<td>93</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>20</td>
<td></td>
<td>166</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50</td>
<td></td>
<td>150</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100</td>
<td></td>
<td>212</td>
<td>—</td>
</tr>
<tr>
<td>Man</td>
<td>5</td>
<td>0</td>
<td>Intravenous</td>
<td>404</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>20</td>
<td></td>
<td>529</td>
<td>132</td>
</tr>
<tr>
<td>Rat</td>
<td>8</td>
<td>0</td>
<td>Intraperitoneal</td>
<td>1223</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>500</td>
<td></td>
<td>1676</td>
<td>343</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1000</td>
<td></td>
<td>1615</td>
<td>403</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2000</td>
<td></td>
<td>1335</td>
<td>407</td>
</tr>
<tr>
<td>TOCP-rat</td>
<td>2</td>
<td>0</td>
<td>Intraperitoneal</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>500</td>
<td></td>
<td>261</td>
<td>260</td>
</tr>
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<td></td>
<td>2</td>
<td>2000</td>
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<td>298</td>
<td>295</td>
</tr>
<tr>
<td>Rabbit</td>
<td>5</td>
<td>0</td>
<td>Intravenous</td>
<td>1129</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20</td>
<td></td>
<td>1210</td>
<td>64</td>
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<tr>
<td></td>
<td>3</td>
<td>100</td>
<td></td>
<td>1020</td>
<td>80</td>
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<td></td>
<td>1</td>
<td>1000</td>
<td></td>
<td>1090</td>
<td>70</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>3</td>
<td>0</td>
<td>Intravenous</td>
<td>1639</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50</td>
<td></td>
<td>1692</td>
<td>146</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>5</td>
<td>0</td>
<td>Intraperitoneal</td>
<td>3488</td>
<td>133</td>
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<tr>
<td></td>
<td>5</td>
<td>500</td>
<td></td>
<td>3230</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2000</td>
<td></td>
<td>3754</td>
<td>192</td>
</tr>
<tr>
<td>Mouse</td>
<td>3</td>
<td>0</td>
<td>Intraperitoneal</td>
<td>4630</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2000</td>
<td></td>
<td>4330</td>
<td>249</td>
</tr>
</tbody>
</table>

The results obtained with guinea pigs and mice were complicated by the fact that the normal plasma contains an ali-esterase which is inhibited by 5 × 10⁻⁶M DINP, and a small amount of another esterase which is not inhibited by 5 × 10⁻⁶M DINP but which is inhibited by E₆₀₀ or by high concentrations of DINP. This DINP-resistant tributyrinase thus resembles the DINP-resistant tributyrinases found in post-heparin plasma from dog, man and rat (Table 2). Nevertheless, normal plasma from the mouse and guinea pig do not exhibit clearing activity (Table 3). After injection of heparin, the plasma acquired a high clearing activity, but the activity of the DINP-resistant tributyrinases did not increase markedly (Table 3).
incubated with various concentrations of E600 before testing the enzyme activities. Again both enzymes were inhibited but in this case the tributyrinase is more sensitive than the clearing factor (Table 5). From these experiments it can be concluded that the clearing factor and the DINP-resistant tributyrinase are different enzymes.

Table 4. Inactivation of the enzymes in post-heparin plasma by exposure to high temperatures

Citrated plasma, obtained from rats 15 min. after intraperitoneal injection of 2000 units heparin/kg. or from a dog 6 min. after intravenous injection of 20 units heparin/kg., was heated in a water bath at 48° or 50° for various periods of time. The activity of the clearing factor in the control and heated samples of plasma was determined in the usual way with lipaseastic dog plasma as substrate. The activity towards tributyrin was determined manometrically in the presence of 5 x 10^-4 M DINP.

<table>
<thead>
<tr>
<th>Species</th>
<th>Temp. (°C)</th>
<th>Time of heating (min.)</th>
<th>Clearing factor</th>
<th>DINP-resistant tributyrinase</th>
<th>Percentage activity remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>48</td>
<td>10</td>
<td>45</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>30</td>
<td>17</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>45</td>
<td>9</td>
<td>32</td>
<td></td>
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<tr>
<td></td>
<td>50</td>
<td>20</td>
<td>8</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>50</td>
<td>30</td>
<td>11</td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>

However, it is interesting to observe that the clearing factor is fairly sensitive to inhibition by E600 and almost completely resistant to DINP (Table 5). In this respect, this enzyme resembles the lipase of pancreas (cf. Aldridge, 1954). However, the relative activities of these enzymes on lipaseastic dog plasma and on olive oil differ considerably. When pancreatic lipase was liberated into the blood stream of rats by the subcutaneous injection of acetyl-β-methylcholine plus eserine (Popper & Necheles, 1943), a marked increase in the rate of hydrolysis of olive oil by the plasma was observed, together with a slight increase in the DINP-resistant tributyrinase activity and the production of a small clearing activity (Table 6). Injection of heparin into rats, on the other hand, produces a large clearing activity and a smaller increase in the rate of hydrolysis of olive oil, together with the liberation of large amounts of the DINP-resistant tributyrinase into the plasma (Table 6).

Table 5. Inhibition of the enzymes in post-heparin plasma by incubation with E600 and DINP

Citrated post-heparin plasma was incubated with an equal volume of water or inhibitor solution for at least 30 min. at room temperature. Subsequently samples were removed to determine the activities of the clearing factor and DINP-resistant tributyrinase in the usual manner.

<table>
<thead>
<tr>
<th>Percentage activity remaining</th>
<th>Concentration of inhibitor in incubation mixture (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>DINP-resistant tributyrinase</td>
</tr>
<tr>
<td>Dog</td>
<td>DINP</td>
</tr>
<tr>
<td>Rat</td>
<td>3 x 10^-3</td>
</tr>
<tr>
<td></td>
<td>3 x 10^-4</td>
</tr>
<tr>
<td></td>
<td>1 x 10^-4</td>
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<tr>
<td>Rat</td>
<td>3 x 10^-3</td>
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<td>3 x 10^-5</td>
</tr>
<tr>
<td>Man</td>
<td>3 x 10^-3</td>
</tr>
<tr>
<td></td>
<td>3 x 10^-4</td>
</tr>
<tr>
<td></td>
<td>3 x 10^-5</td>
</tr>
</tbody>
</table>

DISCUSSION

The fact that heparin, incubated with normal blood in vitro, does not produce clearing activity suggests that the clearing factor must be liberated from the tissues after injection of heparin into the animal. Extracts of normal heart and lung do indeed exhibit clearing-factor activity (Anfinsen et al. 1952; Korn, 1954). The activity of these extracts is

Table 6. Comparison of the effects of injections of heparin and of acetyl-β-methylcholine plus eserine on the enzymes of rat plasma

Male rats weighing approx. 300 g. were injected twice at a 15 min. interval with 0-3 mg. acetyl-β-methylcholine/kg, plus 0-3 mg. eserine/kg. subcutaneously; the rats were killed and citrated blood collected 60 min. after the first injection. Other rats were injected with 2000 units heparin/kg. intraperitoneally and the citrated blood collected 15 min. later. The activities of the clearing factor and the DINP-resistant tributyrinase in the plasma were determined in the usual manner. The hydrolysis of olive oil was measured titrimetrically by the method of Cherry & Crandall (1932).

<table>
<thead>
<tr>
<th>Activity of the plasma enzymes</th>
<th>Clearing factor (ΔE600 μM/100 min.)</th>
<th>DINP-resistant tributyrinase (μmoles acid/ml. plasma/60 min.)</th>
<th>Olive oil hydrolysis (μmoles acid/ml. plasma/60 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance injected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.07</td>
<td>1.8</td>
<td>0.29</td>
</tr>
<tr>
<td>Heparin</td>
<td>0.10</td>
<td>38.7</td>
<td>1.05</td>
</tr>
<tr>
<td>Acetyl-β-methylcholine + eserine</td>
<td>1.1</td>
<td>4.1</td>
<td>5.29</td>
</tr>
</tbody>
</table>
increased in the presence of small amounts of heparin but the heparin is not essential (Korn, 1954); the clearing factor is, in fact, inhibited by larger amounts of heparin (Brown et al. 1953). Moreover, there does not seem to be any correlation between the heparin content and the clearing activity of the blood during shock (Levy & Swank, 1953). In other words, the primary factor involved in the production of clearing activity in vivo is not the activation of the clearing factor by heparin but rather the liberation of this enzyme from one or more tissues (cf. Weld, 1944; Swank & Levy, 1952; Anfinsen et al. 1952; Levy & Swank, 1954).

The results obtained in the present investigation indicate that heparin is also capable of liberating a DINP-resistant tributyrinase from tissues. The origin of this esterase is still uncertain, although preliminary experiments have shown that the heart and lung of the normal rat contain a DINP-resistant tributyrinase together with a DINP-sensitive all-esterase. Further experiments are required in order to establish the mechanism by which heparin and other heparin-like substances liberate clearing factor and the DINP-resistant tributyrinase from the tissues. The results obtained in a recent investigation by Seifter & Baeder (1954) suggest that the liberation of these enzymes might be connected with the effect of hyaluronidase on the ground substance of the tissues.

**SUMMARY**

1. The effect of heparin injections on alimentary lipaemia and on the plasma esterases has been investigated in six species of animals. In all cases the post-heparin plasma contained a factor capable of clearing lipaemic plasma. The injection of heparin in man, dog and rat liberates a tributyrinase into the plasma which is not inhibited in vitro by low concentrations of eserine or diisopropyl p-nitrophenyl phosphate (DINP). The DINP-resistant tributyrinase activity of rabbit, mouse and guinea pig plasma does not increase markedly after the injection of heparin.

2. Both the clearing factor and the DINP-resistant tributyrinase in post-heparin plasma are inactivated by heating at 48–50° or by incubation with diethyl p-nitrophenyl phosphate (E600). However, the tributyrinase is less sensitive than the clearing factor to destruction by heating and more sensitive to inhibition by E600.

3. It is concluded that the clearing factor and the DINP-resistant tributyrinase are different enzymes, both of which may be liberated from the tissues into the blood stream by the injection of heparin.

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**REFERENCES**