THE RELATION BETWEEN BLOOD DESTRUCTION AND
THE OUTPUT OF BILE PIGMENT.

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Many facts point to a close relation between blood destruction and
the yield of pigment in the bile. The assumptions currently made in
this connection by physiologists and clinicians have recently been
summarized by one of us and critically reviewed.¹ A method now
available for total bile collection² renders possible an experimental
test of some of them.

The Pigment Changes Following Bile Loss.

In dogs losing all their bile, significant changes take place in the
hemoglobin percentage of the blood and in the bilirubin content of
the secretion lost. They have been briefly described in a previous
paper in which the methods employed for the care of the animals and
the study of the bile have also been outlined.³ As a rule, for several
days after the operation whereby drainage of the common duct is
effected, the hemoglobin percentage alters but little (Text-figs. 1 and
2), though in general it tends to fall slowly to about four-fifths of
the initial value in the course of a week (Text-fig. 1). The bilirubin
output by contrast falls off briskly from the beginning, and at the
end of the same period of time averages only about half of the first
amount (Text-fig. 3).⁴ There can be no doubt that the large early
amount is sometimes derived in part from extravasated blood. The
yield from dogs in which hemorrhage into the tissues was known to

¹ Rous, P., Physiol. Rev., 1923, iii, 75.
⁴ See also McMaster, Broun, and Rous, Text-fig. 2. Several instances com-
plicated by blood extravasation are there included.
TEXT-FIG. 1. Changes in the hemoglobin percentage expressed in terms of the finding on the 1st day after operation.—Uncomplicated instances.

TEXT-FIG. 2. (See Table I.) Unusually marked postoperative changes in the hemoglobin percentage and bilirubin output. The periods dealt with in Table I, during which the hemoglobin percentage fell rapidly, are included between the bracketed arrows on the chart.
have taken place increased markedly during the period when the hematoma was breaking down (as, for example, in Dog 30 of Text-fig. 6). Yet when careful hemostasis was practised, and at most only a few cubic centimeters of blood left the vessels, the pigment yield halved in from 7 to 10 days (Text-fig. 3).

**TEXT-FIG. 3.** Postoperative changes in the bilirubin output in uncomplicated instances, as expressed in percentages of the yield of the 1st day.

There has been no general recognition of these postoperative changes for the excellent reason that animals in which a bile fistula has been established after the classical fashion are ordinarily not worked with until wound healing has taken place. Brugsch and Retzlaff alone appear to have noted them, remarking as an aside to other matters that during the first 14 days of bile loss there is a falling off in number of red cells and bilirubin output. They furnish no figures on the point. Their dogs were suspended in slings and all the bile was collected.

The later pigment changes vary with the individual. In our animals they were unquestionably affected by certain of the experi-

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The chart shows the percentage of hemoglobin destruction over time. Per cent hemoglobin, per cent bilirubin, and per cent albumin changes are plotted over the x-axis. The y-axis represents the percentage values. The chart includes several data points labeled with letters and numbers. The text at the bottom of the page indicates that the chart should be used with Table I.
mental procedures employed, notably by exercise and by bile feedings. But always in uncomplicated instances, the anemia indicated by the early drop in the hemoglobin percentage persisted, though varied, at times, by intervals of more or less complete recovery. Usually it remained slight. We are inclined to believe that in the two animals in which it became marked (see Text-fig. 4; also McCMaster, Broun, and Rous), there was another factor involved besides loss of the bile. Dog 23 of Text-fig. 4 of the present work had a severe intercurrent bronchitis, and later a gradually developing intestinal obstruction; while Dog 24 of the previous paper occasionally lost blood from hemorrhoids. With mild anemia the hemoglobin percentage as a rule altered but slightly from week to week, as did the bilirubin output also. Every well defined blood change found reflection in a change in this last. In the two dogs just mentioned, with pronounced anemia, remissions occurred at frequent intervals, the hemoglobin percentage rising for a time only to fall again, usually to a lower level; and as it rose and fell so too did the yield of bilirubin. The subjective element can be ruled from account in connection with the findings; for the estimations on blood and bile were made by different workers each unaware of the other's results, while, furthermore, the remarkable correspondence in the pigment changes did not come to attention until the data were correlated, weeks after they had been obtained. The synchronous alterations were slow and wave-like, often as much as a fortnight elapsing between crest and crest. Circumstances have prevented us from following them for more than 3 months.

The character of the anemia was repeatedly studied. Always it was of secondary type, as shown by cell counts and the examination of stained smears. The color index was somewhat, but not greatly, less than the normal; poikilocytosis, though present, was never marked. The number of circulating reticulated cells, which was followed by Robertson's technique, proved to be even less than in healthy dogs.
The inference seems justified that the hematopoietic tissue was less active than usual. Certainly it was putting forth cells somewhat deficient in pigment.

The charts leave no room for doubt that a quantitative relationship of some sort exists between the blood and bile pigments. But is it the relationship generally supposed? The calculations in vogue nowadays on blood destruction as expressed in the yield of bilirubin or urobilin have for basis the assumptions that from the hemoglobin molecule 4 per cent by weight of hematin is derived, and that for every gram of hematin approximately 1 gm. of bilirubin is excreted. Do the data of our experiments accord with such a view?

Stadelmann long ago noted that after injections of hemoglobin the expected rise in the bilirubin content of the bile was incompletely realized. His finding seems to have been wholly lost sight of. Recently Whipple has sponsored the conception that bilirubin has other sources besides the blood, a principal one being the carbohydrates of the food. We have corroborated the observations of Whipple and Hooper on the effects of carbohydrate diet to alter the bilirubin output transiently but have shown that the alteration is referable merely to a changed rate of pigment evacuation, not to a change in the amount manufactured from day to day, which remains unaffected. Whipple and Hooper collected the bile during 6 or 8 hours of each 24. They made interesting observations on the pigment excreted during this time, following injections of the hemoglobin of laked blood, but the circumstances of the work were such that they could reach no conclusion on quantitative relationships. They noted, however, that in anemic animals the bilirubin output for the 6 hour period was sometimes astonishingly small.

The Calculated Pigment Relationships.

As already stated, every considerable drop in the hemoglobin percentage of our dogs, uninduced by extraneous means, was accompanied by a drop in the bilirubin yield. Granting that a lessened hemoglobin

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percentage means a proportionately lessened amount of the pigment in circulation, one can gain an idea from the data as to whether the known amount of bilirubin excreted during the period in which the blood pigment apparently underwent a lessening represents accurately the amount of blood withdrawn from circulation, or, to speak loosely, destroyed. In Dog 20, for example, the hemoglobin percentage fell from 125 per cent to 86 per cent in the course of 10 days. Does this change find exact expression in the bilirubin output?

Several potential sources of error or complication must be considered prior to an analysis along the lines indicated. Alterations in the hemoglobin percentage are known to be only roughly indicative of changes in the total amount of the pigment. And may not a part of the bilirubin from blood suddenly destroyed be excreted so slowly by the liver as to escape reckoning? The charts furnish evidence on the point. They show that whenever there was a sharp decline in the hemoglobin percentage an equally sharp decline in the bilirubin output took place, both reaching their lowest point at the same time. This could not have happened had bilirubin excretion been notably delayed. The possibility that the substance has other sources besides hemoglobin can, in the present relation, be dismissed. If it has such sources these would appear to be negligible in the present relation, as witness the charts. Changes in the diet influence only the rate of pigment evacuation, and this transiently. But exercise, by increasing the rate of blood destruction, may act to increase markedly the total yield. To minimize this disturbing influence the animals were confined in metabolism cages. Noteworthy errors in bile collection and in bilirubin quantitation were ruled out by the methods employed; and only such figures on hemoglobin percentage have been admitted to consideration as were corroborated by the findings of the preceding or subsequent 24 hours. The factor of blood losses masked by replacement must have influenced the results, sometimes, doubtless, to an important extent. For during the periods supplying the data under analysis, in which the hemoglobin percentage fell, there must have been some repair of the blood loss, since the formation of corpuscles can scarcely have ceased altogether. But for present purposes it will be simplest to suppose that it did cease, and that in consequence the fall in hemoglobin represents the actual blood loss, not, as was actually the case, a somewhat greater loss compensated in part by replacement.

In Table I the actual bilirubin output is contrasted with the expected output during four periods (indicated by brackets and arrows on Text-figs. 2 and 4), in which the hemoglobin percentage lessened rapidly and markedly, without loss from the body of blood, bile pigment, or uroblin, in the stools or urine. Dog 23 of Text-fig. 4 was, like the other animals, in excellent condition at the time, giving no sign of the complicating maladies which rendered its later condition poor. The data on Dog 24 to be found in a previous paper have not been tabulated
### Table I.

**Bilirubin Output during an Intercurrent Fall in the Hemoglobin Percentage (Text-Figs. 2 and 4).**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Weight</th>
<th>Period included</th>
<th>Calculated blood amount</th>
<th>Hemoglobin</th>
<th>Bilirubin output</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg.</td>
<td>days</td>
<td>cc.</td>
<td>per cent</td>
<td>per cent</td>
<td>gm.</td>
</tr>
<tr>
<td>23</td>
<td>14(\frac{1}{2})</td>
<td>5</td>
<td>1,334</td>
<td>98</td>
<td>73</td>
<td>25</td>
</tr>
<tr>
<td>25(a)</td>
<td>16</td>
<td>6</td>
<td>1,471</td>
<td>115</td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td>25(b)</td>
<td>16</td>
<td>7</td>
<td>1,471</td>
<td>115</td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td>20</td>
<td>12(\frac{1}{2})</td>
<td>10</td>
<td>1,172</td>
<td>125</td>
<td>86</td>
<td>39</td>
</tr>
</tbody>
</table>
because the animal occasionally bled from hemorrhoids. The losses in total hemoglobin have been calculated from the percentage changes, on the basis that 9.2 per cent of the body weight of the animal consists of blood; 17 that a blood having 100 per cent of hemoglobin by the Newcomer standard we employed has 13.8 per cent of hemoglobin in every 100 cc.; and that hemoglobin yields 4 per cent by weight of hematin. 18 Most authors assume that hematin appears weight for weight as bile pigment, but in the calculations we have preferred to adopt the conservative estimate of Eppinger and Charnas 19 that for every gram of hematin 0.9 gm. of bilirubin is elaborated.

As the four tabulated instances show (Table I), the actual bilirubin output during periods of blood destruction came to but a fraction of the expected output, averaging about one-third of it, despite the circumstance that several elements in our calculations combined to give a minimal figure for this latter. Corroboratory examples could be culled from our unpublished data.

The Actual Pigment Relationships.

Experiments were now begun to measure the amount of blood destruction more exactly. Advantage was taken of the regularity with which anemia follows upon bile loss, and the total hemoglobin of several animals was ascertained 24 hours prior to intubation of the common duct, and again some days later when the hemoglobin percentage had dropped (Text-fig. 5). The decrease in circulating pigment thus measured must be referred in part to hemorrhage at operation; and though, owing to hemostasis, such part was negligible, it introduces an element of uncertainty into the data. For this reason some later estimates of total hemoglobin were made, to be compared with the findings a few days after operation. But in only one of three instances had any demonstrable loss of hemoglobin occurred in the interval (Text-fig. 5). In order to provide a destruction of corpuscles for the purpose of the observations, recourse was ultimately had to treadmill exercise, 20 and to the transfusion of alien blood.

18 The figure for the standard was determined by Dr. J. M. Neill of the Hospital of The Rockefeller Institute, who kindly carried out for us analyses of the pigment content of specimens of dog blood.
In Tables II and III and Text-figs. 5 and 6, the results of all the experiments find place. The dogs had, without exception, the appearance of vigorous health. They were fed for the most part on a mixture of bread and cooked lean meat in fixed proportion, but occasionally received bread and milk, or raw lean meat. The stools, which were clay-colored, were regularly examined for blood (guaiac reaction) and for stercobilin (Schlesinger's test). Several animals that had been destined for the work were discarded because one or both of the pigments was found. Neither occurred in any of the animals employed. The urine, daily searched for urobilin and urobilinogen and hematoporphyrin, was regularly devoid of the first mentioned substances and of abnormal amounts of the last. Whenever bilirubinuria was sufficiently pronounced for quantitation, the amount of bile pigment escaping in this way was determined according to a procedure already described. 21 Such determinations were necessary in but one animal, Dog 19 (Text-fig. 6). Whenever the bile became infected, incubation tests were carried out to find whether the organisms altered bile pigment. This they never did, nor did they prove pathogenic for the animal harboring them. The dogs were the last of a considerable series intubated for bile collection and fared best of all, the laparotomy wound healing by first intention in practically every case. They were sacrificed after from 1 to 3 months of observation, sometimes because of the development of total obstruction from stone in the common duct, and again for reasons of expediency.

The method of Van Slyke and Salvesen 22 was employed for the determination of total hemoglobin, and by one of us familiarized with it through much previous work. 20 The routine observations on the hemoglobin percentage were made by another individual, and it has been reassuring to observe,—as bearing out an assumption upon which Table I is based,—how nearly the percentage changes in most instances correspond with those in the total pigment. To arrive at a figure on the expected output of bilirubin, the current view on the quantitative relationship between hemoglobin and this pigment was adopted, as it had been for Table I. And, as in the case of this table, the influence of the hematopoietic activities to mask the amount of blood destruction, making it appear less than it actually is, has been left from account. In some instances there was a 24 hour period prior to operation during which, of course, no bile could be collected. The actual bilirubin output for the time can only be conjectured. We have supposed it to equal the largest amount subsequently obtained in the same period of time.

Intercurrent Losses of Total Hemoglobin (Table II, Text-Fig. 5).— In Dog 26 the loss in total hemoglobin during the period of the 6 days following operation, together with the 24 hours immediately preceding it, would seem to have been extremely large,—equivalent to that in

about 500 cc. of whole blood,—despite the fact that hemostasis was nearly perfect. The actual output of bilirubin proved to be less than a third of that warranted by such blood destruction. During the next 6 days the loss of blood was less, though still considerable. It was almost exactly indicated by the changes in the hemoglobin percentage, which had not been the case at first. The actual output of bilirubin now came to one-third of the expected output.

Dog 30 lost during 5 days, inclusive of that before operation, an amount of hemoglobin such as had existed in about 300 cc. of the whole blood. The actual bilirubin yield for the period totalled 43 per cent of the expected quantity. When the total hemoglobin was next determined 10 days later, a more moderate loss in it was found. The actual bilirubin output now slightly exceeded the expected one. It goes without saying that the smaller a hemoglobin loss, and the greater the period of time over which it is distributed, the more important will the factor of cell replacement be, as masking the amount of destruction. Gradual losses may be wholly concealed by replacement. When this is the case the daily bilirubin yield will be considerable, although, since the hemoglobin percentage does not alter, none whatever would be expected under the limiting conditions of our calculation in which the element of replacement is ignored. The findings in the second period of Dog 30, in which the actual bilirubin output exceeded the calculated figure, afford a partial illustration of these facts. They are more strikingly evident in the data on Dog 28.

The hemoglobin percentage of Dog 28 fell abruptly during an initial period of 5 days without any noteworthy diminution in the total hemoglobin. The actual bilirubin output for the period many times exceeded the expected one. An error in the first estimate of total hemoglobin may be invoked to explain the anomalous results, and the discrepancy between the changes in percentage of the pigment and its total quantity point to such a probability. But if the claim be allowed, a similar one must be lodged against the findings for the first period of Dog 26, in which a discrepancy of almost as great dimensions, but opposite in direction, was observed.

During the second period of Dog 28 there was almost no change in the percentage and total quantity of the blood pigment. Whatever the daily loss of cells may have been it was practically concealed by
### TABLE II.

**Bilirubin Output during Intercurrent Changes in Total Hemoglobin (Text-Fig. 5).**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Weight</th>
<th>Elapsed period</th>
<th>Cell volume</th>
<th>Plasma volume</th>
<th>Blood volume</th>
<th>Hemoglobin per 100 cc. of blood</th>
<th>Total hemoglobin</th>
<th>Loss of hemoglobin within the body, *</th>
<th>Bilirubin output for period,</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg.</td>
<td>days</td>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>25½</td>
<td>7</td>
<td>1,121</td>
<td>968</td>
<td>2,089</td>
<td>21.12</td>
<td>441.5</td>
<td>105.05</td>
<td>4.38 1.33</td>
<td>Total hemoglobin determined on the day previous to operation.</td>
</tr>
<tr>
<td>26</td>
<td>22½</td>
<td>6</td>
<td>941</td>
<td>837</td>
<td>1,778</td>
<td>15.7</td>
<td>330.5</td>
<td>279.0</td>
<td>46.49 1.94 0.69</td>
<td>After 5 days of bile collection.</td>
</tr>
<tr>
<td>30</td>
<td>19</td>
<td>5</td>
<td>990</td>
<td>935</td>
<td>1,925</td>
<td>16.45</td>
<td>316.2</td>
<td>260.0</td>
<td>51.43 2.05 0.88</td>
<td>12th day of bile collection. Bile infected after the 8th day. No bilirubinuria.</td>
</tr>
<tr>
<td>30</td>
<td>18</td>
<td>10</td>
<td>795</td>
<td>904</td>
<td>1,709</td>
<td>13.67</td>
<td>260.0</td>
<td>233.3</td>
<td>22.37 0.90 1.1</td>
<td>Total hemoglobin determined on the day previous to operation.</td>
</tr>
<tr>
<td>30</td>
<td>17½</td>
<td>10</td>
<td>826</td>
<td>925</td>
<td>1,755</td>
<td>14.81</td>
<td>260.0</td>
<td>233.3</td>
<td>22.37 0.90 1.1</td>
<td>After 4 days of bile collection.</td>
</tr>
<tr>
<td>30</td>
<td>18</td>
<td>10</td>
<td>795</td>
<td>904</td>
<td>1,709</td>
<td>13.67</td>
<td>260.0</td>
<td>233.3</td>
<td>22.37 0.90 1.1</td>
<td>13th day after operation. Bile sterile throughout.</td>
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<tr>
<td></td>
<td>14½</td>
<td>15</td>
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<tr>
<td></td>
<td></td>
<td>5</td>
<td>559</td>
<td>619</td>
<td>1,178</td>
<td>14.83</td>
<td>174.8</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>569</td>
<td>642</td>
<td>1,201</td>
<td>13.95</td>
<td>167.8</td>
<td>2.63</td>
<td>0.11</td>
<td>0.73</td>
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<td></td>
<td>15</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>543.5</td>
<td>746.5</td>
<td>1,290</td>
<td>12.49</td>
<td>161.0</td>
<td>2.76</td>
<td>0.11</td>
</tr>
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<tr>
<td>Total hemoglobin determined on the day previous to operation.</td>
<td></td>
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<tr>
<td>After 4 days of bile collection.</td>
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<tr>
<td>After 7 days of bile collection. Bile sterile throughout.</td>
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</tr>
</tbody>
</table>

*As distinct from the loss incurred in the determinations.
No bilirubinuria in any animal except No. 30, in which an occasional trace was found, as in many normal animals.
replacement. Under such circumstances the actual output of bilirubin could not but greatly exceed the calculated one, as was indeed the case.

\[ X = \text{Hemoglobin per cent as calculated from the reduction in total hemoglobin.} \]

\[ \Delta = \text{Hemoglobin per cent as calculated from the reduction in total hemoglobin and corrected for change in body weight.} \]

\[ \text{--- --- = Actual bilirubin output per hour during the period.} \]

\[ \text{------ = Expected output.} \]

TEXT-Fig. 5. (See Table II.) The actual and the calculated bilirubin yields during periods of intercurrent losses of total hemoglobin.

**Induced Losses of Total Hemoglobin (Table III, Text-Fig. 6).—**

Two dogs (Nos. 28 and 30) employed for the work just discussed were exercised some weeks later on a treadmill for 3 to 5 hours on each of 4 successive days, in order to bring about a rapid destruction of red cells. Experience had shown that exercise of the sort could not be
begun immediately after a determination of total hemoglobin by the CO method without great risk of hemorrhage into the tissues through the puncture openings in the jugular veins; while, furthermore, the dogs were not fitted to run well until all the hemoglobin was once again in a condition to combine with oxygen. For these reasons the total blood pigment was ascertained 18 to 24 hours prior to use of the treadmill. Directly after the animal was taken from the mill on the 4th day, the total hemoglobin was again measured, although the 24 hour period of bile collection which included the final exercise hours did not come to an end until the next morning. It follows that the observations upon the blood were not wholly synchronous with those upon the bile but preceded the latter by about 18 hours on the average. Whether one ignore this fact or make allowance for it in computing the actual bilirubin output for the days of exercise,—and we have done both in Table III,—the result is the same. The actual bilirubin output of the animals amounted to little more than half of the expected one.

The destruction of compatible blood transfused to dogs is known to begin early and to proceed rapidly. The course of events in Dog 19, which was somewhat anemic when given 328 cc. of citrated dog blood containing 51.5 gm. of hemoglobin, well illustrates the rule. The concurrent alterations in hemoglobin quantity and bilirubin output proved highly instructive. In a first period of 2 days, following introduction of the strange blood, there was a considerable destruction of cells, as shown by the alterations in total hemoglobin, though without bilirubinuria or symptoms. The increase in the yield of bile pigment was many times less than it should have been on calculation. Blood destruction proceeded more gradually during the next 7 days, as evidenced both by the day to day reductions in the per cent of hemoglobin and the changes in total amount. Bilirubinuria was still absent, and the general condition of the dog excellent. The actual yield of bile pigment for this period was not greatly less than the expected. Now the state of affairs altered almost over night. The hemoglobin percentage began to fall more rapidly, there was pronounced bilirubinuria, and an anemia developed more marked than that prior to transfusion. There was no tissue icterus, but the animal appeared languid. The causes for the developing anemia have been
TABLE III.

**Bilirubin Output during the Course of an Induced Reduction in Total Hemoglobin (Text-Fig. 6).**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Weight</th>
<th>Treatment</th>
<th>Elapsed period</th>
<th>Erythrocytes</th>
<th>Plasma volume</th>
<th>Hemoglobin per 100 cc. of blood</th>
<th>Total hemoglobin</th>
<th>Loss of hemoglobin within the body*</th>
<th>Bilirubin output for period</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>18</td>
<td>5 hrs. of treadmill exercise on each day except the last, when 3 hrs. only was given.</td>
<td>days</td>
<td>cc.</td>
<td>cc.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>821</td>
<td>1,024</td>
<td>1,845</td>
<td>13.95</td>
<td>257.4</td>
<td>17.4</td>
<td>The total hemoglobin was determined 22 hrs. before beginning exercise, and again 3 hrs. after its close. No bilirubinuria. Bile sterile.</td>
</tr>
<tr>
<td>28</td>
<td>14‡</td>
<td>5 hrs. of treadmill exercise on each day except the last, when 4 hrs. only was given.</td>
<td></td>
<td>531</td>
<td>669</td>
<td>1,200</td>
<td>14.1</td>
<td>109.2</td>
<td>15.05</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>511.5</td>
<td>704.5</td>
<td>1,216</td>
<td>12.35</td>
<td>150.1</td>
<td></td>
<td>The total hemoglobin was determined 22 hrs. before beginning exercise, and again 3 hrs. after its close. No bilirubinuria. Bile infected.</td>
</tr>
<tr>
<td>13</td>
<td>Transfused.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>564</td>
<td>598</td>
<td>1,162</td>
<td>15.8</td>
<td>183.6</td>
<td>The total hemoglobin was determined 2 hrs. before transfusion.</td>
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<tr>
<td>2</td>
<td>656</td>
<td>483</td>
<td>1,139</td>
<td>19.11</td>
<td>217.6</td>
<td>12.58</td>
<td>0.5</td>
<td>0.11</td>
<td>No bilirubinuria. Bile infected.</td>
<td></td>
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<tr>
<td>7</td>
<td>602</td>
<td>532</td>
<td>1,134</td>
<td>16.94</td>
<td>192.2</td>
<td>19.89</td>
<td>0.8</td>
<td>0.505</td>
<td>No bilirubinuria.</td>
<td></td>
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<tr>
<td>8</td>
<td>492</td>
<td>706</td>
<td>1,198</td>
<td>11.58</td>
<td>138.9</td>
<td>48.75</td>
<td>1.95</td>
<td>1.57</td>
<td>Marked bilirubinuria (the 85 mg. of pigment excreted during the period are included in the actual bilirubin output).</td>
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* As distinct from the loss incurred in the determinations.
† Total bilirubin output during the four 24 hour periods of bile collection which cover the days on which the dogs were exercised.
dealt with by Robertson. Prominent among them is marrow inactivity induced by the plethora after transfusion. Because of the temporary lack of need for new red cells, there occurs almost no elaboration of them by the animal while the plethora lasts, and when the strange blood is suddenly made away with through the action of induced antibodies, anemia inevitably develops because the residuum of host corpuscles is small. The expected bilirubin output during

\[ 7.2 \text{ mg.} \]

\[ D_0 30-18 \text{ kg.} \]

\[ 18 \text{ kg.} \]

\[ 30 \text{ kg.} \]

\[ P_0 30-18 \text{ kg.} \]

\[ D_0 30-18 \text{ kg.} \]

\[ 18 \text{ kg.} \]

\[ 30 \text{ kg.} \]

\[ P_0 30-18 \text{ kg.} \]

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\[ D_0 30-18 \text{ kg.} \]

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\[ D_0 30-18 \text{ kg.} \]

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\[ P_0 30-18 \text{ kg.} \]

\[ D_0 30-18 \text{ kg.} \]

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\[ 30 \text{ kg.} \]

\[ P_0 30-18 \text{ kg.} \]

\[ D_0 30-18 \text{ kg.} \]

\[ 18 \text{ kg.} \]

\[ 30 \text{ kg.} \]

\[ P_0 30-18 \text{ kg.} \]
this period of rapid destruction in Dog 19 was large, more than twice that of the days immediately preceding, yet the actual output came nearer to equalling it than previously. Thereafter an abrupt turn for the better in the general condition was noted, the bilirubinuria ceased, the bile became much less pigmented, and recovery from the anemia began. The total amount of hemoglobin was not ascertained again.

DISCUSSION.

In the analysis (Tables II and III) of the data from the last two sets of experiments here discussed, the actual bilirubin output has been contrasted with the expected one which was estimated from the known loss in total hemoglobin as if so much pigment in the form of blood had been taken from a jar and bilirubin derived from it by chemical means. As already mentioned, losses masked by replacement through the hematopoietic activities were disregarded, and of necessity, since they could not be gauged. Despite their potential influence to increase the bilirubin output, this was always less, usually several times less, than the expected quantity whenever a marked and rapid loss of the blood pigment was demonstrable.

These observations show clearly that the quantitative relation existing between blood destruction and the bilirubin output is very different from that generally supposed. Save under special circumstances, which themselves go to prove the rule, the yield of bile pigment was far below what it should have been, to judge from the amount of hemoglobin removed from the circulation. Under the special circumstances referred to, when the body was oversupplied for the time being with the products of blood destruction, as shown by the state of the urine, the actual yield of bilirubin approached the calculated one. Usually the actual yield was less than half of this latter, which was computed on a basis that yielded a minimal figure for it.

Why, in our experiments, did the actual yield of bilirubin fall so far short of the expected one? The quantitative relationships between hemoglobin, hematin, and bilirubin, as such, can scarcely be impeached. Nor do the charts afford indications of any delay in the excretion of bile pigment. Other sources of it there may be besides the blood, but, as has been said, these could have no influence under the conditions of the work. The explanation evidently is that a
part of the pigment from destroyed blood, out of which bilirubin can be formed, undergoes retention within the body,—a possibility suggested by Stadelmann many years ago. That the iron-containing derivatives of the hemoglobin molecule are held for future use has been generally acknowledged. Bilirubin has been thought of as waste stuff. But recently we have brought evidence that some of it is reabsorbed after reaching the intestine. Whether there is a salvage of material by this means for later use in the manufacture of new red cells remains to be determined. But the blood and the bile changes which follow upon loss of the bile must be discussed in the light of such a possibility.

An immediate question in connection with the postoperative blood changes recorded in the present paper is whether they are referable to the loss of pigment in the bile. No other cause for them was evident if one except a not infrequent reddening of the duodenum, unaccompanied by hemorrhage and consequent doubtless on the absence of bile. Anemia developed in every one of eleven animals studied with special reference to the point, most of them extremely vigorous and all kept under conditions and on a diet which suffice in normal dogs for the maintenance of an excellent blood state. The hemoglobin percentage of Whipple and Hooper's long tended fistula dogs remained consistently high. The observations of these authors appear to have been begun a fortnight or more after operation, when the fistula wound had healed. They attribute the well-being of their dogs to liver feedings or the intermittent passage of a little bile into the intestine through some narrow channel in the tissues. The animals had access to the external fistula opening during 16 or 18 hours of each 24 and may well have licked up some of the escaping bile,—though that they obtained much of it seems unlikely. Bile deprivation in our dogs was complete. Furthermore, ours were kept caged because of the influence of exercise upon the bilirubin output, whereas Whipple and Hooper's were allowed to run in a yard daily. Exercise, as Broun has shown, stimulates the hematopoietic tissue. Possibly it is responsible for alterations in hemoglobin metabolism that are sufficient to counteract the tendency to anemia after bile loss. How-

ever this be, the present findings leave no doubt that such a tendency exists; and the demonstrated activity of the body to conserve bile pigment, together with its ability to retrieve a portion of that excreted into the intestine, give reason for the belief that the anemia results from bilirubin loss. Human beings with a bile fistula frequently develop anemia—in one case reported by Balfour and Ross\textsuperscript{24} the hemoglobin had fallen to 30 per cent after 3 years of bile loss, although the physical condition of the individual was otherwise good. But many patients losing all the bile fail rapidly and are the subject of complicating disturbances to which such blood changes as occur may well be referable.

The rapid falling off in the bilirubin output during the 1st week after operation, or, to put the case more directly, the high postoperative yield as compared with the later one, is in some instances clearly the result in part of the destruction of red cells extravasated at operation, as we have pointed out. Possibly some corpuscles are injured by the anesthetic. In any event, the destruction within the body of but a few extra cubic centimeters of blood would suffice, according to current views, to swell markedly the bilirubin total of a 24 hour period. But there is another potential reason for the high output of the 1st day after operation. Under normal circumstances the bilirubin yield by the liver may represent not merely excretion of the pigment but an enterohepatic circulation of it.\textsuperscript{6} With the sudden elimination of the latter factor, on intubation of the animal, the bilirubin yield should fall off.

The postoperative anemia in intubated dogs,—one feature of which is a low color index of the cells,—develops a little later and more slowly than the fall in the bilirubin output. It can be explained, as has just been indicated, by the continued loss of such part of the bile pigment as would normally be saved from the intestine by reabsorption and utilized in the formation of new corpuscles. In this relation the evidence in our experiments for differing degrees of conservation of the pigment from destroyed blood has significance. In the transfused dog, No. 19 (Table III, Text-fig.6), which received alien blood when itself anemic, almost all the pigment derived from

\textsuperscript{24} Balfour, D. C., and Ross, J. W., Arch. Surg., 1921, iii, 582.
the destruction of the 2 days immediately following transfusion was retained, to judge from the bilirubin output. During the next period, with a continuance of blood destruction, the actual yield of bile pigment came closer to the expected one; and in a final period, when the latter was large, approximated it still more nearly. The course of events was exactly what one would have anticipated had the body at first a need for the pigment, hence retaining most of it, and then gradually been supplied with as much and more than was required. In line with this idea of a conserving mechanism, which may on occasion be active in saving pigment or again lack occasion to function, is the fact that in those of our animals in which blood destruction was precipitated by exercise or transfusion, the actual yield of pigment in the bile more nearly approximated the expected one than when the reduction in hemoglobin was not artificially induced. The conditions may well have been less favorable to pigment conservation in the first mentioned series of instances. Whipple and Hooper record experiments showing that in anemic dogs given injections of small quantities of hemoglobin, the bilirubin increase during the next 6 hours was far less than in similarly treated non-anemic animals. They did not follow the changes to completion, but their protocols suggest a pigment conservation in the presence of body need. There was no evidence for this in the few protocols of Brugsch and Yoshimoto,\textsuperscript{25} who concluded that injected hematin was quantitatively represented in the bilirubin output. But much, obviously, will depend on the immediate body need and the amount of pigment given. Brugsch and Retzlaff injected it on many consecutive days, and lumped their findings for the period.

Consecutive observations, extending over many weeks, on the hemoglobin percentage and the bilirubin output (see Text-fig. 4; also McMaster, Broun, and Rous\textsuperscript{8}) bring out the presence of a relationship between the two which, on cursory inspection, would appear to be undisturbed by normal influences other than exercise. But there are hidden factors which act to distort this relationship. Whenever the hemoglobin percentage is stationary or rising, such blood destruction as may be going on is, of course, wholly concealed through the hema-
G. O. BROUN, P. D. McMASTER, AND P. ROUS  

Topoietic activity; and when the percentage falls gradually, the concealment continues, though now it fails to mask completely the destructive process. Under such circumstances the bilirubin yield cannot be an accurate reflection of the percentage alteration. Furthermore, the amount of it put out in the bile will be directly affected by variations in the degree of pigment conservation. It is remarkable that a daily yield which is the resultant of blood changes that are partially concealed and of a pigment conservation which may vary should so nearly reflect gross alterations in hemoglobin as indicated by the percentage value of blood specimens. If one chooses to think not in terms of this latter substance but in those of the cells in which it is lodged, and ignores intercurrent changes in the color index, then the bilirubin yield becomes a more or less accurate expression of cell mortality. On first view it appears to be a strikingly faithful one. When the cell population is large or small, the number of cell deaths should, other things being equal, likewise be large or small, and so too with the bilirubin output. Such is the actual case. Indeed, the variations in output so closely follow those in the hemoglobin percentage as to give the impression that cell deaths are registered practically at once, that is to say within a few hours, in terms of bile pigment. But always one has to reckon with masked blood destruction and pigment conservation as disturbing factors. Here and there in the charts their influence is manifest. Whenever a noteworthy remission from anemia occurred, the increase in cell number evidenced by the mounting per cent of hemoglobin involved, by corollary, an increase in the eventual number of cell deaths. That this number increased even while the repair was going on may be inferred from the rise in the bilirubin yield. But here a curious fact may be noted. The rise did not parallel but actually preceded that in the hemoglobin per cent. Either, as repair began, more cells were destroyed than ordinarily, or else the need for bilirubin conservation had lessened. As a matter of fact, proof has been brought in a previous paper of a special corpuscular wastage during recovery from anemia, traceable to the circumstance that many of the cells placed in circulation by the hematopoietic tissue are unfit to withstand the exigencies of life there. It will further

be seen from the charts that whenever a marked drop in the hemoglobin percentage occurred, uninduced by artificial aids, the bilirubin yield fell simultaneously, but generally to a much more marked extent. The factor of replacement must have been of relatively slight importance at such periods, since the marrow was inactive as proven by the small number of circulating reticulocytes. In four specimen instances (see Text-fig. 4; also the curve in a previous paper) the hemoglobin dropped from 118 per cent to 80 per cent, 99 per cent to 70 per cent, 92 per cent to 76 per cent, and 93 per cent to 62 per cent, whereas the bilirubin decreased from 6.2 mg. to 3 mg. (118 per cent to 57 per cent), 4.4 mg. to 2.2 mg. (99 per cent to 46 per cent), 5.3 mg. to 2.2 mg. (92 per cent to 38 per cent), and 5 mg. to 2.9 mg. (93 per cent to 54 per cent) per hour respectively. An increasing conservation of pigment as the anemia gradually developed would explain these findings.

Instances of the sort referred to, in which the drop in the hemoglobin percentage must have come about in the main through a progressive depletion of the current stock of cells without adequate replacement, and in which the bilirubin output decreased concurrently as a consequence of the lessening of cell population with, by corollary, a lessened number of cell deaths from "natural causes," may be contrasted with those others in which a decrease in blood pigment was induced by extraneous influences (Text-figs. 5 and 6). Here the bilirubin yield mounted while the anemia was developing, as would follow from the excessive mortality in a thinning population of corpuscles. It is evident that the changes in the bilirubin output of intubated animals from day to day should aid one to tell whether a given anemia is the expression of ordinary wear and tear on the corpuscles in the lack of the usual cell replacement, or whether, on the other hand, it results from increased blood destruction despite what the hematopoietic tissue may be doing in the way of replacement.

SUMMARY.

In dogs intubated for the collection of all of the bile, a marked falling off in the yield of bilirubin is regularly to be noted after operation, followed soon by an anemia of secondary character. Though, in the absence of complications, the anemia is mild, it persists despite the
excellent general condition of the animal. Intercurrent changes in the hemoglobin percentage take place from time to time, and these are accompanied by very similar fluctuations in the bilirubin quantity. At first inspection the data strongly suggest that blood destruction finds accurate quantitative expression in the yield of bile pigment. But this is not the case. True, the destruction finds expression in terms of bile pigment and practically at once; and the data support the conception that bilirubin has no other sources besides the hemoglobin of destroyed blood. But our experiments show that the amount of it put forth during the development of an anemia from gradual blood destruction, either intercurrent or induced, is far below that derivable from the net quantity of hemoglobin disappearing from the circulation. The discrepancy is referable to a process of pigment conservation which varies in proportion to the body need.

We have shown in a previous paper that bile pigment may on occasion be absorbed from the intestinal tract. This fact and those reported in the present communication lead one to question the accepted view that bilirubin is mere waste material eliminated by way of the liver. The anemia which develops upon total loss of the bile is not improbably consequent on the pigment loss therewith.

Current methods of computing the rate of normal and pathological blood destruction from the bilirubin (or urobilin) yield are unsound in principle and open to large error in practice. Yet there is no doubt that day-to-day variations in the output of bile pigment result from changes in the blood; and on occasion they provide enlightening evidence of the nature of hemic events.