Previous experiments have shown that the blood of most healthy human subjects yields extracts that raise the activity of the reticulo-endothelial system (R. E. S.) of rabbits, an effect which is ascribed to the presence of a positively restropic factor. In malignant disease this factor appears to be absent and the extracts contain instead a substance that depresses the functional level of the R. E. S. (negative restropic factor). In some instances, however, of both malignant and non-malignant conditions, restropic factors were not detected in the blood (1).

To determine whether a switch-over from a positive to a negative reaction takes place in the course of malignant development in animals, several experiments were carried out. The methods used were the same as those previously described. The presence or absence and the nature of the restropic factors were established by injecting extracts from blood into rabbits of known Congo red index, and the effect was again determined by the Congo red method (2, 3).

Restropic Activity of Blood of Rats and Rabbits: It has been reported previously that positively restropic extract can be obtained from the blood of normal rabbits (1). Experiments have now been extended to the blood of rats, some of which were albinos of the Wistar stock, while others were obtained from the stock of the Royal Cancer Hospital (Free). Blood of healthy animals of both sexes, about three months old, was obtained from the ventricle under ether anaesthesia. Since several cubic centimeters are required for the preparation of restropic extracts to be tested in rabbits, groups of five rats were used in each experiment, the blood being pooled. The methods of extraction and testing were identical with those used in previous experiments. One injection, corresponding to 3 c.c. of blood, was administered. Extracts obtained and tested in this manner showed positive restropic activity. The reaction of one rabbit, injected with an extract of rat blood (Fig. 1 control), illustrates the results, which were uniform. It thus appears probable that the blood of normal rabbits and intact rats contains a positively restropic substance.

Restropic Activity in the Blood of Rats Bearing a Transplantable Tumour: The question whether the appearance of negative restropin in the blood during malignant conditions is a specifically human phenomenon was investigated by examining the blood of rats bearing grafts of the Walker sarcoma. Blood was collected at various stages of tumour development. In one group, which may be described by way of illustration, the tumours had reached dimensions of $2.5 \times 2$ cm. when the blood was collected by ventricular puncture under ether anaesthesia. As in the previous experiment, the blood of five rats was pooled...
immediately on withdrawal. The extract showed a strongly negative restropic activity (Fig. 1). Similar results were obtained in three other groups of rats from which blood was collected (a) when the tumour had become palpable but was still very small, (b) when it had attained dimensions of $3 \times 3.5$ cm., (c) after it had become very large. It would thus seem that negative restropic activity is not restricted to malignancy in man, but appears also in at least one type of malignant tumour in other animals.

**Development of Negative Restropic Activity in Tumour-bearing Rats:** The change from a normal positive restropic activity to the negative effect characteristic of malignancy was studied in rats bearing the Walker sarcoma. Blood was withdrawn in the manner previously described from each of a group of five animals and pooled for testing within three to four days after the graft had been made and from other groups at longer intervals after grafting. Since the rats may require as long as ten days to recover fully from the effects of the ventricular puncture different groups had to be used. The method thus suffers from two disadvantages. Since the rate of development of the tumour and the corresponding change in the restropic activity of the blood may vary individually within any given group, the method yields not an individual index, but only a group index of restropic activity. Secondly, changes in restropic activity cannot be observed continuously in any given group.

The results of the tests are shown in Table I. Three congo red indices are given. The first corresponds to the "base line" of reticulo-endothelial function in the test rabbit; the second shows the rate of Congo red absorption twenty-four hours after the injection of the blood extract, and the third ten days after the injection.

The results may be briefly summarized as follows: Four days after grafting positive restropic activity had disappeared, the blood failing to affect the R. E. S. of the rabbit to any significant degree. In a second experiment, in which the test was made six days after grafting, positive restropic activity was also almost completely extinct, while in a third group, in which the blood was obtained on the seventh day, negative restropic activity had developed, although no tumour was yet palpable. Negative activity was found also in the blood of animals eight and fourteen days after grafting; in one animal of each
of these groups a small tumour nodule could be palpated. The last three groups shown in the table also gave negative restropic extracts, but these animals had definitely palpable or large tumours. Neither typically positive nor typically negative restropic activity was observed in a group of animals which showed no palpable tumours fifteen days after implantation and which subsequently failed to develop tumours. The result of the Congo red test in this particular group is atypical, inasmuch as a distinct rise was observed at the final determination, ten days after the injection of the extract. It is possible that this group yielded an extract of unusual properties and productive of delayed positive response, or that the rabbit reacted abnormally.

It would thus seem that the transplantable tumours used in these experiments cause the disappearance of normal positive restropic activity and the development, after a short neutral period, of negative activity even before a tumour has become palpable.

The question then arises, is the process that terminates in the development of negative restropic activity reversible. Little evidence is yet available, but the results of two experiments may be recorded in which large Walker sarcomas were removed surgically. In two groups of three rats each blood was collected seven and fourteen days respectively after ablation of the neoplasm. At that time no recurrence of the tumour had been noted. The extracts thus obtained failed to affect the R. E. S. of test rabbits, though each of the rats used previously belonged to one of two groups which yielded negative restropic samples eight and fourteen days respectively after tumor implantation.

Restropic Activity of Rabbits Treated with a Carcinogenic Compound: In a previous communication it has been shown (4) that rabbits respond to the continued administration of a carcinogenic compound (1:2:5:6-dibenzanthracene) with a gradual reduction of R. E. S. activity, which is preceded, however, by a rise in this activity if the compound is administered in benzene solution. Although malignant tumours develop but rarely in rabbits exposed to the action of carcinogenic compounds, it seems reasonable to assume that animals thus treated tend to attain a precancerous condition. Hence it seemed of particular interest to study the restropic activity of the blood of such animals as treatment progressed. Such tests could be made on individual animals, for
The observations are presented in Table II. In this table the last three columns refer not to the animal from which the blood sample was taken, but to the test rabbit used for determination of the restropic activity of the blood. This distinction between the donor of the blood and the test animal (recipient) should be borne in mind. It should also be remembered that in this experiment two distinct phenomena are considered, viz. (a) the reticulo-endothelial activity of the donor and (b) the capacity of its blood to affect the R. E. S. of untreated test animals.

The results may be briefly summarised as follows:

1. Control rabbit treated with benzene: A gradual increase of reticulo-endothelial activity took place over a period of six months; the blood of this animal retained the normal positive restropic activity.

2. Control rabbit treated with oil: No change in the reticulo-endothelial activity occurred during seven months of treatment. The blood retained its positive restropic activity and raised the Congo red index of the test animal from 59 to 71. The subsequent fall of the index of the test animal to a level below the "base line" is not to be regarded as specific, as a similar fall has occasionally been observed when extracts from human blood were employed.

3. Rabbits treated for several months with dibenzanthracene dissolved in oil or benzene: Rabbit 17 was tested on five occasions. After four months and again after seven months of treatment reticulo-endothelial activity was found to be diminished and the blood to have lost its positive activity without developing negative restropin. After eight months impairment of reticulo-endothelial function was more pronounced and a distinct negative activity was sufficiently large samples of blood can be withdrawn from a rabbit at fairly frequent intervals. Fourteen experiments have been carried out to date. Five c.c. of venous blood was collected at intervals of about thirty days, and extracts were prepared and injected into an intact test rabbit.

<table>
<thead>
<tr>
<th>No.</th>
<th>Substance Injected</th>
<th>Period of Treatment (Months)</th>
<th>Initial Congo Red Index</th>
<th>Index at Time of Test</th>
<th>Degree of Change in Index *</th>
<th>Before Injection</th>
<th>24 Hours after Injection</th>
<th>10 Days after Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Oil</td>
<td>7</td>
<td>43</td>
<td>45</td>
<td>+ 4</td>
<td>59</td>
<td>72</td>
<td>39</td>
</tr>
<tr>
<td>19</td>
<td>Benzene</td>
<td>6</td>
<td>32</td>
<td>45</td>
<td>+17</td>
<td>48</td>
<td>68</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>Dba/Benz</td>
<td>9</td>
<td>48</td>
<td>25</td>
<td>-48</td>
<td>50</td>
<td>32</td>
<td>59</td>
</tr>
<tr>
<td>11</td>
<td>Dba/Benz</td>
<td>8</td>
<td>50</td>
<td>28</td>
<td>-44</td>
<td>47</td>
<td>36</td>
<td>34</td>
</tr>
<tr>
<td>12</td>
<td>Dba/Oil</td>
<td>8</td>
<td>45</td>
<td>30</td>
<td>-33</td>
<td>43</td>
<td>38</td>
<td>44</td>
</tr>
<tr>
<td>15</td>
<td>Dba/Benz</td>
<td>6</td>
<td>32</td>
<td>44</td>
<td>+38</td>
<td>37</td>
<td>57</td>
<td>37</td>
</tr>
<tr>
<td>14</td>
<td>Dba/Benz</td>
<td>6</td>
<td>50</td>
<td>30</td>
<td>-40</td>
<td>52</td>
<td>43</td>
<td>36</td>
</tr>
<tr>
<td>14</td>
<td>Dba/Benz †</td>
<td>8</td>
<td>50</td>
<td>47</td>
<td>-6</td>
<td>40</td>
<td>31</td>
<td>43</td>
</tr>
<tr>
<td>17</td>
<td>Dba/Oil</td>
<td>5</td>
<td>55</td>
<td>44</td>
<td>-20</td>
<td>36</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>17</td>
<td>Dba/Oil</td>
<td>7</td>
<td>55</td>
<td>32</td>
<td>-42</td>
<td>43</td>
<td>39</td>
<td>52</td>
</tr>
<tr>
<td>17</td>
<td>Dba/Oil</td>
<td>8</td>
<td>55</td>
<td>28</td>
<td>-49</td>
<td>38</td>
<td>26</td>
<td>36</td>
</tr>
<tr>
<td>17</td>
<td>Dba/Oil</td>
<td>9</td>
<td>55</td>
<td>20</td>
<td>-64</td>
<td>45</td>
<td>32</td>
<td>48</td>
</tr>
<tr>
<td>17</td>
<td>Dba/Oil</td>
<td>10</td>
<td>55</td>
<td>28</td>
<td>-49</td>
<td>40</td>
<td>22</td>
<td>34</td>
</tr>
</tbody>
</table>

*Expressed as percentage of initial index. † Test made after interruption of treatment (see previous paper, page 257).
noted in the blood sample. This finding was confirmed with a blood sample withdrawn one month later.

A similar result was obtained in Rabbit No. 7. Five months after the institution of treatment impairment of the reticulo-endothelial function of this donor was distinct and negatively restropic activity had developed in the blood. Similar observations were made in animals 11 and 14.

Two further findings may be mentioned. Rabbit 15 reacted atypically, as has been mentioned in a previous communication (4), failing to show a reduction of reticulo-endothelial activity after treatment with 1:2:5:6-dibenzanthracene (benzene solution) for six months. Rabbit 12 gave a lower Congo red index after eight months treatment than at the beginning of the experiment, but the blood, though losing its positive effect, did not develop negative restropic activity.

No malignant condition was noted in any of the donor animals in these experiments at the time of the test, though it is of course possible that precancerous changes may have been present.

The experiments indicate that the artificial production of a precancerous condition tends to be accompanied by the development of negative restropic activity in the blood, preceded by a period during which positive restropic activity is lost. A quantitative comparison of successive tests is impossible because of the differing "base lines" of reticulo-endothelial activity in the various test animals.

**DISCUSSION**

The present experiments support the view that positive restropic activity is a common characteristic of the blood of healthy subjects, human or animal. They show, further, that negative restropic activity in malignant conditions is not restricted to the human species, where it was first found. Whether negative restropic activity occurs only in malignant conditions cannot yet be stated, though it may be provisionally suggested that negative restropic activity is generally characteristic of malignant conditions and of physiological states favouring malignancy.

Isolated instances of indifferent reaction may be regarded as devoid of significance, for absence of restropic activity may be simulated as a result of experimental errors in the preparation of the extract. In view of the present experiments, in which an indifferent reaction was obtained between the disappearance of positive and the appearance of negative restropic activity, it seems likely that the absence of restropic activity reflects a condition no less definite than either the positive or the negative state. Clearly, the neutral condition may precede the development of a tumour, but there is no evidence that it is restricted to precancerous states; in fact, neutral extracts have been obtained both in healthy human subjects and in patients suffering from advanced malignant disease.

Absence of restropic activity may occur not only as a transitional stage in the development of a neoplasm, but may also be found after its removal, this recurrence of a neutral reaction being the only present indication that a negative restropic state of the blood may be reversed. The existence of neutral
DEVELOPMENT OF MALIGNANCY IN ANIMALS

reactions also demonstrates that neither positive nor negative activity can be referred to a substance invariably present in the blood.

The question whether negative restropic activity precedes or follows the development of a malignant tumour would seem to be decided by the present experiments, for negative restropin was obtained both from the blood of rabbits that showed no clinical signs of neoplastic development and from rats in which no tumour was palpable. The existence of a malignant condition cannot be excluded, however, on clinical evidence. For this serial experiments are required involving complete microscopic examinations of animals at the phase of treatment when negative restropic activity is first found. At present it would seem that in the rat and in the rabbit the development of a tumour to a stage where it is detectable by direct observation may be preceded by the appearance of a negative restropic activity.

These experiments also throw some light on the relationship between the functional level of the R. E. S. and the restropic activity of the blood in the same animal. In considering this question, it must be remembered that we are dealing with quantitative variations of two distinct phenomena. The activity of the R. E. S. is determined directly, i.e., in the animal itself, whereas the restropic activity of the blood is measured indirectly, i.e., in another animal. There is ample evidence that both the basic activity of the R. E. S. and its reactivity to restropic factors vary widely from animal to animal. This variation, together with the necessity of employing two animals, makes it difficult to correlate the activity of the R. E. S. of any given rabbit with the degree and type of the restropic activity of its blood.

On the whole, the present study suggests that the two properties change together following treatment with a carcinogenic agent. Thus, negative restropic activity was noted only after the Congo red index had fallen well below the base line; conversely, positive activity persisted in an animal which did not show a typical response to the carcinogenic agent. The correlation is, however, by no means complete. In the first place, negative restropic activity seems to develop later than the fall of the index; secondly, the change in restropic activity, once developed, seems not to be subject to the fluctuations that occur in the index; finally, it does not seem as if the R. E. S. of the donor of negative restropin fully reflects the presence of negative restropin in the circulation. Although the difference between the initial level of the R. E. S. and its level after prolonged treatment is pronounced and corresponds with that seen in animals that have been treated with negatively restropic extract, this fall is established gradually over a period of several months, whereas the injection of negatively restropic extract takes effect within the space of twenty-four hours. In other words, animals that had been treated with dibenzanthracene developed negative restropin in their blood, but the activity of the R. E. S. did not seem to decline at a corresponding rate.

The causes of this lack of correspondence are now under investigation.

Summary

(1) The blood of intact rats and rabbits yields positive restropic extracts.
(2) The implantation of Walker rat sarcoma is followed by the disappear-
ance of the positive and, at a later stage, by the appearance of a negative restropic factor.

(3) In rabbits treated with 1:2:5:6-dibenzanthracene the positive reaction disappears at a stage when the index has fallen below the base line. Subsequently the blood may develop negative activity, even though no tumour can be detected by direct observation.

**NOTE:** The tumour-bearing animals used in these experiments were received from the Research Institute, Royal Cancer Hospital (Free). The writers wish to express their thanks to Prof. E. L. Kennaway.

**REFERENCES**