TURNOVER OF $^{131}$I-LABELLED FIBRINOGEN IN FEVER

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The use of the $^{131}$I-label for fibrinogen turnover studies has been attempted only sporadically and in the last few years. In an early investigation in this laboratory using iodine labelled whole plasma proteins, fibrinogen was found to have a half-life of 66 hr. in rabbits (Cohen, Holloway, Matthews and McFarlane, 1956). In 1958 Christensen found an average half-life of iodinated fibrinogen in humans of 4·3 days and deduced that approximately half the body fibrinogen pool exists outside the vascular system. In 1959 Hammond and Verel reported a mean half-life of 5·1 days for human $^{131}$I-fibrinogen and claimed over 80 per cent of it to be in the extravascular compartment. Lewis, Ferguson and Schoenfield in 1961 studied $^{131}$I-fibrinogen in 20 normal dogs and found a mean half-life of 58 hr. and a distribution ratio between intra- and extravascular pools of 1·93.

The foregoing results present no clear picture and indeed cast doubt on the validity of the $^{131}$I-label as an indicator of fibrinogen metabolism. McFarlane (1963) has recently shown that if $^{131}$I-fibrinogen is to behave identically in vivo with the unlabelled protein, certain precautions are necessary in its preparation and labelling. When rabbit fibrinogen was iodinated at different substitution levels he found that not more than 0·5 atoms of iodine could be associated with 1 molecule of fibrinogen without altering its biological behaviour compared with that of biosynthetically prepared $^{14}$C-fibrinogen. Experiments reported below were undertaken to investigate the behaviour of $^{131}$I-labelled fibrinogen prepared in this way in normal rabbits and in others after inducing fever.

MATERIALS AND METHODS

Animals

Ten male Sandylop rabbits, bred at the National Institute for Medical Research, received the labelled material. The animals were kept in individual metabolic cages on a standard pelleted diet and on drinking water containing 0·005 per cent NaI. Five days before the main experiment started, the plasma volume and the $^{131}$I-iodide space of these rabbits were estimated by methods already described (Regoezzi, 1963a).

Preparation and labelling of fibrinogen

From the plasma of 2 donor rabbits 220 mg. fibrinogen was prepared by repeated ammonium sulphate precipitation, as described by McFarlane (1963). This amount of fibrinogen was finally contained in 5 ml. and iodinated at a substitution level of 0·5 atoms iodine per molecule of fibrinogen (330,000 molec. wt.) using inactive iodine monochloride as carrier (McFarlane, 1958). The efficiency of iodination was 50 per cent. After dialysing against citrate-phosphate-saline buffer with 5 changes in 3 hr., $\frac{3}{4}$ of the labelled preparation was injected into a 1·5 kg. rabbit for screening. This rabbit was hydrated (30 ml. 5 per cent saline
+ 200 mg. KI intravenously) in order to promote diuresis and thus to reduce the amount of \(^{131}\text{I}\)-iodide in the plasma. After a 21-hr. "screening" period the rabbit was bled and the plasma separated. The 4·88 per cent of non-protein-bound \(^{131}\text{I}\)-activity present at this time in the plasma was reduced to 1·81 per cent by a 1-hr. dialysis against the above buffer. The fibrinogen was then injected into rabbits without further treatment, each of them receiving 7·2 to 8·9 mg. fibrinogen corresponding to 84–104 \(\mu\)c fibrinogen-bound activity.

**Measurements**

The behaviour of the \(^{131}\text{I}\)-fibrinogen was studied over a period of 10 days by means of the following measurements:

*Total body gamma-radiation.*—This was measured in a ring counter as described by Campbell, Cuthbertson, Matthews and McFarlane (1956). Before ring-counting, the bladder was emptied using a rubber catheter.

*Total body protein-bound activity.*—This was derived from total body \(\gamma\)-radiation values by subtracting the total free \(^{131}\text{I}\)-iodide activity present in the body. The latter was calculated from the non-precipitable activity of the plasma samples multiplied by the \(^{131}\text{I}\)-iodide space factor. It was assumed that the \(^{131}\text{I}\)-iodide space of the rabbits did not alter significantly during the experiment. An experimental support of the assumption will be given elsewhere (Regoezzi, 1963a).

Blood samples were taken from the marginal ear vein of the rabbits using 0·01 ml. 38 per cent sodium citrate per ml. of blood as anticoagulant. Correction for the activity removed by sampling and therefore not catabolised, was made in the usual way (Cohen et al., 1956). The samples were counted in a well-type scintillation counter, first the total plasma and subsequently the supernatant after precipitation with 20 per cent trichloroacetic acid and centrifugation. By this means, two further parameters were obtained:

*Intravascular non-precipitable \(^{131}\text{I}\)-activity.*—This was estimated directly.

*Plasma protein-bound activity.*—This was derived from the difference between total and supernatant counts.

*Fibrinogen content of plasma.*—This was estimated by the method of Ellis and Stransky (1961), i.e. 0·5 ml. plasma was diluted to 6 ml. using barbitone-saline buffer (0·1 \(\text{M}, \text{pH 7·2}\)) and divided equally into 2 Beckman cells. To one of them, 0·0148 ml. of a calcium-thrombin solution was added, the other one being used as a blank. The increase in optical density due to conversion of fibrinogen into fibrin was read after 20 min. at 470 \(\mu\) wavelength.

*Fibrinogen specific activity.*—This value was calculated using the following formula:

\[
\text{specific activity} = \frac{\text{protein counts/ml. plasma}}{\text{mg. fibrinogen/ml. plasma}}
\]

**Induction of fever**

In 5 of the rabbits, fever was induced after the 92nd hr. of the turnover experiment. For this purpose, 1 \(\mu\)g. of an international pyrogen reference preparation supplied by the Department of Biological Standards, N.I.M.R. (Humphrey and Bangham, 1959) and 0·1 ml. of a typhoid-paratyphoid A and B vaccine supplied by the Wellcome Research Laboratories, Beckenham, England, were alternately injected i.v. at 12-hr. intervals until the end of the experiment. By this means, a rise in rectal temperature up to 39·5°–40·5° was obtained for periods of approximately 6 hr. immediately following each injection. The rectal temperature of untreated rabbits under the same conditions did not exceed 39°.

**RESULTS**

Observations on normal rabbits were obtained from 5 rabbits over a 10-day period and from another 5 rabbits over a 4-day period, \(i.e.\) until fever was induced. Results given by a rabbit which was generally representative of the group, but was exceptional in one particular feature, are illustrated in Fig. 1. The semilogarithmic plot of plasma protein-bound activity \((t_i = 58 \text{ hr.})\) resulted in a straight line suggesting a steady fibrinogen turnover. The fibrinogen specific activity curve (dotted line) in this particular rabbit but not in the others could be resolved into
2 exponential components. This was not due, however, to a change in the catabolic rate, but to a continuous increase of the plasma fibrinogen level from 2-4 mg./ml. up to 3-0 mg./ml. in the first 4 days followed by a return to the original value by the seventh day. The total body γ-radiation curve extrapolated at zero time to 105 per cent, the total body protein-bound activity curve (t₁/₂ = 60 hr.) only to 90 per cent showing that approximately 15 per cent of the total body γ-radiation came from non-protein bound ¹³¹I still present in the body water (shadowed area). Results showing the behaviour of ¹³¹I-fibrinogen in the 10 rabbits are summarised in the Table.

Practical conclusions arising from these observations on total body activities are discussed further below. For comparison with fibrinogen similar investigations were carried out with ¹³¹I-rabbit-albumin using the same experimental techniques to explore whether total body ¹³¹I-iodide behaves in the same way. Results of a typical ¹³¹I-albumin turnover experiment are shown in Fig. 2.

As can be seen from the shadowed area, there is a small transient accumulation of ¹³¹I-iodide in the first few days, possibly due to the catabolism of a few per cent of denatured material. Subsequently, total body γ-radiation and protein-bound activity curves had the same slope. The small area between these two curves (4 per cent) reflects the lower catabolic rate of albumin and indicates, incidentally, that calculation of albumin catabolism based on measurements of total body γ-radiation are permissible.
### Table.—Equilibrium Distribution and Catabolic Rate of $^{131}$I-fibrinogen in 10 Rabbits

<table>
<thead>
<tr>
<th>Experiment</th>
<th>F/4</th>
<th>F/5</th>
<th>F/6</th>
<th>F/7</th>
<th>F/8</th>
<th>F/9</th>
<th>F/10</th>
<th>F/11</th>
<th>F/12</th>
<th>F/13</th>
<th>Mean</th>
<th>Std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt. (kg.)</td>
<td>2.57</td>
<td>3.30</td>
<td>3.16</td>
<td>3.00</td>
<td>2.87</td>
<td>2.80</td>
<td>3.08</td>
<td>3.17</td>
<td>3.15</td>
<td>3.28</td>
<td>3.03</td>
<td>$\pm$ 0.22</td>
</tr>
<tr>
<td>Intravascular fibrinogen mass (mg.)</td>
<td>284</td>
<td>180</td>
<td>316</td>
<td>255</td>
<td>234</td>
<td>239</td>
<td>209</td>
<td>333</td>
<td>276</td>
<td>279</td>
<td>$\pm$ 60</td>
<td></td>
</tr>
<tr>
<td>Distribution ratio (R) (intra- to extravascular pool mass)</td>
<td>3.50</td>
<td>4.62</td>
<td>4.49</td>
<td>4.88</td>
<td>2.59</td>
<td>5.64</td>
<td>3.09</td>
<td>5.83</td>
<td>4.74</td>
<td>3.30</td>
<td>4.27</td>
<td>$\pm$ 1.09</td>
</tr>
<tr>
<td>Half-life of intravascular fibrinogen (hr.)</td>
<td>57</td>
<td>59</td>
<td>58</td>
<td>66</td>
<td>65</td>
<td>58</td>
<td>70</td>
<td>72</td>
<td>53</td>
<td>64</td>
<td>.</td>
<td>62</td>
</tr>
<tr>
<td>Half-life of total body protein-bound activity (hr.)</td>
<td>58</td>
<td>60</td>
<td>60</td>
<td>70</td>
<td>68</td>
<td>61</td>
<td>72</td>
<td>75</td>
<td>56</td>
<td>66</td>
<td>.</td>
<td>56</td>
</tr>
<tr>
<td>Catabolic rate* (per cent/day)</td>
<td>37.5</td>
<td>34.3</td>
<td>35.0</td>
<td>30.4</td>
<td>35.5</td>
<td>33.7</td>
<td>31.4</td>
<td>27.0</td>
<td>38.0</td>
<td>33.8</td>
<td>33.7</td>
<td>$\pm$ 3.3</td>
</tr>
</tbody>
</table>

* $= \frac{0.693 \times 24}{t_1\text{(plasma)}} \times \frac{1 + R}{R}$ where $R =$ distribution ratio.
Fibrinogen Catabolism in Febrile Rabbits

In all 5 rabbits in which fever was induced a prompt hyper-fibrinogenaemia was observed. The levels rose in the first 24 hr. to 165 per cent (range: 158–174 per cent) of the normal fibrinogen level and continued to rise during the following 3 days but at a slower rate up to 207 per cent (180–260 per cent). From the 7th day a decline set in although the plasma fibrinogen content was still significantly above normal. Results under these conditions are shown in Fig. 3.

The start of the pyrogenic treatment is marked by an arrow. Up to this time the behaviour was normal (Fig. 1). After induction of fever, an accelerated rate of fall was evident in the fibrinogen specific activity curve. The curve of plasma fibrinogen concentration behaved like a reflection of the fibrinogen specific activity curve, whereas the slope of the plasma protein-bound activity curve was constant. A convexity appeared on the total body activity curve coincident with the febrile phase. Comparison with the protein-bound activity curve, the slope of which was unchanged, shows that this was clearly due to accumulation of activity in non-protein form in the body water. Similar results were obtained in all 5 rabbits.

DISCUSSION

The data support earlier conclusions (McFarlane, 1963) concerning the importance of measuring the $^{131}$I-iodide activity of the body in fibrinogen turnover experiments. Presumably because of the relatively rapid rate of catabolism of this
protein, $^{131}$I-iodide accumulated to significant levels in the plasma and body water as shown by the area of the shaded portions in the figures. When $^{131}$I-albumin was injected on the other hand, the accumulation of $^{131}$I-iodide was much less. Other work has shown that the level of retained $^{131}$I-iodide in this kind of experiment cannot be accurately predicted even when the same preparation of fibrinogen is injected, since the renal excretion rate for radio-iodide in rabbits is very variable (Regoeczi, 1963b). Significantly lower levels of non-precipitable activity in the plasma (and in the body) can be obtained by adding excess inactive chloride to the drinking water (McFarlane, 1963), but this was not considered necessary in these experiments.

It appears that additional information on intravascular fibrinogen turnover may be obtained if both protein-bound counts/ml. plasma and fibrinogen specific activities, i.e. protein-bound counts/mg. fibrinogen, are measured. The reason is that the protein-bound counts/ml. plasma depend only on the catabolic rate, provided the plasma volume remains constant, whereas specific activity values are influenced not only by fibrinogen catabolism but also by its synthesis rate. Thus if a change occurs in the slope of the specific activity curve then comparison with the counts/ml. curve will help to decide whether the change which occurred primarily affected the rate of synthesis or the rate of catabolism (see Matthews, 1961). In these experiments comparison of protein-bound activities/ml. plasma with fibrinogen specific activities in Fig. 3 shows, that in spite of large alterations
in the fibrinogen pool brought about by fever, the fractional catabolic rate remained remarkably constant.

Humphrey and McFarlane (1954) have made what may be a related observation, namely that the fractional rate of catabolism of pneumococcus antibody globulins in actively immunised rabbits was the same irrespective of whether the animals had 3 mg./ml. or 30 mg./ml. of circulating antibody.

Since the mechanism of plasma protein catabolism is still largely unknown, it becomes too speculative to discuss here how this constancy may be maintained.

It was noted in these experiments that fibrinogen specific activities seemed to be susceptible to somewhat larger random variations than those of albumin. This may be due to the smaller pool of fibrinogen in the plasma or to its normally higher turnover rate, or both. It is also possible that the mechanism controlling fibrinogen synthesis is unduly sensitive, e.g. to trauma or even simple blood withdrawal. As illustrated in Fig. 1, 1 out of 10 normal rabbits reacted during the first few days by increasing its rate of synthesis and no reason for this exceptional reaction was apparent.

**SUMMARY**

The metabolic behaviour of *in vivo* screened $^{131}$I-fibrinogen was investigated in 10 rabbits.

Radioactivity liberated by breakdown of the protein is measurable in the body water, larger amounts being found than when similarly labelled albumin was catabolised.

In 5 of the rabbits fever was provoked by toxin injections and resulted in hyperfibrinogenaemia. Fibrinogen synthesis was considerably increased but no detectable change occurred in its catabolic rate.

In the febrile phase the rate of elimination of radioactivity from the animals was reduced due to retention of $^{131}$I-iodide in the body water.

**REFERENCES**


