CCXL. STUDIES IN THE SECRETION OF MILK FAT

I. THE EFFECT OF INANITION ON THE BLOOD LIPOIDS OF THE LACTATING COW

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DURING the past few years the work of Lintzel [1934], Blackwood [1934], Graham *et al.* [1936] and Maynard *et al.* [1938], based on the principle of simultaneous arterial and venous blood sampling, has shown that the only lipid fraction of the blood used by the mammary gland for milk fat production is the neutral glyceride fraction and not that comprising the phosphatides or cholesteryl esters. At first sight this might seem to suggest that for the secretion of milk fat the gland simply selects from the blood the particular type of glycerides which it requires and that these are then allowed to diffuse into the milk. But it is clear from the peculiar composition of the fat itself and also from the fact that the respiratory quotient of the actively lactating mammary gland has now been found by Graham *et al.* [1938] to be considerably greater than unity, that more complex processes such as the synthesis of fat from carbohydrates are also undoubtedly involved. It would therefore appear that there are at least two main precursors of milk fat in the blood, the neutral glycerides on the one hand and some form of carbohydrate material on the other, and so it seems reasonable to suppose that the composition of the milk fat produced by an individual animal at any particular time will depend on the extent to which each of these precursors contributes towards its production. It was felt that further evidence regarding the biochemical and physiological processes involved in milk fat secretion might be obtained by a detailed study of both the blood and milk fats of animals kept under such experimental conditions as would be expected to alter the proportions in which these two precursors were used by the gland. One possible method of achieving this end would be to cause in some way a marked alteration in the lipid concentration of the blood of the cow in the hope that an increase or decrease in the level of triglycerides in the plasma would considerably alter the proportion of the milk fat arising from this particular fraction of the blood. Consequently, it was decided to investigate the problem by experiments involving inanition and certain other procedures which might be expected to cause a considerable alteration in the relative availabilities of the two precursors. The present paper and the communication which follows it are concerned only with the inanition work, the first dealing exclusively with the blood lipoids and the second with the milk fat. The effect of inanition on the general composition of the milk has already been discussed by Smith *et al.* [1938].

EXPERIMENTAL

The blood and milk fat samples analysed in the present work were obtained in the course of an experiment undertaken by Dr S. Morris to determine the effect of inanition on the metabolism of lactating and non-lactating ruminants. The animals used were three non-pregnant Ayrshire cows in the sixth to seventh month of their lactation period. After a few weeks, during which they had been receiving a normal well-balanced diet, food was withheld from two of them for a period of 12 days, after which free access to food was again permitted, water being given freely at all times. Under these circumstances Cow no. 1 appeared to present a case of simple inanition throughout the 12 days, but with Cow no. 2 severe symptoms of milk fever occurred in the second day of the fast, and although they rapidly disappeared after inflation of the udder, their occurrence must be taken into consideration as a possible complicating factor. For Cow no. 3 the experiment was discontinued on the seventh day of the fast because by that time the condition of the animal was not satisfactory, a fact which may well have been due to a marked deficiency in depot fat as revealed by subsequent *post mortem* examination. At suitable times before, during and after inanition, blood samples were taken from one of the mammary veins, and composite daily milk samples were collected for the isolation of the fat.

Extraction of the blood lipoids

About 200 ml. of blood were centrifuged for 45 min. and the plasma was separated from the corpuscles. The plasma was then allowed to drop in a fine stream into about 15 volumes of alcohol-ether (3:1), the amount of solvent made up to 20 volumes and the mixture left for a few hours at room temperature with occasional shaking. It was then filtered, aliquots of the filtrate were removed for the colorimetric estimation of phosphorus and a known volume of the filtrate, usually about 2 l., was reduced to dryness on a water bath *in vacuo* under an atmosphere of nitrogen. The residue so obtained was extracted several times with chloroform and the extracts were filtered from the relatively large amounts of blood inorganic salts which accumulated at this stage. The corpuscles were similarly treated except that they were first mixed with an equal volume of water to cause haemolysis and the mixture was finally made up in 25 volumes of alcohol-ether. 20 volumes of solvent were used for the plasma and 25 for the corpuscles, since it has recently been shown by Boyd [1936, 1, 2] that these are the minimum proportions which can be used with safety if the fat extraction in both cases is to be as complete as possible.

Analysis of the blood lipoids

Owing to the fact that the volume of blood available in most animal experiments and also the concentration of lipoids in the blood are both so exceedingly small, accurate analysis of the blood lipoids has always been a matter of considerable difficulty. In the present work, for reasons outlined later, it was decided not to employ any of the usual micro methods, but rather to take blood samples of such a size (about 200 ml.) that the ordinary standard macro procedures, suitably adapted for use with unusually small amounts, could be applied to the resulting lipoids. This was possible in the present work since the withdrawal of such large samples of blood is without ill effect on the cow. For every sample, therefore, fatty acids and total unsaponifiable matter were determined by direct weight, cholesterol by weighing the digitonide, iodine values by the

Fig. 1. The effect of inanition on the lipid phosphorus content of the blood.
●—● Cow no. 1. ○—○ Cow no. 2. △—△ Cow no. 3.

Table I. *The analytical data for the corpuscle lipoids (mg./100 ml.)*

Days of fast	Total fatty acids			Total unsaponifiable matter	Cholesterol	Cholesterol content of unsaponifiable matter
	Wt.	I.V.	Mol. wt.			
Cow no. 1						
Before	2	—	63	354	—	—
	1	374	60	360	227	183
	0	400	63	379	222	186
During	5	381	60	367	225	186
	10	406	59	349	222	192
	11	359	60	363	216	186
After	28	355	63	359	217	174
	30	363	62	342	188	165
Cow no. 2						
Before	8	388	67	380	228	190
	5	404	65	363	207	180
	1	400	67	377	215	183
During	2	397	66	391	236	200*
	6	372	61	341	214	178
	8	386	56	—	220	176
	12	371	59	350	205	188
After	38	362	59	367	201	167
	40	378	62	340	213	171
	44	371	60	359	222	172
Cow no. 3						
Before	2	432	64	336	207	196
	1	413	63	398	216	187
	0	408	64	334	206	193
After	6	389	59	364	214	196

* Day on which Cow no. 2 suffered from milk fever.

bromine absorption method and molecular weights by titration. Many precautions are necessary if accurate results are to be obtained, but as it is hoped that a separate communication on the details of the procedure will shortly be published, only the barest outline of the methods need be recorded here.

Corpuscles. As the corpuscle lipoids consist almost entirely of free cholesterol and phosphatides it was felt that nothing would be gained by the preliminary separation of the phosphatides. The solvent was therefore removed from the chloroform solution, obtained as already described, and the residue saponified directly by heating it for about 30 min. with 20 ml. alcohol and 2 ml. 40% KOH, to which 20 ml. benzene were added to render the phosphatides more soluble. The fatty acids and unsaponifiable matter were then isolated, washed with water and finally made up to known volumes in light petroleum, from which suitable aliquots were taken for analysis. The results for lipoid P as estimated in the original alcohol-ether extract are shown in Fig. 1, while the remaining values are recorded in Table I.

Plasma. After the removal of the solvent from the chloroform solution of the plasma lipoids, the residue was dissolved in about 10 ml. of ether and transferred to a centrifuge tube and the phosphatides were precipitated with 30 ml. acetone. After standing at room temperature, the mixture was centrifuged, the clear supernatant liquid poured off and the residue washed with acetone. It was then dissolved in ether and reprecipitated. Finally, both the phosphatide and non-phosphatide fractions were made up to known volumes and analysed. That almost a complete separation of the two fractions had been obtained was indicated by the facts that the P content of the non-phosphatide fraction was not more than 3% of the whole and was often considerably less and that no more than the merest traces of cholesterol remained in the phosphatides. For this precipitation the use of $MgCl_2$ and a temperature of 0° are frequently recommended in the literature in order to assist the separation of the phosphatides, but it was found in the present work that these extra details were unnecessary, provided that really pure ether and acetone were available, and, as there is a very serious risk of precipitating some of the cholesteryl esters and neutral glycerides along with the phosphatides at such a low temperature as 0° , and as Mg ions may tend to cause difficult emulsions at a later stage, the simpler procedure outlined above was found to be the safest and most efficient.

After the P estimation, the phosphatide fraction was saponified as already described for the corpuscles. Total fatty acids and unsaponifiable matter were then isolated and analysed. For the non-phosphatide fraction, total lipoids and free cholesterol were first estimated in suitable aliquots before the remainder was saponified. The variations in concentration of the different plasma lipoids for the three animals are shown in Figs. 1, 2 and 3, while the molecular weights and iodine values of the acids are arranged in Table II.

One of the chief difficulties in analysing blood lipoids at the present time is that no method has yet been found for separating the cholesteryl esters from the neutral glycerides, these fractions, which together form the two main constituents of the non-phosphatide fraction, having very similar solubility properties. It is only possible to estimate the amount of fatty acids originally present as triglycerides by calculation. This was done here by assuming that the mean molecular weight actually found for the total non-phosphatide fatty acids was also the mean molecular weight of the acids esterified with cholesterol, which, as the latter greatly predominate, would give a very close approximation to the truth. From the amount of glyceride fatty acids so estimated it was possible to calculate the amount of glycerol which must have been required for

Fig. 2. The effect of inanition on the plasma lipids of Cow no. 1.

Fig. 3. The effect of inanition on the plasma lipids of Cow no. 2.

Fig. 4. The effect of 6 days' inanition on the plasma lipoids of Cow no. 3.

Table II. *Analytical data for the plasma lipoids not shown diagrammatically in the Figs.*

		Fatty acids				Total cholesterol content of unsaponifiable matter %	Sum of fatty acids, unsaponifiable matter and glycerol as % of original non-phosphatide fraction
Days of fast	Phosphatide		Non-phosphatide				
	I.V.	Mol. wt.	I.V.	Mol. wt.			
Cow no. 1							
Before	2	64	333	134	295	99	97
	1	66	359	136	295	94	95
	0	59	335	135	293	94	95
During	5	62	320	136	295	91	96
	10	65	340	125	304	96	94
	11	62	359	134	291	96	90
After	23	62	322	137	294	85	94
	28	74	323	129	294	91	94
	30	74	340	136	295	92	94
Cow no. 2							
Before	8	60	342	139	304	90	99
	5	71	331	142	305	96	99
	1	60	318	137	312	97	99
During	2	68	332	149*	306	97	97
	6	69	388	137	303	94	97
	8	70	333	140	304	91	99
	12	58	370	137	297	93	94
After	38	63	355	130	301	88	92
	40	57	347	131	302	90	94
	44	65	348	130	304	90	88
Cow no. 3							
Before	2	77	333	140	293	98	98
	1	69	323	143	289	97	96
	0	65	366	142	293	98	97
After	6	59	370	143	294	99	93

* Day on which Cow no. 2 suffered from milk fever.

their esterification. Then, by adding together the values found for total fatty acids, total unsaponifiable matter and those calculated for glycerol, it was possible to estimate the percentage of the original non-phosphatide fraction which was accounted for by the various constituents. It will be observed from Table II that this figure was usually well over 90 %, a fact to which reference is made later.

DISCUSSION

The work of Maynard & McCay [1929] and of Porcher & Maynard [1930] has shown that the blood lipid level of ruminants under normal circumstances changes extremely slowly and that variations in it tend to be but slight. In fact with non-lactating cows Aylward & Blackwood [1936] found no definite or consistent alteration in the total fatty acid content of the blood even after a fast of 10½ days, while later results of Aylward *et al.* [1937] showed that although for two days after a drench of "labelled" fat the blood of the cow became gradually enriched with the "labelled" constituents of the drench, the actual lipid level itself was not measurably affected. It was therefore obvious that if the small changes in composition or in amount which may be expected in the blood fat of ruminants were to be detected, the methods adopted for analysis would have to be as searching and as accurate as possible. For this reason it was felt that the micro procedures now commonly in use such as that of Bloor [1928; 1929], were not applicable to the present experiments as they would not deal with sufficient material for the thorough examination which the author had in view. Nor was another type of method such as that published by Stoddart & Drury [1929] of much value here, as it depends on the assumption that the mean molecular weight of the fatty acids is always a given value from which their amount is calculated, whereas actually in the present work, the constancy or variability of these particular molecular weights before and during inanition was one of the properties which it was desired to investigate. It was therefore decided that the macro procedure, suitably adapted, would be the one most likely to give the best results. Although this method is somewhat long and laborious, it was believed that its application to a few large blood samples would be of much greater value than more numerous but less reliable analyses carried out on very much smaller amounts.

Phosphorus. The lipid P results shown in Fig. 1 are typical of the general effect of inanition found for the other lipid fractions of the blood. In the corpuscles the average figure of 16.2 mg. per 100 ml. for all three cows prior to the fast fell by less than 5 % to 15.4 during inanition, and a few weeks after re-alimentation it had tended to return towards the original value. That it never quite reached the initial figure may well have been due in part to the presence of small amounts of plasma still remaining in the corpuscles, for the latter were not washed with saline prior to their extraction as it was felt that, by prolonging the process of separation unnecessarily, more errors might be introduced than were actually avoided and that any plasma still remaining in the corpuscles would be too small to invalidate the general conclusions in any way.

In the plasma very much greater changes were observed than in the cells. The mean initial figures of about 10 mg. were reduced by almost 40 % to 6 mg. during inanition, and strangely enough this low level persisted even 6 weeks after free access to food was again permitted. In the work of Aylward & Blackwood [1936], the lipid P level in whole blood was found in most cases to rise immediately after food was withheld rather than to fall, but this unexpected observation can now be explained by the facts that almost half the lipid P in whole blood is

contained in the corpuscles, that its level in the corpuscles is not much affected by inanition and that the proportion of corpuscles in the blood as shown by Smith *et al.* [1938] increases from about 30 to over 40% in the initial stages of starvation. It is therefore only later in the fast that an appreciable reduction in the phosphatide content of whole blood is noticeable. It is of interest to observe that the total decrease in the lipid P of whole blood found for dry cows by Aylward & Blackwood [1936] was about 10% as compared with the much larger figure of 25% which can be calculated for lactating cows in the present work, notwithstanding the fact now well established that phosphatides are not precursors of milk fat.

Corpuscles. One of the main objects of analysing the lipoids of the corpuscles was to find whether their level or nature was materially affected by very abnormal conditions, for if no more than minute changes could be observed under such a prolonged period of inanition, it is highly improbable that they would ever change appreciably under any normal circumstances to which the cow might be subject. Consequently, it would be most unlikely that variations in milk fat secretion, whatever their cause, could ever be traced to changes or temporary peculiarities in the lipoids of the cells. If this were in fact found to be so, extension of the corpuscle side of the work in future experiments of this type would be quite unnecessary.

Before discussing the results it is essential to mention that the complete extraction of the lipoids from corpuscles, even by the procedure recommended by Boyd [1936, 2], is by no means easy, nor can it readily be done with identical results for different samples taken at the same time from the same batch of corpuscles. This difficulty can doubtless be attributed to the fact that the cells contain such extremely small amounts of very complex phosphatides in the presence of relatively large quantities of proteins, with which they may even be loosely combined. It is therefore not surprising to find considerable variation in the fatty acid levels before inanition as recorded in Table I, in spite of the fact that there was probably little actual change in the pre-fast period. There is, however, sufficient consistency to show that on the whole there was a reduction of some 6 or 7% in the total fatty acids as a result of inanition and that this decrease persisted for at least 6 weeks after re-alimentation. Thus with Cow no. 2 an original mean of 397 mg. fell to 371 mg. by the 12th day of the fast and was still close to this value 40 days later. That it did not show any tendency to return towards the original figure in the post-fast period, as was the case with the corpuscle P, was again probably due, in part at least, to the presence of small amounts of plasma still remaining in the corpuscles. As these small residual amounts of plasma had a higher ratio of fatty acids to lipid P than was the case for the corpuscles themselves, they would have a greater effect on the figures for corpuscle fatty acids than on those for the lipid P.

With regard to the nature of the corpuscle fatty acids, the iodine values in Table I suggest a very slight reduction in the degree of unsaturation during inanition. Thus, the mean initial figures of 62, 66 and 64 for the three cows fell to 60, 59 and 59 respectively towards the latter part of the fast, and showed a slight tendency to return towards the normal values in the post-fast period, but it is doubtful if such small variations have any significance. Unfortunately, from the molecular weights found for the acids little can be concluded. It appeared during the course of the experiments that the estimation of these particular molecular weights by titration is by no means a simple matter and that this is not entirely due to the very difficult end points which were encountered. The figures obtained ranged from 334 to 391 and possess at least two points of

interest: firstly, the great and irregular variations, and secondly, the magnitude of the values as a whole. From other so far unpublished work by the present author, it may be assumed that the corpuscle phosphatides are much more complex than is generally realized, but even if their main constituents were C_{20} , C_{22} and C_{24} acids, the mean molecular weight of all the acids would not be expected to be much greater than about 340. In fact one experiment on a much larger scale than the present has suggested that the value for corpuscle phosphatide fatty acids from a pooled sample of bovine blood is somewhat under 330. It is hoped that future work on the nature of these phosphatides will throw more light on this aspect of the subject, but at present it can only be stated that the variations in molecular weights as quoted in Table I are probably due to the difficulty of isolating the substances in a pure state and of making the actual determinations, and not to any significant change in the acids themselves.

Turning now to the cholesterol and total unsaponifiable matter, it is again seen from Table I that the alteration in level during inanition was in all cases negligible, but 30 or 40 days after the fast the cholesterol appears to have been somewhat reduced. Thus, taking Cow no. 1 as an example, the pre-fast figure of about 185 remained almost unchanged at a mean of 188 during starvation but then fell by some 9% to 170 in the post-fast period. The reason for the lack of change during the fast and the decrease following re-alimentation cannot at present be given, but taking the results as a whole and comparing them with those of the plasma, these changes in level may be regarded as exceedingly slight.

As the unsaponifiable portion of blood lipids is frequently assumed to consist almost entirely of cholesterol it was of considerable interest to estimate the actual proportion of each sample which consisted of this compound. It is clear from Table I that for Cows nos. 1 and 2 the mean figure for the amount of cholesterol in the unsaponifiable matter was 85% and for Cow no. 3, 92%, but the individual samples showed too much variation amongst themselves for any conclusion to be drawn as to the effect of inanition upon them. It is clear, however, that in every sample of unsaponifiable matter there was an appreciable amount of material soluble in light petroleum not precipitated by digitonin and not altered by resaponification. From these results as a whole it may therefore be concluded that the corpuscle lipoids of the lactating cow are not readily inclined to alter either in their nature or in their concentration even during prolonged inanition.

Plasma. Unlike the corpuscles, the plasma showed large reductions in the level of the various lipid constituents as a result of starvation. With Cow no. 1, for example, there was a decrease during the fast of about 26% in ester cholesterol, an initial figure of 176 mg. falling to just below 130 mg., while in the post-fast period this value fell still further to 94 mg., a total decrease of 47%. Similarly with the phosphatide and non-phosphatide fatty acids, original figures of 143 and 177 became 94 and 119 towards the end of the fast and diminished further after re-alimentation to mean values of 83 and 105 respectively. These figures represent a total reduction of 45 and 40% in the phosphatide and non-phosphatide fatty acids between the pre- and post-fast periods. The fact that these values were actually lower some weeks after free access to food had been permitted than they were when food was completely withheld is of considerable interest, though no definite explanation can at present be given. It may be that on re-alimentation, fat was so rapidly being used for replenishing the animal's depleted storage depots and for milk secretion that the level in the plasma could not be maintained above this low figure.

With Cow no. 2 the effects of inanition were complicated on the 2nd and 3rd days by the occurrence of milk fever, which was found to be accompanied by hyperlipaemia. Thus, as shown in Fig. 3, there were increases in the ester cholesterol, phosphatide and non-phosphatide fatty acids of 32, 25 and 14 % respectively, initial values of 169, 149 and 178 increasing to 223, 198 and 203. After the symptoms of milk fever had disappeared, however, these increases were followed by considerable decreases analogous to those found for Cow no. 1. Thus for ester cholesterol the value of 223 fell to 117 by the last day of the fast and was reduced to 100 in the post-fast period, a total decrease equal to 41 % of the initial pre-fast figure. Similarly the phosphatide and non-phosphatide fatty acids fell to 70 and 118 by the end of the starvation period and were as low as 85 and 106 some weeks after re-alimentation had been established, figures which are equivalent to decreases of 44 and 40 % respectively between the initial and final values. Again in Fig. 4 (Cow no. 3) it is confirmed that inanition causes a definite decrease in the various fatty constituents of the plasma, but for this cow the decrease only amounted to about 13 % for ester cholesterol, 30 % for phosphatide fatty acids and 18 % for those from the non-phosphatide fraction by the 6th day of the fast when the experiment was discontinued. It is of interest to notice from the figures just cited that in all cases, whether the lipid level rose or fell, the phosphatide fatty acids tended to be affected to a somewhat greater extent than the others, perhaps owing to a greater degree of metabolic activity on the part of these particular acids.

Further reference to Figs. 2, 3 and 4 shows that there is also a very definite reduction in free cholesterol and in triglyceride fatty acids as a result of inanition. For Cow no. 1, for example, initial figures of 45 and 43 respectively were reduced to 38 and 21 by the 11th day of the fast and to mean values of 25 and 29 in the post-fast period, final decreases which are equivalent to 44 and 33 % of the original pre-fast figures. The corresponding reductions in free cholesterol and glyceride fatty acids for Cow no. 2 were 59 and 36 %, and for Cow no. 3, after a fast of only 6 days, they were 22 and 35 % respectively. The values for glyceride fatty acids in both Figs. 2 and 3 appear to be characterized by greater irregularities than any of the other lipid constituents, and although this may actually have been the case in the plasma, it may well have been due mainly to the fact that these values were obtained, as already described, by calculation from the figures found for free and total cholesterol and for total non-phosphatide fatty acids. Any unavoidable errors therefore in the estimation of these latter fractions may have combined in several cases to give somewhat greater errors in the relatively small values calculated for the triglyceride acids. This suggestion gains support from the fact that no such irregularities occurred in the values for free cholesterol, which are almost equally small but which were found by direct estimation.

One point of considerable interest which may be stressed at this stage and which is very obvious from Figs. 1-4 is the extraordinary tendency in the plasma of the cow for all the various lipid constituents, with the possible exception of the triglycerides, to behave alike under different conditions. Thus in milk fever, the rise in phosphatide fatty acids was accompanied by similar rises in cholesterol esters and free cholesterol, while again during simple inanition they all decreased in much the same way, with the result that the general composition of the total lipoids tended to remain the same although their level in the plasma changed. This tendency to a constant composition for the lipoids of cow's plasma is exceedingly important, for, if general, it means that in large scale experiments to discover, for example, whether there is any correlation between the characteristic blood

fat level of an animal and its milk fat production, it might be possible in some sections of the work to estimate only lipoid P and cholesterol, both of which can readily be determined, while the more laborious and less accurate estimation of the fatty acids could be omitted, at least in a preliminary survey.

With regard to the effect of inanition on the actual nature of the various lipid fractions, it may be concluded, just as for the corpuscles, that there was extremely little change. For the phosphatide fatty acids, the iodine values and molecular weights recorded in Table II show too much variation amongst themselves for any definite conclusion to be drawn, except that, considered as a whole, they do not suggest any noticeable alteration as a result of fasting in the general type of fatty acids present. Here again, as with the corpuscles, considerable difficulty was experienced in estimating the molecular weights of these particular fatty acids, so that the variations shown for them are thought to be due mainly to the experimental difficulties involved rather than to changes in the acids themselves. Curiously enough no such difficulties were encountered in estimating the molecular weights of the non-phosphatide fatty acids, for which the values were much more consistent. Thus with Cow no. 1, for example, the molecular weights of the phosphatide acids varied between 320 and 359, whereas for those from the non-phosphatide fraction, they all fell within the limits of 291 and 304, 7 out of the 9 being between 293 and 295. Why there should be this particular difficulty with the acids from the blood phosphatides is not at present clear, but it is hoped that future work will help to elucidate the problem. It is interesting to observe that the non-phosphatide fatty acids of Cow no. 2 had consistently higher molecular weights than those of the other two cows, the mean value being 305 for no. 2 as compared with 295 and 292 for nos. 1 and 3. Some individual variation is obviously possible in this respect.

A study of the data given in Table II for the iodine values of the non-phosphatide acids again suggests that inanition must have caused very little change, if any, in the nature of the acids. It is only with Cow no. 2 that any slight alteration can be seen, for on the day when this animal suffered from milk fever the initial mean iodine value of 139 rose to 149. It then fell again to 138 during the latter part of inanition and ultimately to 130 some 6 weeks after re-alimentation. It would appear therefore that for this animal hyperlipaemia was accompanied by an increase in unsaturation, suggesting the mobilization of unsaturated acids in the emergency caused by milk fever, and that hypolipaemia in the post-fast period was accompanied by a decrease in unsaturation, but at the most these changes were small.

It is a well known fact that it is possible for the iodine values and molecular weights of two mixtures of fatty acids to be the same in spite of differences in the composition of the mixtures. Consequently, where possible, it is advisable, as a third check, to estimate the amounts of saturated acids in the mixtures by the Twitchell separation process. Owing to the small amounts of acids available in the various samples in the present work it was only possible to apply this separation on a micro scale to pooled samples of the plasma non-phosphatide acids from Cow no. 1. The saturated acids so estimated amounted to 11.2% of the total acids before and during inanition and to 12.4% in the post-fast period. These figures may possibly be a little lower than the true values, as they were obtained on very small amounts of material, but they confirm the absence of any appreciable change as a result of the fast.

The proportion of cholesterol in the unsaponifiable matter of the plasma was somewhat higher than for the corpuscles. For example, for Cow no. 2 the figure for the plasma samples varied between 88 and 99% with a mean of 96, whereas

in the corpuscles the limits were 78 and 91 with a mean of 84. Only during the post-fast period of the experiment were any appreciable changes noticed in these particular figures for the plasma. Thus with Cows nos. 1 and 2, pre-fast means of 96 and 94 % both remained almost unaltered during inanition but fell later to 89 %. It can scarcely be said, however, that such small decreases are significant.

In Table II it will be observed that for each sample the non-phosphatide fatty acids and unsaponifiable matter determined by direct weight have been added to the small amount of glycerol calculated to be present (usually about 3 mg./100 ml. plasma), and the sum has been expressed as a percentage of the original non-phosphatide fraction. The great majority of the figures so obtained are found to fall between 94 and 99 % suggesting that the amount of water-soluble fatty acids in the non-phosphatide fraction of the plasma is exceedingly small, if, indeed, they occur there at all. This finding is not in any way at variance with that of Stewart & Hendry [1935] who were able to suggest by other means that the water-soluble fatty acids of whole blood did not exceed 3 % of the total fatty acids.

SUMMARY

1. The effect of inanition on the blood lipoids of three lactating cows has been investigated in detail as part of a scheme for the study of certain problems connected with milk fat secretion.

2. Only very slight decreases of considerably less than 10 % were observed for the lipid levels in the corpuscles, during and after a 12-day fast, whereas for the plasma, general reductions of as much as 40 or 50 % took place and these low levels persisted even 6 weeks after free access to food had again been permitted.

3. Neither in the corpuscles nor in the plasma could any appreciable change be detected in the actual nature of the lipoids as a result of inanition, although there was a tendency for greater unsaturation in the plasma fatty acids to accompany milk fever symptoms.

4. It was shown that if any water-soluble fatty acids are present in the non-phosphatide fraction of the plasma of lactating cows the actual amount must be exceedingly small.

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