BRITISH MEDICAL JOURNAL

LONDON SATURDAY OCTOBER 26 1940

THE DIAGNOSTIC VALUE OF PREGNANDIOL EXCRETION IN PREGNANCY DISORDERS

BY

CUTHBERT LESLIE COPE, F.R.C.P.

(From the Nuffield Department of Medicine, Oxford)

The substance pregnandiol, which is very similar in chemical structure to progesterone, is found in the urine only when a corpus luteum or a placenta is actively functioning in the body. It is thus present only during the luteal phase of normal menstruation and during pregnancy, and very strong evidence has now been accumulated, notably by Venning and Browne (1937), that it is an excretion product of progesterone metabolism. The observations leading to this conclusion have been confirmed in the main by me (Cope, 1940b) and have been summarized briefly elsewhere (Cope, 1940a). Pregnandiol is excreted in the urine as a compound with glucuronic acid, and the resultant complex, sodium pregnandiol glucuronide, can be relatively easily isolated and estimated by direct weighing, full details of the method having been published by Venning (1937, 1938).

The excretion of this complex might thus be expected to throw valuable light on disturbances of pregnancy, especially those associated directly or indirectly with abnormal hormonal metabolism. During the past two years we have carried out pregnandiol analyses on a variety of abnormal forms of pregnancy in order to determine so far as is possible the conditions in which such analyses provide useful clinical information. In this work we have been chiefly concerned not with the theoretical implications of the data collected but with the establishment of the place of pregnandiol analysis as a diagnostic aid. The conclusions here considered are based on the study of over 100 cases of pregnancy, of which some 75 were seriously abnormal. It is hoped that from such a relatively small series of cases the significance of pregnandiol analyses can be broadly outlined, though it is realized that far more data must be collected before their significance can be firmly established. If our own conclusions can stimulate others to collect such data they will have served their main purpose.

Normal Behaviour of Pregnandiol

Before abnormalities of pregnandiol excretion can be interpreted the normal behaviour of this substance must first be understood. The following are the main points essential to the interpretation of results.

Although pregnandiol is excreted, isolated, and weighed as a glucuronide complex, its excretion is always expressed in terms of milligrammes of actual pregnandiol, this being 320/536 of the weight of the complex. For clinical purposes it is the total daily excretion which is of importance, the actual concentration in the urine being of little significance. The total amount of pregnandiol excreted during the whole luteal phase of normal menstruation varies from 3 to 60 mg. Maximal excretion usually occurs five to seven days before

the onset of bleeding, and may be from 1 to 6 mg. a day, the latter figure being exceeded only rarely. Pregnandiol disappears from the urine before bleeding starts, but if pregnancy occurs and the period is suppressed then excretion continues. During the first few weeks of pregnancy the daily excretion remains small, the output often not exceeding that excreted during the peak days of a menstrual cycle. Since pregnancy is the only condition so far known in which amenorrhoea is associated with the continued excretion of pregnandiol the fact has already been used as a method of diagnosing early pregnancy. As pregnancy proceeds the daily output of pregnandiol increases, to reach a maximum in the eighth and ninth months, but after delivery it falls to zero in a few days. The height to which pregnandiol excretion rises varies considerably in different pregnancies. The recorded figures of reliable observers vary from 30 to 120 mg. a day in the last six weeks of pregnancy. Venning (1938) gives a mean figure of 75 mg. a day, but our own series of normal pregnancies varied from 30 to 110 mg., with a mean of 55 mg. There is no evidence that the lower figures are necessarily associated with any abnormality of the pregnancy. In the chart is shown a typical excretion curve throughout a

Chart showing pregnandiol excretion throughout normal pregnancy. Shaded area = range of variation of combined figures of Venning (1938) and Wilson and Randall (1938). Upper heavy curve = our own normal case. Lower interrupted curve = case from Hain (1940).

pregnancy in which collection of all twenty-four-hour urine specimens is known to have been practically complete. The range of variation of a series of normal cases is shown in the shaded area. With such a wide normal variation it is clear that no clinical significance can be attached to even relatively large deviations from the mean value, and consequently pregnandiol assays for clinical purposes do not require to be very accurate.

Estimation of Pregnanliol Excretion

Since the significant figure is not the concentration of pregnandiol but the excretion in twenty-four hours, it is necessary to make a complete twenty-four-hour collection of urine, of which the whole or a part is analysed. It is very desirable to have a check on the completeness of collection of urine specimens, and for this purpose we have used the excretion of creatinine. Estimation of the total creatinine content of the specimen is easy, and this will usually exceed 1,000 mg. a day. If the creatinine content of the supposed twenty-four-hour specimen is below this it may be incomplete, and if below 800 mg. should be considered almost certainly so. In such circumstances an approximate figure for pregnandiol excretion can be obtained by correcting the observed excretion to correspond to a creatinine excretion of 1,000 mg. a day.

Full details of the method of estimation of pregnandiol have been published by Venning (1937; 1938), to whose papers those interested are referred. It consists essentially in extracting the urine several times with butyl alcohol, distilling off this alcohol, and dissolving the residue in N/10 soda. This solution is again extracted with butyl alcohol, which is distilled off. The residue from this second distillation is dissolved in a small volume of water and the pregnandiol glucuronide precipitated by addition of excess of acetone. This precipitate is again dissolved in a small quantity of water and re-precipitated by acetone, the product thus purified being finally dried and weighed. In our hands this method has proved far superior both in convenience and in accuracy to any other which we have tried, and has therefore been used exclusively in this work.

The quantity of urine to be analysed must depend on its pregnandiol content. Generally 1,000 c.cm. or the day's output, whichever is less, is desirable before the twentieth week, and 500 c.cm. in the second half of pregnancy. It is wise to practise the method first with late pregnancy urine containing much pregnandiol, and later with a similar urine diluted with a pregnandiol-free urine so that familiarity with the white crystalline or flocculent precipitate may be gained. Blood or albumin in the urine makes the analysis more difficult—the former by yielding a dark greenish pigment in the final stages which may obscure the presence of small quantities of pregnandiol, the latter by favouring the development of emulsions during the butyl alcohol extraction that are difficult to break up. Such urines are best avoided until experience has been gained with the method on uncontaminated specimens. We have found it advantageous to saturate the urine with salt before extracting with butyl alcohol. This alcohol, which is distilled off during the analysis, may be used again for subsequent extractions without further purification, thereby effecting a great economy of material when many analyses are being made. All acetone used for precipitation of pregnandiol may also be dried with calcium chloride, distilled, and used again. In the method as developed by Venning the extracted pregnandiol glucuronide is re-precipitated from aqueous solution by acetone in order to ensure reasonable purity of the final product. We have carried out this second precipitation in every case and consider it highly desirable if fallacious results are to be avoided. Small quantities of impurity which may be mistaken for pregnandiol are often brought down in the first precipitation. They usually fail to appear in the second precipitation, but in doubtful cases a melting-point determination will decide. This should exceed 245° C. in ordinary urines, but in heavy albuminuria the final product is frequently less pure and has a lower melting

point. These precautions are, however, usually only necessary when the amount of pregnandiol is small. When it is large the bulk of the first precipitate is sufficient evidence of adequate pregnandiol excretion, and then a second precipitation is not essential for many clinical purposes.

Pregnanliol Excretion in Pregnancy

In the first few months of pregnancy estimation of pregnandiol excretion can be of value in several ways.

1. Diagnosis of Pregnancy

Demonstration of pregnandiol in the urine in a case of amenorrhoea must be regarded as strongly suggestive of pregnancy, for no other condition is so far known in which the two are associated. It is necessary, however, before making this diagnosis, to be certain that the pregnandiol excretion is not the normal prelude to a fresh menstrual bleeding. To be of value estimations must be capable of detecting as little as 3 mg. a day, and the whole twenty-four-hour output must therefore be extracted. Wilson and Randall (1939) and Hain and Robertson (1939) have both used the test successfully for pregnancy diagnosis, but sufficient data are not yet available to determine how it compares in reliability with the Aschheim-Zondek reaction. Absence of pregnandiol from the urine of a woman with recent amenorrhoea usually means that she is not pregnant. We record two cases in which this was of diagnostic value.

Case 13.—A woman aged 39 became pregnant for the first time in February, 1939, but had an incomplete abortion in May requiring evacuation of the uterus. Menses occurred in June and July, but she returned in November, 1939, with a history of four months' amenorrhoea. She was believed to be pregnant again, and was entered as an ante-natal patient. The urine, examined because of her history of previous abortion, contained no pregnandiol, and it seemed probable either that she was not pregnant or that the foetus had died. This view was not accepted by the ante-natal department, but as the urine sixteen days later still contained no pregnandiol an Aschheim-Zondek test was ordered; this was negative. On admission to hospital no signs of pregnancy were found, and a diagnosis of pseudocyesis was made. The uterus contained a moderate-sized fibroid.

Case 78.—A woman aged 35 was admitted with a history of three months' irregular uterine bleeding with occasional brown discharge. The uterus was palpable above the pubes and was irregular. Fibroids were diagnosed, but there was a possibility of associated pregnancy. The urine was therefore examined for pregnandiol, but contained none on two occasions. At operation the absence of pregnancy was confirmed.

We have stated that absence of pregnandiol from the urine *usually* means that the patient is not pregnant. The qualification is necessary because cases do occur in which for a short time pregnandiol is absent. We believe they are uncommon, but their frequency can only be determined by study of a larger series of pregnancies. Our experience suggests that chronic nephritis can prevent the excretion of the pregnandiol complex, but it may also be absent when no renal lesion can be demonstrated. Browne, Henry, and Venning (1939) have noticed its absence for short periods in early pregnancy, and we have recently seen a woman in whom it was absent at the nineteenth and twenty-first weeks. After a threatened abortion at this time pregnancy proceeded normally, pregnandiol excretion at the twenty-fifth week being within normal limits (47 mg. a day).

This latter case (No. 34), the only one of its type we have encountered, has necessitated our advising a rather greater caution in interpreting the absence of pregnandiol than we had hoped would be necessary. It emphasizes the fact that results must be interpreted in close association with the clinical findings, but this is true of

many diagnostic tests which are nevertheless of great value when properly applied. Absence of pregnandiol should never be regarded as significant unless it is demonstrated on two or more occasions.

2. Threatened Abortion

Absence of pregnandiol from the urine of a woman with signs or symptoms of threatened abortion is evidence of abnormality and is an indication for repetition of the determination on one or more subsequent occasions. While its absence from a single collection of urine is usually significant some cases do occur in which this absence is of only short duration and does not seem to be of serious import. Browne, Henry, and Venning (1939) record one such case in which disappearance of pregnandiol from the urine on the forty-ninth day was followed by a continuation of normal pregnancy to full term and a rapid return of normal pregnandiol excretion; and our own case (No. 34), mentioned above, is another example. Care must therefore be taken in drawing conclusions from the result of a single analysis. If, however, pregnandiol is persistently absent at two or more determinations this is very suggestive of one of three possibilities: either abortion is inevitable, or the products of conception have been already partially or completely evacuated, or, finally, the foetus has died without expulsion, producing a condition of "missed abortion." Distinction between these three possibilities must be made by clinical means. The value of the pregnandiol estimation in threatened abortion lies not so much in aiding the diagnosis as in calling attention to the fact that serious abnormality is present, requiring closer investigation. The subsidence of acute symptoms is too often regarded as evidence that the threat of abortion has passed; but this is not always justified, and such cases may return weeks later with signs of retained products or of intra-uterine death. A negative pregnandiol assay in the urine will frequently enable an earlier diagnosis to be made at the time of acute symptoms. Treatment of a case of threatened abortion with no pregnandiol excretion should not be regarded as complete until either it is certain that the uterine contents have been evacuated or definite clinical evidence is obtained that the foetus is alive, the latter alternative occurring much less often. The following two cases illustrate the prognostic value of pregnandiol assays.

Case 25.—A woman aged 37, with two healthy children aged 14 and 12 years, was admitted with a history of twenty weeks' amenorrhoea and intermittent, irregular vaginal bleeding during the past three months. Previous menstrual history was normal and there had been no miscarriages. The uterus was the size of an eighteen weeks' gestation, the cervix was soft and closed, and there was blueing of the vagina. The foetal heart was not heard. With rest and sedatives symptoms subsided, but pregnandiol was absent from the urine on the three occasions when it was sought. In spite of this evidence of persisting abnormality she was sent home and wheat-germ oil was prescribed. A month later she had to be readmitted because of recurrence of bleeding. The uterus was now the size of ten to twelve weeks' pregnancy, and a large clot was passed. Evacuation of the uterine contents removed clot and small pieces of placental tissue. No foetus was found.

Case 9.—A woman aged 24, with one child, was admitted with a history of three months' amenorrhoea and slight vaginal bleeding at intervals during the past two weeks. There was abdominal pain "like that of menstruation." She had had a similar slight bleeding at the third month of her previous pregnancy, which had nevertheless gone to full term. The uterus was enlarged to the size of ten to twelve weeks' pregnancy; the cervix was completely closed, but there was an erosion round the external os which bled readily on swabbing. After a fortnight of treatment with rest and sedatives and a single injection of 5 mg. of progesterone her

symptoms subsided, but a slight brown discharge began. Pregndiol was absent from the urine on the five occasions during this time when it was sought, and thus made extremely probable a diagnosis of uterine death. An Aschheim-Zondek test was reported "negative, but hormone concentration is higher than in a non-pregnant woman." She was nevertheless sent home, but had to be readmitted one month later because of persistence of brown discharge. After rupture of the membranes a placenta of four months' development was removed. No foetal elements were seen.

In both these instances pregnandiol analyses gave very strong support to diagnoses which from the available clinical evidence could only be tentative. We have come to believe that persistent absence of pregnandiol excretion in a case of threatened abortion should be regarded as evidence suggesting that abortion is inevitable even if the classical signs of inevitability are lacking, for such signs are no more than indications that expulsion of uterine contents has already begun. But if pregnandiol is present in the urine in threatened abortion it is unjustifiable to conclude that abortion will not occur. Our series contains three cases which aborted in spite of high pregnandiol excretion figures, and Browne, Henry, and Venning (1939) report a similar case. We believe that this will prove to be not uncommon. If pregnandiol is present in the urine even in amounts as low as 3 mg. a day conclusions as to prognosis cannot safely be drawn. It is important, therefore, to distinguish between a small excretion and its complete absence, and for this reason the quantity of urine analysed should be the full twenty-four-hour output if possible.

3. Incomplete Abortion

Since pregnandiol is believed to be derived from the placenta rather than from the foetus its excretion might be expected to continue when fragments of placental tissue have been retained. We have studied six cases of incomplete abortion with symptoms sufficient to require admission to hospital; we found no pregnandiol in five, the sixth showing a doubtful trace. It seems, therefore, that the retained fragments are usually either too small or too damaged to provide pregnandiol in recognizable amount. This will presumably be influenced by the time that has elapsed since abortion took place.

4. Death of Foetus

When intra-uterine death is suspected on clinical grounds absence of pregnandiol from the urine provides strong support for the diagnosis, and we have found this to be the condition in which pregnandiol assays can be of greatest value. But here again the converse is not true, and a normal pregnandiol excretion is not evidence that the foetus is alive. If foetal death is suspected and pregnandiol is nevertheless found in the urine it is helpful to repeat the estimation at weekly intervals. A progressive fall in pregnandiol excretion is suggestive, though this may be difficult to demonstrate, since daily fluctuations are sometimes large. But if the excretion falls to zero the evidence is almost conclusive.

We have observed 20 cases at a time when there was clinical reason to believe that the foetus had died. In all these the death of the foetus was confirmed subsequently. Of these 20 cases, 6 had a complete absence of pregnandiol when first seen and thereafter, and in a further 3 it fell to zero while under observation. In 7 of the remaining cases pregnandiol excretion was definitely below normal limits, but not absent, and in the other 4 excretion could not be regarded as abnormal. Thus in 45% of these cases the absence of pregnandiol strengthened the diagnosis of foetal death. The low or normal excretion in the other 11 cases did not provide any diagnostic aid, since even when pregnandiol excretion

was low it was not considered justifiable to conclude that the foetus had died or was dying. Nor in any of these was clear evidence obtained of progressive fall in excretion, though more extensive observation would no doubt have revealed it in some. This, however, would have entailed a greater number of analyses than is likely to prove feasible for routine clinical purposes.

Our early experiences with the use of pregnandiol excretion in the diagnosis of intra-uterine death were so encouraging that we were able by this means to diagnose it correctly in two cases in which it had not been suspected. It was only later that the previously mentioned anomalous Case 34 was encountered and a greater caution in the interpretation of results had to be advised. It is clearly of great importance that the frequency of occurrence of such cases as this apparent exception should be determined on a large series. In another case we were encouraged, because of a rise in pregnandiol excretion, to cast doubt on a diagnosis of intra-uterine death although the radiologist had reported "definite evidence of death." In this instance a premature child was born alive, though it died soon after.

We believe that when further experience has been gained pregnandiol excretion will come to have a recognized place in the diagnosis of intra-uterine death, arousing the suspicion in some cases, strengthening it in others, and possibly even confirming it in some.

Interfering Factors

There is reason to hope that increasing understanding of the factors affecting pregnandiol excretion will enhance its diagnostic value. There is evidence that both chronic nephritis and toxæmia of pregnancy can seriously interfere with this excretion. We have seen one woman with renal functional impairment due to chronic nephritis who excreted no pregnandiol one day before delivery of a healthy living child at full term. In a second case of chronic nephritis pregnandiol excretion fell to zero at the twenty-seventh week and, in view of our experience of the previous case, we deemed it unwise to diagnose foetal death, though this had in fact occurred. Weil (1938) has reported that in toxæmia of pregnancy pregnandiol excretion may be absent, but I (Cope, 1940a) have been able to collect a consecutive series of 10 toxæmic cases showing no serious interference with pregnandiol output. Since this series was collected, however, we have seen a woman with pregnancy toxæmia without nitrogen retention in whom no pregnandiol excretion was present. Pregnanndiol excretion will therefore probably prove unreliable as an aid to diagnosis in cases of pregnancy associated with either chronic nephritis or toxæmia of pregnancy.

Isolated observations on gravid women suffering from pyelitis, hypochromic anaemia, syphilis, hyperemesis, and severe diabetes have not revealed any interfering effect of these conditions on pregnandiol excretion.

Pregnanndiol and the Aschheim-Zondek Test

Pregnanndiol excretion provides information comparable to that of the Aschheim-Zondek and Friedman tests. This might have been expected, since both pregnandiol and the urinary gonadotrophin on which these tests depend are apparently derived mainly from the placenta. But measurement of pregnandiol excretion has several advantages over the biological tests. It does not require a licensed laboratory or facilities for housing animals, but can be done by persons unskilled in biological methods in any laboratory possessing a balance and means of centrifuging at low speeds quantities of fluid up to 500 c.cm. Being a gravimetric chemical analysis results are accurate, may be expressed numerically, and are not subject to the varia-

tions inherent in all biological reactions. Its main disadvantages are that it is rather more time-consuming than the biological tests and that it requires the collection of a larger quantity of urine, especially during the first few months of pregnancy. It is not strictly essential for clinical purposes, however, that the urine collection be a complete twenty-four-hour one provided the volume is adequate, for a simple creatinine estimation will indicate roughly but sufficiently what proportion of the day's urine output a given specimen represents.

Pregnanndiol excretion enables pregnancy to be diagnosed as early as the Aschheim-Zondek test, though its reliability for this purpose has still to be investigated on a large series of cases. It is probably a less sensitive indicator of retained active placental fragments than the biological tests. In two of our cases of incomplete abortion the Aschheim-Zondek reaction was recorded as "negative but higher than non-pregnant," although no pregnandiol was detected in the urine. Unfortunately relatively few of the cases whose pregnandiol excretion we have studied have also had Aschheim-Zondek tests, but the detailed figures recorded by Browne, Henry, and Venning (1939) indicate that gonadotrophin excretion may continue at normal levels in cases of missed abortion in which pregnandiol has fallen to a low figure. This suggests that fall of pregnandiol excretion may prove a more reliable indicator of foetal death than the biological reactions based on gonadotrophin excretion.

We have seen two cases in which there was gross discrepancy between the pregnandiol excretion and the Aschheim-Zondek reaction.

Case 28.—A woman aged 29, with three children, was admitted with a history of two months' irregular uterine bleeding following an abortion. Incomplete abortion was diagnosed, curettage carried out, and scanty scrapings obtained. She returned home, but bleeding persisted and she was readmitted one month later. The uterus was larger than previously and vaginal blood clot contained a few hydatidiform villi. The urine contained no pregnandiol, but the Aschheim-Zondek test was positive in undiluted urine, negative in a dilution of 1 in 100. Evacuation of the uterus removed only small pieces of necrotic tissue. Bleeding continued and total hysterectomy was finally performed. The Aschheim-Zondek test one day before this operation gave a weak positive in 1 in 100 dilution. Diagnosis of chorion epithelioma was confirmed by the pathologist.

Case 33.—A woman aged 32 was admitted with a history of intermittent uterine bleeding. Diagnosis of threatened or incomplete abortion at nine weeks' pregnancy was made. The Aschheim-Zondek test was strongly positive, but the urine contained only a doubtful trace (0.7 mg.) of pregnandiol a day. The uterus was curetted, and early secretory endometrium but no placental tissue was obtained. She was discharged, but was readmitted two weeks later with symptoms of ectopic gestation. At laparotomy this was found to be ruptured but adherent to omentum. The Fallopian tube contained chorionic elements; the placental site and the decidual reaction were poor. The ovary contained a corpus luteum of pregnancy but was oedematous.

Although definite conclusions cannot be drawn from these two examples they are nevertheless of interest. In both of them incomplete abortion was originally diagnosed, the Aschheim-Zondek test was strongly positive and pregnandiol excretion absent, and uterine curettage at first revealed nothing significant. We had been led to hope by Case 28 that the association of a strongly positive Aschheim-Zondek test with absence of pregnandiol might prove of diagnostic value in chorion epithelioma; but Case 33, although giving a similar initial history, shows that this hope was unjustified.

Weakly positive Aschheim-Zondek reactions are often obtained in menopausal women, and may lead to a suspicion of pregnancy. In such cases it seems probable

that pregnandiol excretion will help to remove doubt, for while the occurrence of pregnandiol excretion may be merely the normal prelude to a fresh menstrual bleeding, its persistence at a subsequent estimation a fortnight or more later renders this very unlikely and a diagnosis of pregnancy almost certain.

Summary

Pregnandiol is a product of corpus luteum and placental metabolism. Its excretion in the urine is relatively easily measured in any biochemical laboratory. An attempt has been made to assess the diagnostic value of pregnandiol excretion in abnormal pregnancy. Its complete absence is nearly always evidence of serious abnormality, suggesting in early pregnancy the imminence of abortion and in late pregnancy the death of the foetus. The significance of a low pregnandiol excretion still remains uncertain. A normal pregnandiol excretion is not evidence that abortion will not occur or that the foetus is still alive. Both chronic nephritis and toxæmia of pregnancy may interfere with pregnandiol excretion. Deviations from the normal excretion in these conditions do not necessarily mean that the gestation is abnormal. Pregnanndiol excretion seems assured of a place as a diagnostic aid in obstetric disorders.

REFERENCES

- Browne, J. S. L., Henry, J. S., and Venning, E. H. (1939). *Amer. J. Obstet. Gynec.*, **38**, 927.
 Coppe, C. L. (1940a). *Lancet*, **2**, 158.
 — (1940b). *Clin. Sci.*, in the press.
 Hain, A. M. (1940). *J. Endocrinol.*, **2**, 104.
 — and Robertson, E. M. (1939). *Lancet*, **1**, 1324.
 Venning, E. H. (1937). *J. biol. Chem.*, **119**, 473.
 — (1938). *Ibid.*, **126**, 595.
 — and Browne, J. S. L. (1937). *Endocrinology*, **21**, 711.
 Weil, P. G. (1938). *Science*, **87**, 72.
 Wilson, R. B., and Randall, L. M. (1938). *Proc. Mayo Clin.*, **13**, 813.
 — (1939). *Ibid.*, **14**, 8.

EFFECT OF REPEATED INTRAVENOUS INJECTIONS OF BILIRUBIN ON ERYTHROPOIESIS IN ANAEMIC DOGS

BY

RICHARD R. BOMFORD, D.M., M.R.C.P.

Assistant Physician, London Hospital; late Rockefeller Travelling Fellow and Assistant Resident Physician at the Hospital of the Rockefeller Institute for Medical Research, New York City

It is well known from animal experiments that regeneration after bleeding occurs more rapidly if a solution of the haemoglobin removed is reinjected into the anaemic animal (McMaster and Haessler, 1921; Miller and Rhoads, 1934). To explain this observation it has sometimes been assumed that as the injected haemoglobin is broken down some product or products are retained and re-utilized in the formation of new haemoglobin. The experiments of Hawkins *et al.* (1931, 1939) have shown, however, that this does not happen in the case of the pigment portion; since under experimental conditions in dogs with bile-renal fistulae 90 to 100 per cent. of pigment injected as haemoglobin is excreted in the urine, although at the same time an amount of new haemoglobin equivalent to 90 to 100 per cent. of that injected appears in the circulation. Other observations recorded in the literature and reviewed below indicate that injections of bilirubin do have an effect on erythropoiesis. If this effect is not due to an actual utilization of bilirubin in the formation of new haemoglobin it must be due to some other and at present unknown action of bilirubin on erythropoiesis.

Previous Observations

Patek and Minot (1934) reported the results of treatment with bile pigment in nine cases of chronic hypochromic anaemia. A concentrated almost pure preparation of beef bile pigments was given by mouth in doses

varying from 1.2 to 2.5 grammes daily. This preparation given alone caused no reticulocyte response, but there was an average increase of approximately 7 per cent. in the amount of haemoglobin in about ten days. After a reticulocyte response had occurred as a result of the administration of a suboptimal dose of iron the addition of the bile-pigment preparation caused a second response. In one patient who had been treated with iron for fourteen months without reaching a haemoglobin level higher than 73 per cent. this rose to 85 per cent. in sixteen days when bile pigment was given in addition to iron. These observations seem to indicate that bile pigment given by mouth can in some manner facilitate either the absorption or the utilization of iron. It is open to question, however, whether they also show, as their authors claim, that bile pigment can be absorbed from the gastro-intestinal tract and utilized in the building of haemoglobin.

There are a number of articles in German describing experiments on the effects on erythropoiesis of bile and blood pigments given by mouth and by injection. Verzar (1929), for instance, claimed that various blood pigments, including haemoglobin, bilirubin, and porphyrins, as well as various organ extracts, were erythropoietic. He found that single doses of bilirubin given by mouth or by injection to rabbits caused a transient erythrocytosis in some animals—usually animals with a red cell count in the lower part of the normal range; an erythropenia was, however, produced in animals with a higher count. Regeneration after bleeding was said to occur more rapidly in animals given bilirubin intravenously or by mouth, but the diagrams illustrating this are hardly convincing and the results were apparently not consistent. Fellingner (1932) also found that a single parenteral injection of bilirubin caused an increase in the red cell count of normal rats in about half the animals so treated; and this occurred especially in young animals and in animals with lower red cell counts than the remainder. Continued administration led to a more prolonged increase, which ceased when the medication was discontinued. Regeneration after bleeding was said to be more rapid in animals treated with bilirubin than in animals not so treated; but in this instance again the protocols are not entirely convincing.

Zih (1939) found that the effect of intravenous injections of bilirubin in normal men depended on the dose given. A single dose of 10 to 30 mg. produced an erythrocytosis of short duration, but 150 to 300 mg. produced an erythropenia. Biliverdin gave a similar result, while haematoporphyrin had very little effect. A solution of 15 grammes or more of commercial haemoglobin taken by mouth also produced an erythrocytosis.

The effects of bilirubin on haematopoiesis are therefore, according to these authors, somewhat complicated. There is some evidence that bilirubin in small doses produces erythrocytosis, but that in larger doses it produces erythropenia. Erythrocytosis is most often seen in animals which have initially a red cell count in the lower part of the range of normal, and erythropenia may be seen in those with a higher red cell count. These authors have speculated as to the significance of the effects observed and have even ascribed to bilirubin the function of an erythropoietic hormone concerned in the maintenance of normal blood values. Some of their experiments are, however, open to criticism in that they were performed on small animals on which accurate blood counts are difficult, and the changes observed, which were slight, were sometimes not consistent. The experiments were of short duration, and too often red cell counts alone are recorded.

The object of the experiment reported here was to discover the effect of intravenous injections of bilirubin, given