rough surfaces with its claws and thus lever its body backwards and upwards and make control difficult. Thinner boards might be broken by an adult bird.

In the crush the bird is controlled by one or more assistants applying their hands to the ischial bones and pushing the bird forward, while others push him downwards by applying their hands to the sacral region, and at the same time another assistant lifts the right wing. The operator stands on the left side of the bird, and during the operation of inserting the needle into the vein and collecting the blood he presses with his fore-arms on the bird's back, thereby also helping to prevent its moving upward. Occasionally a bird will flop down and refuse to get up. It is best then to drag it out of the crush and to press it down so that it cannot get up while the blood is being drawn. At least three men are required to keep an adult ostrich down. Attempts were made to control birds in the crush by tying ropes round them to obviate the use of many assistants, for at least four are required to control a restless bird. This method, however, proved undesirable, for the feathers were easily dislodged and the skin was badly chafed by the ropes.

The semi-wild birds from which blood was collected in the Bredasdorp district, when once they were caught, struggled much less than the six birds kept at Mariendahl, and they did not very vigorously resist the drawing of blood from the jugular vein ; no doubt they were too scared to do so. Of the six Mariendahl birds five soon became used to being handled and scarcely resented the insertion of the needle, so that less assistance was required. But the remaining bird, No. 6 , always put up a struggle.

In an adult ostrich the brachial vein measures

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about 6 mm. in diameter, and a hypodermic needle about 4 cm. long and with a bore of about 1 mm. in diameter was found quite suitable for insertion into the vein of a fullgrown bird. For younger birds a needle with a diameter of 0.7 mm. was used. The wings were used alternately when fairly frequent collections were made, and sometimes the blood was obtained from the jugular in order to eliminate the possibility of contamination by inflammatory products. But it would appear that phlebitis is not easily induced in the ostrich. It was never once observed and it is surprising how soon the puncture wound, or indeed any wound, heals in the ostrich.

In the adult bird the skin overlying the vein is fairly thick and its colour is blue like that of the vein ; the vessel cannot, therefore, be seen until the blood flow is slightly impeded by gentle compression, which is also necessary in order to obtain a free flow of blood. When the needle is inserted it should be pushed in at a very acute angle and in a direction against the flow of blood, so that its point is at least 0.5 cm. from the point of entry into the vein ; otherwise the opposite wall of the vessel may be penetrated or if not deeply enough inserted, it may slip out if the bird struggles slightly.

A 20% solution of lithium citrate and powdered heparin, which are again referred to under "Preventing Coagulation of the Blood", were used as anticoagulants. Lithium citrate was used in the early part of the work, but was later replaced by heparin, and the blood was collected in bottles with necks about 0.5 centimetre in diameter and graduated as described by Neser (1923). Each bottle contained such an amount of the lithium citrate solution, or of the powdered heparin, that when the bottle

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was filled to the mark on the neck, there was 1 part of lithium citrate to 199 parts of blood, or 1 part heparin to 5000 parts of blood. It is not always easy to fill the bottle accurately to the level of the mark but since such a small proportion of anticoagulant is used a slight error in the level is not significant when a fair quantity of blood is drawn. The blood was, however, always collected in such small amounts and at such intervals that the observations may be regarded as always having been made on normal blood.

According to the observations by Neser (1923), the rate of the circulation is a very important factor in the distribution of the red cells, a sluggish peripheral circulation resulting in a concentration of the red cells in the periphery and the reverse in the jugular vein. He considered the jugular vein in domestic mammals the best source from which to collect blood, and his reasons may with advantage be repeated here :

1. "The whole circuit is comparatively short and the flow of blood is fairly rapid.

2. The circulation of the head is relatively larger than that of any other accessible part of the body, and for this reason the relative change in the blood for a given activity will be least.

3. There is little, if any, obstruction to the flow of blood in the jugular vein owing to the action of gravity. Stagnation is, therefore, impossible here under normal circumstances, and the blood is consequently thoroughly mixed."

Ponder (1934), on the other hand, remarks : "In spite of occasional statements to the contrary, it seems to be established that the red cell count is the same in normal arterial, venous, and freely flowing capillary blood, and that it is the same in normal blood taken from central and peripheral vessels (Rud, 1922 - '23, McCay, 1928)".

The wing vein of the ostrich may, for the reasons

/mentioned .....

mentioned by Neser, be considered more suitable than the jugular, for the circulation is much shorter in the wing than in the neck, while the other two reasons are equally applicable to the wing vein. Besides, as already stated, blood is more easily obtained from this source.

Counts and percentage volume determinations were made from blood extracted from the jugular and also from the wing vein, but they differed only within limits of error, so that, both for theoretical and practical reasons, the brachial vein may be considered the most suitable in the ostrich.

PREVENTING COAGULATION OF THE BLOOD.

In testing ostrich blood to determine its coagulation time, it was observed that clotting is usually much delayed, and often samples of whole blood, still uncoagulated, were discarded days later. Usually samples did not coagulate for several hours after they were collected but sometimes it did happen that coagulation occurred within an hour. The great advantage of this unusual property of ostrich blood was that observations could also easily be made on whole blood and these could be compared with those made on blood containing anti-coagulants.

Bainbridge and Menzies (1919) state : "The blood of birds contains no platelets, and will not clot, if it is drawn directly from a blood vessel without contact with the tissues. If, however, it is allowed to flow over the adjacent tissues in its passage from the vessel, it will coagulate readily."

This statement requires modification, for, though the blood of the ostrich does not readily clot, this is not

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true of fowl blood. The writer has on many occasions drawn blood-by means of a syringe-direct from the hearts of fowls according to the method of Sloan and Wilgus (1930), and also from their wing veins, and coagulation usually occurred within a short period, even when the inside of the syringe barrel was coated with vaseline. According to Dukes (1934) the period of coagulation for fowl blood is 4½ minutes, and Johnson and Connor (1933) who determined the coagulation time of the blood of 12 fowls found that it varied from 1 minute 10 seconds to 14 minutes 15 seconds with an average of 6 minutes 21 seconds. Kaupp (1929) states the period to be 30 seconds. It is, of course, generally agreed that, though platelets as seen in mammalian blood do not occur in avian blood, the thrombocytes in bird s blood serve the same purpose as the platelets.

Ostrich blood may sometimes clots quickly, especially in warm weather, once it has been in contact with the tissues or if brought in contact with blood that has already coagulated. It was also noticed that the blood coagulated more readily when the bottles were filled slowly because of obstruction in the needle or when a needle with a very fine bore was used.

It would appear that the prolonged coagulation time of ostrich blood cannot be correlated with a low calcium content of the blood. From table (29) it will be noted that the calcium content of ostrich blood averages 10.1 Mgms. per 100 c.c. blood. This figure is high compared with the values given for some animals whose blood clots very readily. For example - to give only a few figures as recorded by Dukes (1934) - the calcium content of the blood of the ox is 7.1Mgms. per 100 c.c. and the coagulation time 6½ minutes, the corresponding figures for the dog being 6.6Mgms. and 2½ minutes and for the cat 5.5Mgms.

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and 1 to 3 minutes. Calcium values, reported by Knowles (1934) for hens not in lay, vary from 8 to 12.3 mgms per 100 c.c. blood.

As coagulation of samples sometimes did take place before it was possible to complete the observations, it was necessary to use an anticoagulant. Neser used a 7½% sodium citrate solution as anticoagulant in the proportion of 1 part anticoagulant to 9 parts of blood, and the writer's intention was to use, also in the same proportion, an isotonic solution of a suitable anticoagulant so that true percentage volume readings could be obtained. It was, however, soon realised that, whereas it is possible to fill up the bottle containing the anticoagulant to a definite mark when collecting blood from a docile animal like the horse, it is difficult to do so when extracting blood from an ostrich. As a rule, the birds are restless, and as the operator has to collect blood from the under side of the wing while usually standing on the opposite side, it is often impossible to fill up the bottle exactly to the mark. This necessitates the subsequent addition of saline ~~solution~~ or the removal of blood, and therefore additional calculations have to be made.

It was, therefore, decided to find an anticoagulant of which such a minute quantity need be used, either in solid or liquid form, that corrections need not be made even if the bottle is filled up slightly above or below the mark. Sodium citrate, potassium citrate, lithium citrate sodium oxalate, potassium oxalate and sodium fluoride were tried in solid form. But it was found that when these were used in such quantities as would invariably prevent coagulation, slight haemolysis sometimes resulted, as was evidenced by tinged plasma when the blood was centrifuged. The aforementioned salts in powder form were therefore

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considered unsatisfactory, as the degree of haemolysis could not be controlled. It was finally found that 0.5 c.c. of a 20% solution of lithium citrate to 99.5 c.c. of blood invariably prevented coagulation and never caused any haemolysis. Moreover, as lithium citrate is a salt that does not occur in the blood, corrections need not be made in chemical analyses of the blood.

The lithium citrate solution, however, causes slight crenation of the cells, and consequently a true percentage volume reading is not obtained. But it will be shown under "Relative Volume of Corpuscles and of Plasma" that by applying a small correction factor to percentage volume readings obtained with citrated blood, such readings may for practical purposes be regarded as identical with those of whole blood.

Heparin, which was unprocurable in South Africa at the time when it was first required, was tried later and proved an even more suitable anticoagulant, for when used in the proportion of 1 mg. to 5 c.c. blood as was used by Wiseman and Bierbaum (1932) for human blood, it also invariably prevented coagulation. Haemolysis was never observed and differences, when present in the percentage volume readings of whole blood and of heparinised blood, were insignificant.

The aforementioned workers state : "After weighing out this quantity a few times to visualise the approximate volume involved the quantity of heparin added to each tube may be estimated without disturbing the accuracy of the test". This procedure was adopted by the writer and proved satisfactory.

PREPARATION OF SMEARS.

Opinion differs considerably as to the most satisfactory method of making blood smears suitable for differential counts. According to Neser (1923) the distribution of the leucocytes is often most irregular when the smears are prepared by the slide method, viz., by placing a drop of blood on one slide and causing it to spread by pushing it with the edge of another slide. He states that as a rule the leucocytes are evenly distributed throughout the smear by his coverslip method in which the blood is spread between a slide and a coverslip, instead of between two coverslips, as is customary in determinations on human blood. However, he does not record differential counts made from smears prepared in both ways, indicating the differences obtained in the counts. De Kock (1931) slightly modified Neser's method by substituting a squarely cut portion of slide glass for the coverslip and states that the method gave more uniform results than smears prepared in any other way. Many other workers also favour the coverslip method.

On the other hand, Schilling (1935) describes only the slide method. Wirth (1931) is of opinion that the distribution of leucocytes is as even with the slide method as with the coverslip method. Lucia and Lucia (1928) give counts made in different ways from smears prepared by the slide method and state :

"It was found that the counts in every case fell within one standard deviation of the group. If the probability of variation around the mean (standard deviation) is taken into consideration, no advantage can be ascribed to the coverslip over the slide method, as far as distribution of cells is concerned."

Kolmer and Boerner (1931) describe both methods, and their only comment is: "The coverslip method is preferred

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by many clinical pathologists, although it tends to produce rupture of the leucocytes." In other text books consulted usually both methods were also described, but no preference was mentioned. The writer also consulted several well-known haematologists, and they prepare smears for differential counts only by the slide method.

Messrs. Arthur Thomas & Co., Philadelphia U.S.A. have placed on the market a "Differential Counting and Staining Outfit" invented by Hausser. With this outfit, smears are prepared as in the coverslip method, the blood being spread with a small rectangular block of glass on a specially thick glass slide. The following remarks from the firm's 1931 Catalogue edition, in which the outfit is advertised, may be quoted here : "It is difficult to obtain blood smears of uniform thickness over a large area with any technique when ordinary microscopic slides and coverglasses are used, because of the curvature in the surfaces of both. It has been stated that this usually amounts to a full wave-length in a linear distance of 1 mm. Since a wave-length is about 600 microns, and a blood corpuscle measures about 10 microns, it is evident that, while at one point absolute contact may be possible, there may be sufficient unequality of surface at a distance of only 1 mm. from such a point to pile up a layer of not less than sixty corpuscles, when smears are so made."

Therefore it does not appear to have been definitely established yet that the coverslip method gives a more even distribution of the leucocytes or in any case a so much more regular distribution as to be of practical importance.

Smears may be easily and satisfactorily prepared, by the coverslip method, from the blood of animals which can be brought into a laboratory or sheltered place in

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which the air can be kept free from dust, but in the case of the ostrich this is not so easy. Usually ostriches are not stabled, but are allowed to pick up their ~~own living~~ food on the veld, and they can only with difficulty be driven into a stable. Under ordinary field conditions, therefore, smears have to be prepared in the open, and as the surface of the glasses have to be scrupulously clean in the coverslip method it is difficult to prepare such smears on a windy day, as the dust so quickly settles on the glasses. Moreover, the numerous fine scales on the feathers of the ostrich easily become dislodged and settle on the glasses.

The coverslip method of Neser as modified by De Kock was tried and, apart from the above-mentioned disadvantage associated with the coverslip method when applied to the ostrich, the writer often had to make a number of smears before a satisfactory one was obtained even on quiet days when the surfaces of the glasses were as clean as could be expected. The slides were always cleaned with bichromate of potash and nitric acid and kept before use in absolute alcohol but owing to the unevenness of the slides - though high quality polished slides were always used - the blood would not spread properly on many of them, and consequently the smears were too thick. Smears could, therefore, not always be prepared quickly with the result that the flow of blood from the needle had to be unduly prolonged - a matter of considerable inconvenience when dealing with a restive animal. Apart from the loss of blood, the bird soon becomes uneasy and the needle is either dislodged or the blood-flow ceases and the needle has to be reinserted.

Thin smears from ostriches can be easily prepared under field conditions by the slide method described by Neser (1923), slightly modified as indicated below;

A platinum loop is attached by means of sealing-wax or other suitable adhesive to the end of the slide used for spreading the blood and one of the corners of the opposite end of the same slide is cut off, so that the length of the spreading edge is about 1.5 centimetres . (Fig.2).

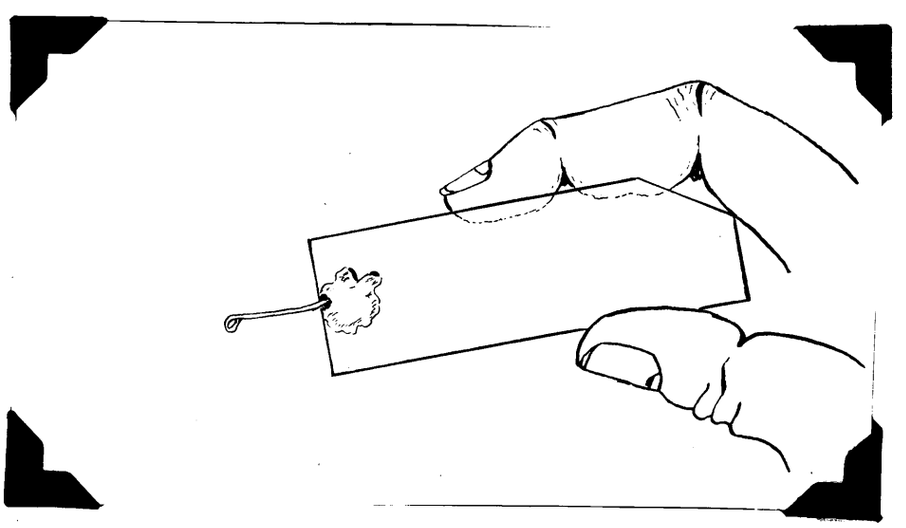

Fig. 2.

The slide to which the loop is affixed is held at its edges with the thumb and forefinger and about midway between its ends, and a drop of blood is then taken up with the loop. After the drop has been deposited on to another slide held in the left hand, the slide carrying the platinum loop is simply tilted and with its narrow edge the blood is spread in the customary manner of pushing the spreader toward the other end of the under slide.

The process of first putting the loop down and taking up the slide for spreading the blood is thus eliminated and the smear is therefore prepared more quickly. In making ~~blood~~ smears from ostrich blood in which not

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only the thrombocytes but also the leucocytes clump very soon after the blood is drawn, a delay of some seconds in spreading the blood may vitiate the making of a smear satisfactory for differential counts.

By using a narrower spreader the blood is prevented from spreading over the long edges of the under slide and thus leucocyte counts can be satisfactorily made diagonally across the smear, as the edges of the smear can be included in the fields examined. The size of the loop used for taking up the blood was always such that the length of the smear was about 3·5 cm. when the drop had been spread and as already indicated the breadth was about 1·5 cm. Dispensing with the use of the loop by taking up blood as it issues from the needle with the edge of the spreader proved unsatisfactory, for usually too much blood adhered to the edge with the result that the smears were too thick.

STAINING OF SMEARS.

May-Grunewald and Giemsa stain (as used by Pappenheim), Giemsa's, Leishman's, Jenner's and Wright's stain were all tried as recommended in text books and by individual workers. Wright's stain was finally adopted.

Staining with Wright's stain entails little work and the smear is usually ready for examination within ten minutes. This stain gave as good and uniform results as any of the others, and proved more satisfactory than some of them for the purpose of differentiating between the lymphocytes and the monocytes. The stain was prepared as prescribed by Carleton (1926) and used as recommended by Slider and Downey (1929).

In properly stained smears the cytoplasm of the red

/cells.....

cells ~~shows~~ show a fawn colour and sometimes fine azurophil granules can be seen in the monocytes. With some samples of stain the diluted stain need only be left on the smear for 5 minutes whereas with others 10 minutes or more are necessary. Experience is the best guide as to how long to stain and to wash.

Pepper and Farley (1933) state : "The difficulty with methylene azur-eosin stains is to obtain the proper balance of eosin and basic stain. Muddy blue preparations are the result of too much alkali in the stain. To keep the stain at just the proper reaction is difficult."

For diluting Wright's stain McJunkin (1920) uses, instead of distilled water, a buffer solution made from monopotassium phosphate and dibasic sodium phosphate. This solution has a pH of 6.4 and will absorb excess alkali without change in the reaction of the solution.

The best results were usually obtained when Wright's stain was used immediately after the smears were prepared.

CELL MEASUREMENTS.

The cells were measured with a Leitz ocular micrometer that had been carefully calibrated, and their measurements are given under "Morphology of the Blood". Ponder (1924) states that this method has been used with variations for the last seventy years and that nearly all tables of the average sizes of erythrocytes contain values determined in this way. He considers that at times results so obtained may have attached errors amounting to 10 per cent. and he remarks as follows: "When a body so small as the

red cell is examined under the microscope owing to the fact that there is a limit of resolution to even the most perfect optical system the edge of the body is not seen distinctly but as a blurred band, the true edge being located somewhere in this "spurious disc" its position depending on the refractive indices of cell and surrounding fluid respectively. However carefully the system is adjusted a blurred band of at least 0.25u will replace the cell edge and if no special precautions are taken the band may be as wide as 0.4u".

There were no facilities for applying the photographic method described by Ponder (1934) and this author states in his work (dated 1924) that Pyper's (1919) diffraction method cannot be applied to avian red cells because of their oval shape.

MORPHOLOGY OF OSTRICH BLOOD.

The cells in the blood of the ostrich closely resemble the corresponding types of cells in the blood of the fowl, and they may be best considered by first reviewing the literature on the morphology of fowl blood.

Most investigators are agreed that the following types of cells are present in the normal blood of the fowl : red cells, thrombocytes and five varieties of white cells, viz., lymphocytes, monocytes, cells with polymorphous nuclei and spindle-shaped acidophil granules in their cytoplasm, cells with polymorphous nuclei and round acidophil granules in their cytoplasm, and basophiles.

Gibbs (1934) refers to a "polymorphonuclear leucocyte without rods and granules" as also being present in normal fowl blood in addition to the abovementioned types of cells, while several workers saw no monocytes, and the findings of those who do record the presence of monocytes

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differ considerably. Thus, to quote only some of the workers listed in table (2) :-

Marthin (1907), Hedfeld (1911), Burckhardt (1912) and Kleineberger and Carl (1912) found no monocytes in fowl blood. According to Wirth (1931), Steen (1913) is of opinion that they do occur, but only seldom, Salomon (1919) records 1&2 per cent., Fritsch (1920) 2 per cent. and Romer (1921) 1.4 per cent. Wirth (1931) records 2 per cent. On the other hand, Ellerman and Bang (1908) counted 23 per cent., Schmeisser (1915) 19.4 per cent., Breusch (1928) 9.2 per cent., Forkner (1929) (using supravital technique) 17.1 per cent. and Cook and Dearstyne (1934) (using supravital technique) 4 to 33 per cent.

These discrepancies serve to emphasise the difficulty of differentiating between lymphocytes and monocytes in ordinary stained preparations of fowl blood; in ostrich blood it is as difficult.

There can be no dispute about the presence of the elements which Sugiyama (contributions to embryology 97 undated) and others consider to be thrombocytes, but Blain (1928) states that he identified no structures corresponding to the platelets of mammals and he does not mention the thrombocytes.

Haematological terminology also gives rise to some confusion, entirely different cytological entities being called by the same name, or the same cytological entity by a variety of terms. Especially is this true of the cells with the spindle-shaped acidophil granules and those with the round acidophil granules, as will be observed from Magath's (1934) table given below.