apply to the destructive action of the spleen on glucuronides.

The figures recorded in Tables 3 and 4 bring out the interesting point that some of the glucuronide excreted in response to menthol is insoluble in ether. Since *l*-menthylglucuronide is easily soluble in ether, this may mean that a small proportion of the menthol may be transformed into a compound forming an ether-insoluble glucuronide. Possible transformations are either a dehydrogenation to a benzenoid compound containing a phenolic hydroxyl group, it being known that phenolic glucuronides are insoluble or only slightly soluble in ether, or an oxidation of either side chain with the formation of an aliphatic hydroxyl or carboxyl

group. These types of compound also yield glucuronides which may be insoluble or only poorly soluble in ether.

SUMMARY

1. Figures are presented for the glucuronidase content of certain organs of the rabbit and the rat.

2. In the rabbit the normal output of glucuronic acid and the percentage conjugation of *l*-menthol with glucuronic acid are both increased for the first two weeks after splenectomy and then fall back to the level for the intact animal by the fourth week. The function of spleen glucuronidase is discussed in the light of these results.

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The Uptake of Oxygen by Blood *in vitro*

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In some recent experiments (Ramsay, 1944b) it was observed that the oxygen capacity of fresh blood often appeared lower than the value obtained from the same blood after a treatment involving reduction with titanous citrate or tartrate. This confirmed similar results obtained by Ammundsen (1941), who used carbon monoxide instead of oxygen and treated the blood with sodium hyposulphite ($\text{Na}_2\text{S}_2\text{O}_4$) instead of a titanous salt. The present author, however, also observed that if the blood was allowed to stand in air, the apparent oxygen capacity increased until it approached the 'total' value obtained initially after reduction. This communication reports a somewhat fuller investigation of the phenomenon, which resembles that encountered by Kallner (1945), who found that the carbon monoxide capacity also increased during exposure of blood to

the air, but that the initial 'low' value was not observed at all if the pressure of carbon monoxide used for the saturation was 150 mm. instead of the 25 mm. originally recommended by Van Slyke & Hiller (1928).

METHODS

Specimens. Since blood from horses gave the most striking results in the earlier investigation, attention was concentrated on that species, but observations were made on other animals, principally sheep, cattle and pigs, and also man. In most of the animals blood was drawn from the external jugular vein into flasks containing oxalate, and suitable precautions were taken to prevent the access of air until the experiments were begun. Except in the case of the pig bloods, this was usually within 5–10 min., although occasionally the delay was reduced to 3 min. Tests showed, however, that a delay of up to at least 3 hr. did not seem to affect the general trend of the results.

Apparatus for aeration (or exposure to gas mixtures). The usual method of exposing blood to air in a mechanically rotated tonometer is not suitable for use with horse blood, which may show a sedimentation rate in Westergren tubes of over 3 mm./min. Moreover, even with bloods having a normal slow sedimentation rate this method of oxygenation is subject to small but significant errors (Roughton, Darling & Root, 1944). In the present work, therefore, treatment of blood with air or other gas mixtures was done in a vessel in which the blood was less subject to sedimentation and related errors, and which was easier to manipulate than a tonometer. The vessel, which is similar to, but simpler than that employed by Brooks (1935), is illustrated in Fig. 1. The large aperture is closed with a one-holed

Fig. 1. Vessel for the aeration of blood samples.
Overall length about 10–11 cm.

stopper carrying a short glass tube of 1 mm. bore. This serves as an exit for the gas, saturated with water vapour at the appropriate temperature (all experiments were done at room temperature), which is passed in through the small side-tube at a rate of 5–10 l./hr. The vessel is shaken mechanically as rapidly as it is possible without causing serious foaming of the contents. It is unnecessary to interrupt the stream of gas during the removal of samples, which can be done within a few seconds of the cessation of shaking by withdrawing the capillary glass tube and inserting the tip of a pipette. As a means of equilibrating blood with air or any other gas mixture this method appears less efficient than the rotating tonometer, because the latter presents a larger and continually changing surface of blood to the atmosphere. This deficiency, even if it be real, is not material to the present experiments.

Blood-oxygen determinations. The usual manometric Van Slyke technique was used, saponin in the ferricyanide reagent being replaced by 0.15–0.20% oleyl sodium sulphate. Too great an excess of this substance not only causes stopcocks to leak, but also impairs greatly its haemolytic action. The correction for dissolved oxygen was that recommended by Sendroy, Dillon & Van Slyke (1934).

Total haemoglobin. This was determined by the method of Ramsay (1944*b*). To avoid low results, the Van Slyke apparatus was washed as a routine before each analysis, once with 1% (v/v) nitric acid and then once with 2.5% (w/v) $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$. Probably the nitric acid inactivates by oxidation traces of sulphur compounds retained by the mercury from the $\text{Na}_2\text{S}_2\text{O}_4$ used in the previous analysis. The sodium phosphate washes out the residual nitric acid and leaves the apparatus at a faintly alkaline reaction, which is least harmful to haemoglobin during the early stages of the analysis.

Iron. This was determined by the method of Ramsay (1944*a*), with one minor alteration. It has been found that

overheating in the digestion process leads to very low results. The error cannot be due to loss of iron, since it is decreased or eliminated by prolonged boiling of the digest after dilution, followed by an extremely slow titration. Neither can the error be due to phosphate, since low results engendered by the presence of relatively large quantities of that ion are not corrected by boiling. Since it has also been found that the presence of traces of HClO_4 does not falsify the titration, and thus that the removal of the last trace of this acid is not vital, overheating of the digest is now strictly avoided by terminating the digestion immediately on the disappearance of the yellow colour described before. The digest is boiled for a few seconds after dilution, and is then cooled to room temperature before the titration is done.

RESULTS

Preliminary investigations (Ramsay, 1944*b*), which now seem perhaps to have been inadequate, suggested that 15 min. aeration should suffice for the saturation of blood with oxygen. In that work, the time of aeration was therefore 15 min., but after it was observed that the oxygen capacity, particularly of horse blood, appeared to increase when the blood was allowed to stand exposed to the atmosphere, it became of interest to determine the effect of a more vigorous exposure of blood to the air. Samples of horse blood fresh from the animal were shaken with air for periods of time far in excess of the 15 min., and the apparent oxygen capacity was determined at intervals. The results were variable, but seemed to fall broadly into three groups, although no sharp differentiation could be made between one group and the next. The groups comprised respectively those bloods in which oxygenation was rapid and complete, those in which it was rapid but not quite complete, and those in which it was very slow. The completeness of oxygenation was judged by comparing the apparent oxygen capacity with the experimentally determined total haemoglobin (as vol. $\text{O}_2\%$). One example from each of these groups is illustrated graphically in Fig. 2, where the oxygen taken up at any given time is expressed as a percentage of the total possible. Oxygenation was never complete in 5 min. This observation is important in connexion with later experiments. Occasionally, aeration for 20–30 min. resulted in 100% oxygenation, but it was more usual for oxygen to be taken up more or less rapidly until 96–98% saturation was reached, after which the curve flattened out, either partially or altogether. In a few cases it proved necessary to aerate the blood for upwards of an hour before oxygenation approached completion. These experiments have been repeated with blood specimens from man, sheep, and a few cows and pigs. Minor differences among the species were noticed. Sheep and cattle bloods took up oxygen more rapidly than human bloods, and the majority of these seemed

more rapid than the horse bloods. It is difficult to fit in the pig bloods, since they were unfortunately all 2 or 3 days old (they were kindly posted to me by Prof. E. J. King, Hammersmith), but a few of

Fig. 2. Rate of uptake of oxygen by three different horse bloods.

them required an exceptionally long aeration period for oxygenation. The most marked and characteristic species difference noticed may be summarized by stating that all sheep and cattle bloods examined were at least 99% oxygenated after 30 min. aeration, whereas figures as low as 96–97% were not uncommon with human and horse bloods, and there were rare cases in which the deficiencies were considerably greater. The analytical error is low enough for these differences to be considered highly significant.

It was claimed by Conant, Scott & Douglass (1928) and confirmed by the author (Ramsay, 1944b) that after evacuation and treatment with titanous tartrate or titanous citrate, blood might be completely oxygenated by aeration for as short a period as 5 min. That oxygenation is indeed complete in such cases is shown by the fact (see Table 2) that each milligram-atom of iron in the experimental blood samples combines, under such treatment, with almost exactly 22.40 ml. of oxygen. This observation stands in marked contrast to those described in the preceding paragraph. The explanation offered by Kallner (1945) for some apparently similar phenomena is that fresh blood contains carbon dioxide,

which exerts some inhibitory effect on the uptake of carbon monoxide (or presumably oxygen, although Kallner did not discuss this gas). Exposure to air removes carbon dioxide, with the result that blood treated in this way should take up carbon monoxide more completely, as is of course the experimental observation. No evidence was given in support of this theory, and the following series of experiments was designed not only to illustrate the striking change in properties which results from previous aeration of the blood, but also to test whether Kallner's hypothesis would serve as an explanation of the behaviour of venous blood *in vitro* towards atmospheric oxygen.

A fresh blood sample was divided into two portions. Portion X was exposed to a vacuum for a time shown by experiment to be sufficient for the removal of practically all oxygen and carbon dioxide. It was then aerated for 5 min., and its apparent oxygen capacity determined (see Table 1, line (i)). In the meantime, portion Y was aerated direct, and its apparent oxygen capacity determined after 5 min. (line (ii)) and 60 min. (line (iii)). Portion Y was then deoxygenated and decarbonated *in vacuo*, and re-aerated for 5 min., after which the apparent oxygen capacity was again determined (line (iv)). Total haemoglobin and iron were also determined, and expressed as vols. of O₂%, the assumption being made in the case of iron that 55.84 mg. of iron combine with 22.40 ml. of oxygen. These experiments were done on the blood of horses and of sheep, since these two species provided the extremes of behaviour when blood samples were simply shaken in air. The results of two representative experiments on each species are given in Table 1.

Table 1. *Oxygen uptake of blood after various treatments*

Treatment of blood	Apparent oxygen capacity (vol. of O ₂ %)			
	Horse A	Horse B	Sheep A	Sheep B
(i) Evacuation and 5 min. aeration (portion X)	13.09	13.60	11.69	11.57
(ii) 5 min. aeration (portion Y)	13.91	13.57	16.11	13.26
(iii) 60 min. aeration (portion Y)	15.89	15.01	18.18	13.43
(iv) 60 min. aeration, evacuation, and 5 min. aeration (portion Y)	16.03	15.31	16.64	13.41
(v) Reduction with Ti (total oxygen)	16.15	15.39	18.20	13.52
Calculation from Fe (55.84 mg. Fe = 22.40 ml. O ₂)	16.38	15.29	18.40	13.68

On examination of Table 1 it is evident that 60 min. aeration, as might be expected, gives a close approximation to the actual total possible oxygen capacity (compare line (iii) with lines (v) and (vi)). Some horses provided exceptions to this rule, the discrepancies in such cases ranging up to about 4%. As is shown by line (ii), 5 min. aeration of fresh blood is quite insufficient for thorough oxygenation. Moreover, and this is important from the point of view of Kallner's hypothesis, evacuation of fresh blood, followed by 5 min. aeration, still does not lead to complete oxygenation (see line (i)). On the other hand, if the blood is thoroughly aerated and then evacuated before 5 min. aeration, oxygenation is usually complete (compare line (iv) with line (iii) and line (v)). One or two sheep bloods behaved exceptionally during these re-aeration experiments, in that they were not completely oxygenated in 5 min., although the oxygen taken up was always more than when the fresh evacuated blood was aerated for the same period. No explanation can be offered, but it was noticed in these cases that the early stages of oxygenation appeared to be sluggish; that is to say, the change in colour of the blood from blackish to bright red was unusually long delayed.

DISCUSSION

When it is borne in mind that the total haemoglobin determination is not affected by previous exposure to air, and that this determination requires only 5 min. aeration, it is interesting to note that treatment of the blood with a reducing agent such as titanous citrate or tartrate increases the rate of uptake of oxygen, under the conditions of these experiments, to much the same extent as exposure of the blood to air for an hour. The experiments of Kallner (1945) suggest the possibility that the same may be true of the treatment with $\text{Na}_2\text{S}_2\text{O}_4$ in the carbon monoxide method for total haemoglobin.

An attempt was made to confirm these experimental results by substituting treatment with oxygen-free nitrogen for exposure to a vacuum, but it was unfortunately found (cf. Mathison, 1911) impossible to remove either oxygen or carbon dioxide completely from blood by this process alone.

It may be noted in passing that the rate of uptake of oxygen from air containing 5% carbon dioxide was not greatly different from the rate when ordinary laboratory air was used. Such slight differences as were sometimes observed may have been due to alterations in acidity. This is slight circumstantial evidence against the hypothesis of Kallner, but a more decisive refutation of the suggestion that his hypothesis might apply to these oxygen experiments may be gleaned from Table 1. Evacuation, which was shown experimentally to remove practically all carbon dioxide, only increases the rate of uptake

of oxygen if it is preceded by thorough oxygenation. The presence of carbon dioxide can therefore not be regarded as a decisive factor.

One possible explanation of the results in Table 1 is that oxygen and the reducing agents may alter the permeability of the erythrocyte to oxygen. Although this seemed unlikely, because the work of Hartridge & Roughton (1927) suggests that the time lag imposed by the cell membrane is of the order of 0.01–0.1 sec., some experiments were done to test this point. It was thought that a comparatively gross experiment would serve to detect a gross effect, so the rate of uptake of oxygen by fresh blood was measured in both the presence and the absence of the least quantity of cetyl trimethyl ammonium bromide required to bring about complete haemolysis in a few seconds. The results, plotted graphically for two typical bloods in Fig. 3, are again given as percentages of the total possible oxygen uptake.

Fig. 3. Rate of uptake of oxygen by two specimens of blood, measured in both the presence and the absence of cetyl trimethyl ammonium bromide. $\odot$ Points for unhaemolyzed blood. $\times$ Points for haemolyzed blood.

It is evident from Fig. 3 that haemolysis by cetyl trimethyl ammonium bromide does not increase the rate of oxygen uptake as measured in these experiments. Hence, since complete removal of the cell membrane is without effect, it seems unlikely that permeability of that structure can be a factor of importance. It should be stated that the method of expressing the results was chosen in view of the confusing fact that in the haemolyzed blood the total haemoglobin was always 2–5% lower than in the untreated blood. This was doubtless caused by the denaturing action of the haemolytic detergent.

The belief is now fairly general that normal blood may contain ferrihaemoglobin in amounts too small to be detected by ordinary spectroscopic methods, such as that of Wendel (1938). Even if gasometric evidence be laid aside, there is still support for this belief in the spectroscopic measurements of Havemann, Jung & Issekutz (1939) and the more recent work of Paul & Kemp (1944). Examination of Table 1 suggests that the results summarized there would be consistent with the hypothesis that fresh venous blood contained larger amounts of ferrihaemoglobin than have hitherto been suspected, and that this disappeared fairly rapidly when the blood was exposed to the atmosphere. Such disappearance is known to take place in cases of experimental and pathological ferrihaemoglobinaemia (Campbell & Morgan, 1939; Cox & Wendel, 1942). This theory is not easy to test. Direct spectroscopic observations may not be very reliable, but efforts have been made to obtain some indirect evidence. Unpublished experiments in this laboratory have shown that two molar proportions of KCN inhibit practically completely the reduction of ferrihaemoglobin by even a ten-fold excess of ascorbic acid. Consequently, it was thought worth while to try the effect of KCN on the rate of oxygenation of fresh venous horse blood. No effect whatever could be detected, whereas it would be reasonable to suppose that a high concentration of ferrihaemoglobin in the blood would have led to a slowing of the rate of oxygenation in the presence of KCN, which might have retarded the rate of disappearance of ferrihaemoglobin. For similar reasons it was thought possible to test the hypothesis by observing the effect of sodium or ethyl iodoacetate, since these substances, by reacting with sulphhydryl groups, might also decrease the rate of reduction of ferrihaemoglobin. Neither of these substances, however, had any effect whatever on the rate of oxygenation. These experiments are unfortunately subject to criticism on the ground that the red cell may not be sufficiently permeable to either cyanide or iodoacetate for any effect to take place in the short time available. Even on theoretical grounds, however, the disappearing ferrihaemoglobin theory is unlikely to be correct. If it were, then, since arterial blood is at least 95% saturated with oxygen (98.5%, according to the recent work of Drabkin & Schmidt, 1945), it would be necessary to assume that the circulating corpuscle accumulated a considerable proportion of ferrihaemoglobin which reverted more or less completely to ferrohaemoglobin each time the corpuscle passed through the lungs. Since the possibility of influence by carbon dioxide has been excluded by experiment, the only factor altering the proportion of ferrihaemoglobin would thus be the pressure of oxygen. Provided that the redox potential lies within certain limits, it is a simple

matter to give a physico-chemical argument demonstrating the theoretical possibility of this (some related phenomena were discussed by Conant & Fieser, 1924), but the fact that a high proportion of the intracorpuseular glutathione appears to be in the reduced condition (Woodward & Fry, 1932; Prunty & Vass, 1943) makes it extremely unlikely that the redox equilibrium is at a level suitable for the operation of such a mechanism. In the absence of definite favourable evidence, then, it seems impossible to maintain the hypothesis that the behaviour of fresh venous blood on exposure to air is due to the disappearance of ferrihaemoglobin. It must be emphasized, however, that in the absence of detailed knowledge of the intracorpuseular redox equilibrium, this criticism need not necessarily apply to the small residual oxygen- and CO-capacity discrepancies noted by Ramsay (1944b) and by Ammundsen (1941). The few larger discrepancies noted by the author in samples of horse blood were probably due to incomplete oxygenation, undetected because of those variations in the rate of oxygen uptake which are the subject of the present discussion.

Since blood is normally almost completely saturated in its passage through the lungs, it seems unlikely that this work has any significance for the living animal. If current figures for the oxygen content of arterial and venous blood be accepted (and there seems to be no reason to doubt them), then most of the circulating haemoglobin must spend the greater part of its time at least partially oxygenated. Hence, since the experiments described here show that thorough aeration is necessary to prevent the reaction between haemoglobin and oxygen from becoming sluggish, conditions in the living body should tend to prevent sluggishness. Indeed, it seems difficult to avoid stating the possibility that the observed sluggishness may develop after the blood is drawn, while it is kept, even for a short time, in a partially oxygenated condition. One is reminded of the observation, put on a sound footing by Brooks (1935) that ferrohaemoglobin is much more rapidly oxidized to ferrihaemoglobin by oxygen at low pressures than at pressures sufficient to permit the existence of a large proportion of oxyhaemoglobin. The stabilizing effect of oxygen may be of general application.

There is a suggestion in the figures given in Table 1 that both the 'total' haemoglobin and the oxygen capacity after 60 min. aeration are in good agreement with the total iron of the blood. Since such agreement is a matter of some importance in connexion with the selection and adjustment of standard haemoglobin methods, it has been thought worth while to add Table 2, in which the experimental oxygen, both 'total' and after 60 min. aeration, calculated for 55.84 mg. experimental iron,

is given for each of ten consecutive bloods, six from horses and four from sheep. Details are given in order that the values may be compared for individual specimens. The theoretically perfect result is, of course, 22.40 ml. in every case. Some similar experiments were done on the equivalence of Van Slyke oxygen and iron in 25 human bloods by Jope, Jope & O'Brien (1943). The results of these workers, recalculated to the same basis, are included in the table for comparison.

Table 2. *Equivalence of experimentally determined oxygen and iron*

(Theoretical value 22.40 ml. O₂/55.84 mg. iron.)

Exp.	Van Slyke oxygen after 60 min. (ml. O ₂ / 55.84 mg. Fe)	'Total' oxygen (ml. O ₂ / 55.84 mg. Fe)	Jope <i>et al.</i> (1943)
1 H*	22.71	22.76	—
2 H	21.75	22.10	—
3 H	22.00	22.55	—
4 H	21.72	22.54	—
5 H	22.03	22.00	—
6 S	22.16	22.19	—
7 H	21.99	22.18	—
8 S	22.38	22.41	—
9 S	22.13	22.20	—
10 S	22.25	22.40	—
Mean	22.11	22.33	22.11 (25 results)
Difference of mean from theoretical	-1.3%	-0.3%	-1.3%
Range	21.72-22.71	22.0-22.76	20.49-23.34
S.D. as % of mean	1.3%	1%	3.3%
S.D. of Fe method	0.5%	0.5%	†
S.D. of O ₂ method	0.5%	0.5%	†

* H=horse, S=sheep.

† Methods stated by the authors to be reproducible to within ± 0.2 - 0.5% .

It appears from this table that the total oxygen capacity, measured after reduction, corresponds very closely indeed to the total iron of the blood. There are no discrepancies outside the limits of experimental error, and the mean, 22.33 ml. of O₂/55.84 mg. of Fe, indicates that virtually the whole of the blood iron is in combination as ferrohaemoglobin or some substance which combines with oxygen after treatment with a suitable reducing agent. Much of the so-called serum iron may be haemoglobinoid, at least in origin, since it is not uncommon to find plasmas or sera which, although not abnormal in colour, show oxyhaemoglobin bands when a sufficiently thick layer is examined spectroscopically. Moreover, it has recently

been suggested that physiological intravascular haemolysis may occur during exercise (Gilligan, Altschule & Katersky, 1943). The Van Slyke oxygens after aeration for 60 min. also agree well with the iron, although there is a little more scatter, and the mean is lowered by one or two results which suggest either that even 60 min. aeration of these bloods was inadequate, or else that they did actually contain small amounts of ferrihaemoglobin. The summary of their results published by Jope *et al.* (1943) indicates that some of their values must have been 7% below their mean, or a full 8% below the theoretical value. It seems possible that in such cases oxygenation was incomplete.

That the apparatus used by the author for aeration is an efficient oxygenator is shown by the fact that after aeration and complete deoxygenation *in vacuo*, blood may be completely oxygenated in 5 min. It therefore seems justifiable to draw to the attention of analysts the fact that the rate of uptake of oxygen by blood is variable, and may on occasion be much slower than has been generally believed. Distinct species variations can be detected, but perhaps the most important practical point is that in all cases the rate is made more rapid by thorough oxygenation of the blood. This suggests that the converse may be true: that the keeping of blood in a low state of oxygenation may have an adverse effect on the rate at which it will subsequently take up oxygen. These difficulties may be avoided if the blood is treated with a suitable reducing agent, since oxygenation may then be carried out in 5 min. It is unfortunate that none of the possible explanations of these observations has so far proved satisfactory.

SUMMARY

1. A simple apparatus for the oxygenation of blood or its treatment with any gas mixture is described. Its advantages over the rotating tonometer are discussed.

2. When blood is aerated in this apparatus, a period of 15-60 min. is usually required for 98-100% oxygenation. The previous observation of frequent small deficiencies in oxygen capacity is confirmed.

3. If the blood has been freshly oxygenated, and then deoxygenated *in vacuo*, only 5 min. aeration is usually required for reoxygenation, as in the titanous citrate or tartrate method for total haemoglobin.

4. The following possible explanations are discussed: presence of carbon dioxide, presence of ferrihaemoglobin, alterations in the permeability of the erythrocyte to oxygen. It is found necessary to reject all of these.

5. Very good agreement is observed between total iron and total haemoglobin or oxygen capacity after 60 min. aeration.

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The Component Acids of Cow Colostrum Fat

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Though much work has been done on the composition of milk fat, little attention has so far been paid to the fat secreted during the first few days after parturition. Baldwin & Longenecker (1944a) studied the fatty acid distribution in the fat of human milk during the first few days of lactation and found that it was different from that of mature milk fat. The results of these workers indicated that as the period of lactation progressed from the first day's colostrum to mature milk, the amount of the acids of greater molecular weight than C_{18} decreased and the amount of C_{12} and C_{14} acids increased. Later, Baldwin & Longenecker (1944b) investigated the colostrum fat of a single cow collected during the first 4 days after parturition and reported that its fatty acid composition was very similar to that of a typical butter fat. The results obtained were only from samples collected from a cow of unspecified breed and comparisons were made with the results of the analysis of typical butter fat by Hilditch & Longenecker (1938). It is possible that if a sample of butter fat from the same cow had been collected and analyzed at a later period some differences might have been observed. Apart from these investigations no work appears to have been carried out on this subject. In the present study attempts were made to collect colostrum fat from some of the common breeds of cattle in India and the results obtained are now presented.

METHODS

The colostrum from which the fat was extracted for the present studies was collected from four different breeds of cows, namely, Sindhi, Gir, Tharparkar and Sahiwal. Milking was done twice a day. In the case of each animal material collected during each 24 hr. after parturition was mixed together, diluted with water and the cream separated. Butter fat was prepared direct from the cream by the 'boiling off' method. The butter fat prepared from each 24 hr. collection from every animal was first analyzed for its general chemical characteristics. The collection of butter fat was continued during the first 15 days of lactation and samples from the first 5 days and the tenth day of lactation were used in the present studies.

During the dry period the cows were receiving 3 lb. of a concentrate mixture (wheat bran, ground-nut cake, grain husk and grain in the ratio of 4:2.5:2.0:1.5) in addition to $\frac{1}{2}$ lb. each of extra grain husk and ground-nut cake. Four weeks before calving pregnant animals were fed 3 lb. of the usual concentrate mixture mentioned above with 3 lb. of wheat bran. For the first 4 days after calving the animals received 8 lb. wheat bran, 1 lb. of *jaggery* (crude cane sugar), with a roughage consisting of 55 lb. green grass and 3 lb. ragi straw. From the fifth day of lactation onwards the animals were fed, together with the usual amount of roughage as mentioned above, different amounts of the common concentrate mixture according to milk yield.

Representative composite mixtures of the colostrum fat during the initial days of lactation were obtained by mixing equal quantities of the fat collected from the different cows