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ULTRAMICROSCOPIC PARTICLES IN NORMAL HUMAN BLOOD

By A. C. FRAZER and H. C. STEWART[1]

From the Physiology Department, St Mary's Hospital Medical School, London

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The apt description "Motes floating in the sunlight", was given by Edmunds [1877] to the particles in normal serum seen under dark-ground illumination. Some twenty years later, Muller [1896] called these same bodies "Haemokonien" (blood dust). There is little doubt that these particles are identical with the molecular base of the chyle, which was described by Gulliver [1847]. Gage & Fish [1924] carried out investigations into the absorption and assimilation of fat using dark-ground examination as a method of observation. They not only confirmed the presence of these particles but also added much to the knowledge of their behaviour and variations. McDonagh [1927], and more recently Peters [1936], have studied the changes in the dark-ground picture in pathological sera.

The object of our experiments is to confirm and amplify the findings of these earlier workers, and thus to define clearly the variations that may occur in the ultramicroscopic picture of normal blood. When the normal picture is clearly established, the factors responsible for these variations must be investigated. Finally, it is our intention to determine what part, if any, these particles may play in the defence mechanism of the body.

Experimental

Many preliminary experiments were made to determine the most suitable technique for obtaining particle counts. Blood was examined (i) as whole blood freshly shed, (ii) as plasma citrated, oxalated, and centrifuged, and (iii) as serum obtained after periods of incubation ranging from ½ hour to 24 hours. As a result of these investigations a standard technique was adopted, which gives accurate results and particle counts identical with those of freshly shed blood. Capillary blood is used as this

[1] Sir Halley Stewart Research Fellow.

gives results similar to those obtained with arterial blood. The blood is collected from the finger or ear, and this is taken as being representative of the state of the blood throughout the arterial side of the systemic circulation. The venous blood is similar to the capillary blood if the particle counts are low, but when they are high there is a marked difference.

The blood is collected into a fine capillary tube, made from alkali-free glass, and incubated for 2 hours at 37° C. The resultant serum is drawn off with a fine glass pipette, and a small drop is placed on a specially prepared slide, mounted, and immediately examined. The slides used are 1·0–1·2 mm. thick, checked by passing them through a micrometer screw gauge, and the cover slips are gauge 0. It is important that the slides should be as free as possible from scratches and defects, as these will interfere with the dark ground. To clean the slides they are boiled in chromic acid, washed with distilled water, stored in alcohol, and wiped dry just before use. All glassware used in these experiments is similarly treated.

The specimen is examined under dark-ground illumination, using a Cardioid Condenser, 1/12 apochromatic objective, and ×20 eyepiece. For visual purposes the illumination is supplied by a 100 W. pointolite or a ribbon filament lamp. For photography an arc lamp is essential. After much experimentation, the illuminant chosen is a 10 amp. arc lamp fitted with Conradty carbons and a variable resistance, through which 24 or 34 amp. may be passed. The current is alternating, but pure carbons give a reasonably steady crater, and, by overloading, a very intense illumination can be obtained for a short time.

With the arc lamp as a source of light, and since only black and white objects are being photographed, there is no advantage in using panchromatic plates. In fact, orthochromatic plates are more satisfactory in every way. Using these plates and the illumination described, photographs have been taken with exposures up to 1/100 sec. Owing to the active Brownian movement of the particles, a rapid exposure is necessary. Arrest of movement appears to be complete at an exposure of 1/25 sec. Cinephotographs of the particles have also been prepared, using a similar technique.

Normal dark-ground picture

In examining a specimen, there are three definite planes that can be focused. Two consist of still particles which are adsorbed to the coverslip or slide and should not be numerous in a good preparation. Between these will be seen a layer of moving particles, and it is this plane, where active Brownian movement can be seen, which is examined.

The particles are of two types, dull and bright. No alteration of lighting or focus will change a dull into a bright particle or vice versa. Examination with polarized light and various colour filters does not affect the picture. It would therefore seem to be an inherent property of the particle that determines to which class it belongs. The bright particles are arbitrarily divided into large and small. The small brights show active movement, are seen more frequently and in greater numbers. Under certain circumstances large bright particles, which have a "sticky" appearance and move sluggishly, may be seen. The dull particles are uniform in size, show active Brownian movement and are usually more numerous than the brights. Their actual size is difficult to compare with that of the brights owing to the difference of refractility, but they appear definitely smaller. The size of the particles is about $\frac{1}{2}\mu$ as gauged by direct measurement, by comparison with objects of known size in the field, and from the dark-ground appearance of fine emulsions of known composition.

The particles will continue in active movement on the slide for some hours and there is but little tendency to clump in normal serum. A few clumps of two or three particles are occasionally seen, but they do not appear to have any special significance. Other bodies may be present, such as red blood corpuscles, leucocytes, blood platelets, threads of fibrin, and very occasionally bacteria. With improving technique these become rarities.

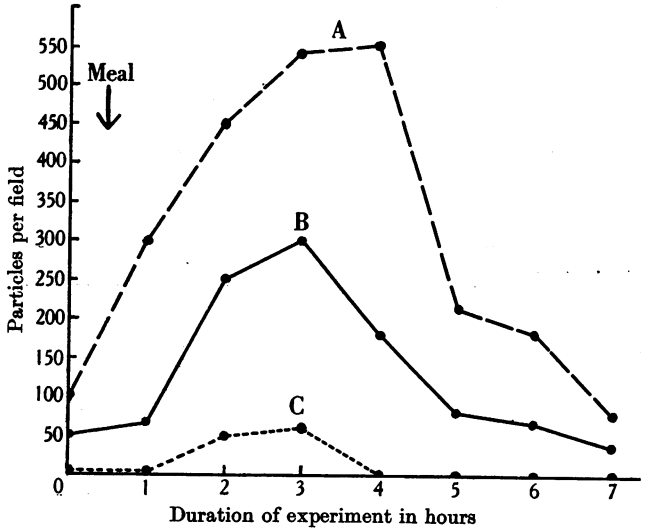
The particles are counted in each specimen. In sera containing only a few particles this is simple, but after a meal it becomes increasingly difficult. In all cases the counting is carried out by three observers whose readings are averaged. If there is any marked difference in the individual results, a recount is made or a new specimen is prepared. The estimated error is not greater than 10 p.c. With sera containing large numbers of particles an eyepiece mask is used. A segment of 60° is cut out from the mask. The first observer thus counts 1/6 of the field, the next rotates the eyepiece and counts another sector. The individual figures are compared and averaged, and the result obtained is multiplied by the eyepiece factor. In this way a remarkably accurate count of a full serum can be made. With the use of this technique a number of human experiments have been done.

The effect of a single meal upon the particle count

Blood was collected in the morning before any food was taken. A normal breakfast was eaten, and specimens were collected at hourly intervals for 7 hours. From the counts obtained curves were constructed

in which the numbers of particles in the standard field described form the ordinates, and the time intervals at which the specimens were taken, the abscissæ. A typical curve is shown in Text-fig. 1.

There is a marked rise in both bright and dull particles occurring within an hour of ingestion, which reaches a maximum in about 2 hours. This increase rapidly declines until the original level is reached in about



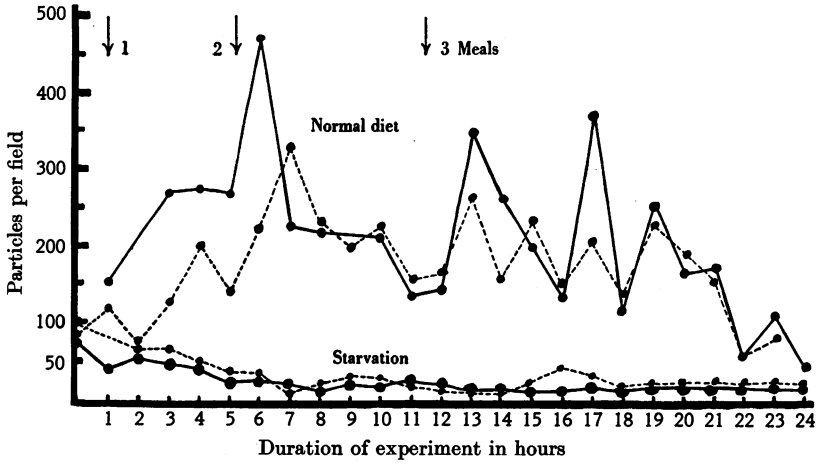
Text-fig. 1. Serum particle curves from one human subject. The meal was a normal breakfast, no further food being taken during the experiment. Curve *A* shows dull particles, curve *B* small bright particles, curve *C* large bright particles. The curves show the relative proportions of these three types, which appear to be constant in all experiments.

4 hours. This effect has been obtained on every occasion after the ingestion of a mixed meal and is in complete agreement with the findings of Gage and Fish. The two photographs (Pl. I, figs. I and II) show the minimum and maximum counts in such an experiment.

The changes in the particle count during 24 hours with a normal mixed diet [Frazer & Stewart, 1936*a*]

Two subjects on a normal mixed diet were used. Blood was collected every hour throughout the day and night. The curves obtained (Text-fig. 2, top two curves) are similar from the two subjects and show clearly the

increase in the particles that occurs after each meal. During the last part of the curve, the number of particles shows a steady fall to the basic level throughout the night.



Text-fig. 2. Serum particle curves of four human subjects. The two upper curves show variations with the normal three meals as indicated by arrows. The two lower curves show the basic level that persisted during 24 hours' starvation.

Particle counts during 36 *hours' starvation*

Blood was collected at hourly intervals from two subjects undergoing complete starvation for 36 hours, and specimens for the last 24 hours of this period were taken for the curves (Text-fig. 2, two lower curves) to compare with those of the last series. During the starvation period only water was taken, and the subjects pursued their normal occupations during the day (attending lectures, etc.). The curves show a fall to the basic level, which is maintained with but little variation throughout the 24 hours. The basic level is reached in about 20 hours after the last meal, when the post-absorptive period of the meal may be considered to end. More recently experiments have been made on subjects starving for longer periods, up to one week. These experiments will be the subject of another discussion, but they show a similar basic level throughout the starving period.

Variations in particle counts with fatty and non-fatty foods

Two subjects *A* and *B* were used in this series. *A* took a fatty breakfast followed by a non-fatty lunch; *B* took a non-fatty breakfast and a fatty lunch. The curves obtained in such experiments are shown in

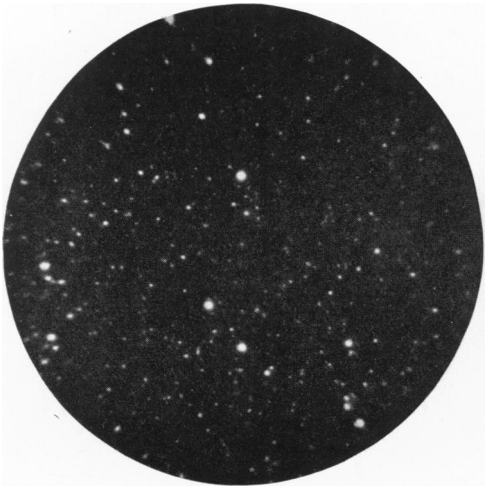


Fig. I.

Fig. II.

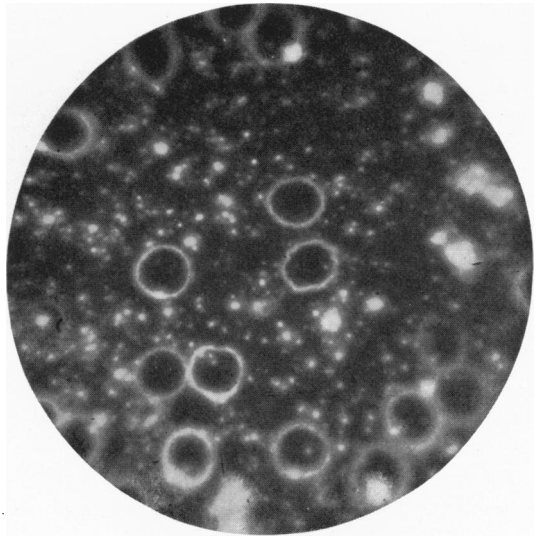


Fig. III.

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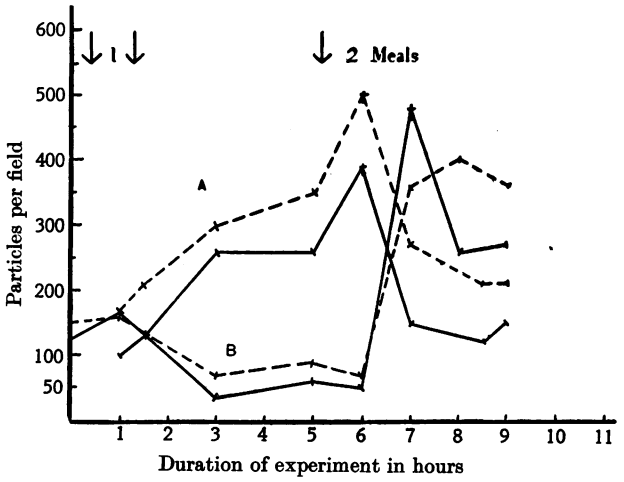
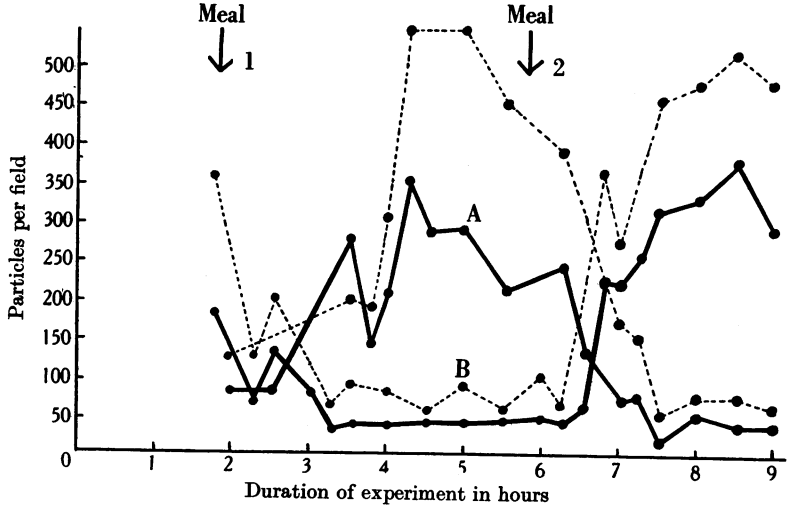
Text-figs. 3 and 4. The curve *A* in Text-fig. 3 shows a marked rise after breakfast and a small transient rise after lunch. Curve *B*, on the other hand, shows no rise after breakfast but a prolonged rise after lunch. It has



Text-fig. 3. Serum particle curves from two human subjects. *A* had a fatty breakfast and a non-fatty lunch and shows initial rise only. *B* had a non-fatty breakfast and a fatty lunch giving delayed rise only. Interrupted lines: dull particles; continuous lines: bright particles.



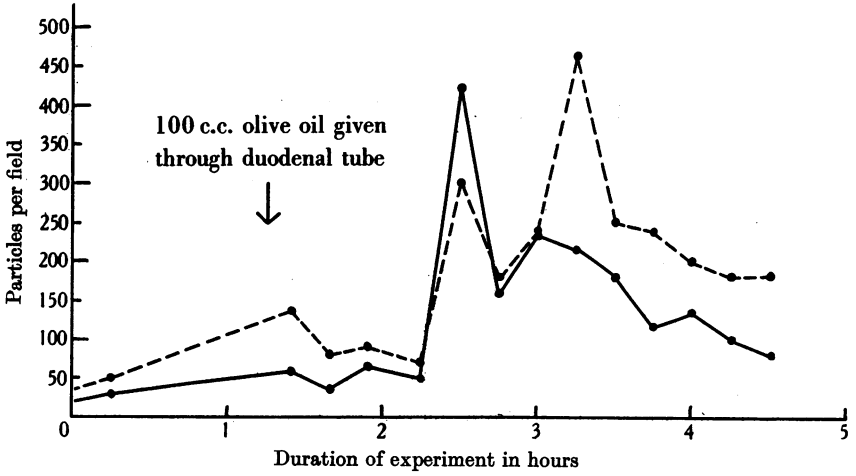
Text-fig. 4. Serum particle curves from two human subjects. *A* had a normal breakfast and a pure carbohydrate lunch. *B* had a non-fatty breakfast and a normal lunch. Interrupted lines: dull particles; continuous lines: large and small bright particles together.

been found consistently that it is only possible to get a rise in the particle count after ingestion of fatty foods. A pure carbohydrate meal caused a very rapid fall in the particle curve in Text-fig. 4.

The effect of carbohydrate and protein food on the particle curve

Pure carbohydrate meals have been taken in the form of potatoes and sugar, and there is no rise in the particles following such a meal. Indeed there is often a sharp fall in particles after carbohydrate food as already shown in Text-fig. 4.

Pure protein food, the whites of eggs, gelatin, and similar foods have been taken in large quantities, and the particle curves studied for 7 hours after ingestion. In no case has there been any increase in the number of particles beyond the ordinary basic variation.



Text-fig. 5. Serum particle curves from one human subject. After administration of fat by duodenal tube. Interrupted line: dull particles; continuous line: bright particles.

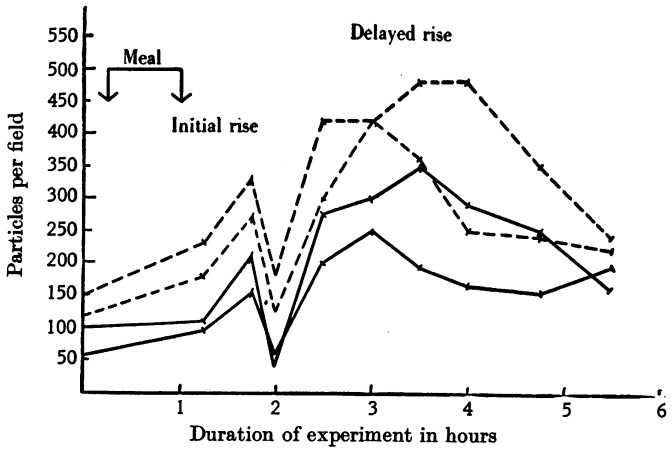
Administration of fat by duodenal tube

A duodenal tube was passed and 100 g. of olive oil, roughly emulsified in 40 c.c. of dilute sodium carbonate (*p*H 9·5), were injected through the tube. The curve obtained (Text-fig. 5) shows a marked increase in the number of particles starting about 50 min. after the injection of the oil.

Dissociation of component parts of the curve [Frazer & Stewart, 1936*b*]

Examination of Text-fig. 3 shows a rise in curve *A* following a non-fatty meal. This rise occurs immediately upon ingestion of food and lasts only a short time. In curve *B*, on the other hand, there is no immediate

rise, but about an hour after ingestion there is a marked increase in particles lasting several hours. The origin of the particles in this latter case would seem to be fat absorbed from the fatty lunch taken by *B*. The only source of particles for the transient rise in curve *A* must be fat which was absorbed from the fatty breakfast and passed into the circulation owing to the intestinal movements arising from the ingestion of the non-fatty lunch.



Text-fig. 6. Serum particle curves from two human subjects, showing initial and delayed rise after a meal. Interrupted lines: dull particles; continuous lines: bright particles.

If this is so, a post-ingestive particle curve consists of two parts, an initial rise and a delayed rise.

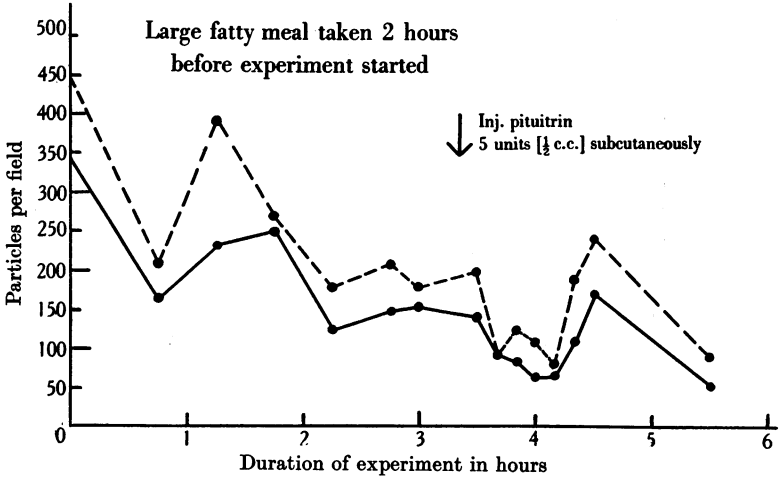
The initial rise is due to the stimulus to intestinal movements derived from the ingestion of food, and the consequent passage of chyle, laden with fat from the previous meal, into the blood stream. It will occur therefore with either fatty or non-fatty meals, provided that the previous meal contained fat; it occurs directly after ingestion, and the rise is comparatively small.

The delayed rise is due to fat which has been absorbed. It does not appear until at least 1 hour after ingestion, it only occurs after a fatty meal, and it is of greater size and duration than the initial rise. Considering Text-fig. 3 again, curve *A* shows after lunch the initial rise only. Curve *B* illustrates the delayed rise due to the fatty lunch, but no initial rise as the previous meal was non-fatty.

To investigate this point further, a more careful analysis of the simple curve was made. Specimens were collected at 10 min. intervals after the ingestion of food. The curves obtained with two subjects (Fig. 6) show a

definite initial rise immediately after ingestion, followed by a fall before the delayed rise occurs. It will be seen that the initial rise is easily missed if specimens are not collected immediately after ingestion, and at short intervals.

To demonstrate the influence of gut movements in causing the initial rise, experiments have been made with various aperients. The subject takes a fatty meal, the curve is followed, and when the main rise due to the absorption is over, the aperient is taken. Fig. 7 shows the effect of

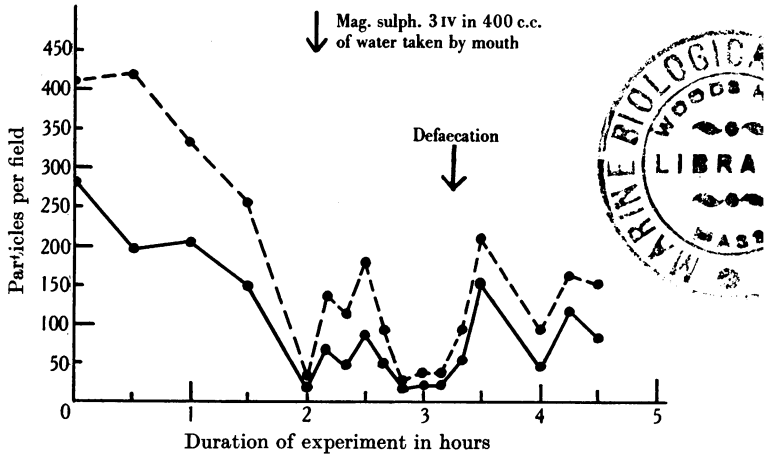


Text-fig. 7. Serum particle curves from one human subject. After the particles from the large fatty meal taken previously had reached a constant level, 5 units of pituitrin were injected subcutaneously. Interrupted line: dull particles; continuous line: bright particles.

pituitrin. There is a marked delay in the action, possibly due to the intense constriction at the site of injection. Another possible factor, accounting for this delayed action, is the influence of the posterior lobe of the pituitary on deposition of fat. It is suggested that pituitrin causes a lowering of blood fat, and in this curve there is certainly a definite fall before the rise due to gut movements.

The last experiment has been repeated using magnesium sulphate as the aperient. The curve obtained under these conditions is shown in Text-fig. 8. This curve shows the rise following the ingestion of the salts. There is no rise if the previous meal was non-fatty. The curve in Text-fig. 8 has a double rise following the saline; the first is due to stimulation on ingestion, since the 4 drachms of magnesium sulphate were taken in a

large bulk of water, and the second rise is due to the active gut movements set up when the saline reached the intestine.



Text-fig. 8. Serum particle curves from one human subject. The magnesium sulphate was given after the rise in particles from a large fatty breakfast, taken 5 hours before, had reached basic level. Interrupted line: dull particles; continuous line: bright particles.

Simultaneous estimation of neutral fat, cholesterol, and ultramiscroscopic particles in human blood

The experiments, so far, show a rise in particles to be associated with absorption of fat from the intestine. It should therefore be possible to correlate the rise and fall in blood fat with that of simultaneous particle counts. To investigate this point experiments have been carried out, of which the following is an example:

A subject took a generous mixed meal, and the time taken to eat this was half an hour. About 10 c.c. of blood were then withdrawn by venupuncture at intervals of 1, 2, 3 and 5 hours from the start of the meal. Some of each specimen was immediately withdrawn into capillary tubes for particle counts. The remainder of the blood from each specimen was allowed to clot, and 3 c.c. of the resultant serum were used from each tube for determining the blood fat.

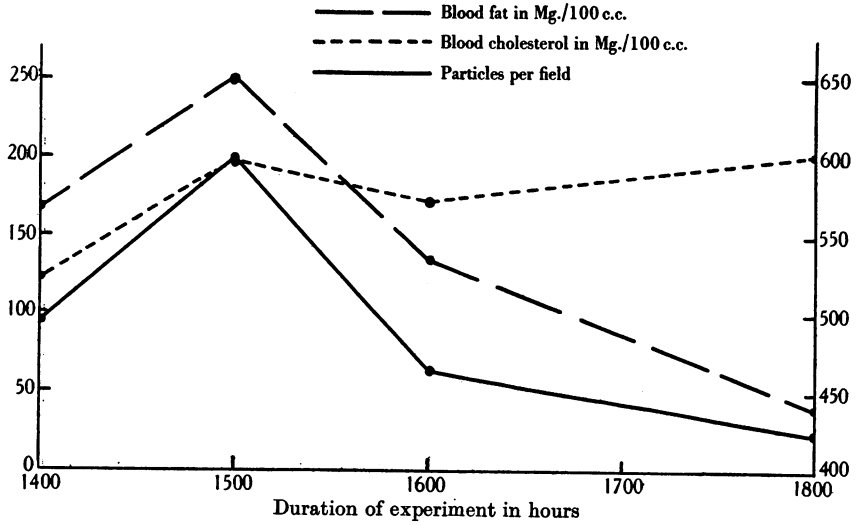
These fat estimations were made by a gravimetric modification of Bloor's [1928] method, and the cholesterol content of each specimen was determined by the colorimeter [Myers & Wardell, 1918].

We have modified these methods somewhat to suit our particular problem. Extraction of the serum is carried out according to Bloor's technique. A petroleum ether aliquot is then evaporated to dryness *in vacuo* in a flat dish and the resulting residue is weighed. This

gives the total fatty material. The residue is then treated according to the Myers & Wardell technique and the cholesterol estimated. This figure is then subtracted from the total weight of fatty material to obtain the amount of fat present. Further details of the modifications are in course of publication.

The results of this experiment are shown in Text-fig. 9.

It will be seen that the blood-fat curve runs parallel to that of the particle counts, whereas the blood-cholesterol curve remains up, and is still rising an hour or more after the other two have returned to the basic level.



Text-fig. 9. Simultaneous curves from one human subject, after a meal taken at 13·00 hours, showing the relation between blood fat, blood cholesterol, and serum particle counts. The blood fat in mg./100 c.c. is read on the right-hand scale, the blood cholesterol in mg./100 c.c. and the serum particles per microscope field, are both read on the left-hand scale.

Thus, the blood-fat curve rises and falls with that of the particle counts, and the timing of the basic levels and peaks of these two curves coincide exactly.

Discussion

These experiments confirm the observations of various workers that there are particles in normal blood, which can be seen by dark-ground illumination. They can even be seen in circulating blood, by dark-ground illumination of the mesentery or the web of a frog's foot. They are well seen in freshly shed blood (Pl. I, fig. III) and they do not increase in numbers on keeping the serum. For these reasons, it is concluded that

these particles are a normal constituent of the blood present in the circulation.

It is not easy to establish the composition of the particles by chemical means, for it is extremely difficult to collect a specimen for analysis free from contaminants. From our experiments the particles only increase after a fatty meal; carbohydrates and proteins do not affect the particle counts. Simultaneous blood-fat estimations show curves parallel to the particle curves. For these reasons it is concluded that the main mass of the particle is fat. It is probable, however, that the fat particles are enclosed in an adsorbed protein film. They appear to carry a negative charge, as shown by cataphoresis experiments.

Gage & Fish have termed these particles chylomicrons. In our discussions we shall retain this term only for particles whose origin is undoubtedly the chyle, but for particles such as are found in the basic level in starvation, where the source is not the chyle, we shall use the term lipomicrons.

That there is a marked rise in the chylomicrons after a meal is undoubted. The rise is due in part to fat eaten within the previous 12 hours, which passes into the circulation due to gut movements resulting from ingestion of food, but mainly to the actual absorption of fat. The time relationships are as follows: the initial rise starts within 15 min. of ingestion and ends within an hour; the delayed rise starts within $1\frac{1}{4}$ hours of ingestion and is completed by $4\frac{1}{2}$ hours.

When there is complete starvation and the post-absorptive period of the last meal is over, the particle counts reach a basic level. This number of lipomicrons is maintained throughout a period of starvation for at least 1 week.

No simple dietary changes or starvation will cause clumping, or any other abnormal behaviour, of the particles. The only effects observed are changes in the relative and absolute numbers of the particles in the blood.

Summary

1. The technique and apparatus used for estimating particle counts, and the method of construction of particle curves are described.

2. The particle counts in serum are increased by the ingestion of fatty food.

3. The particle counts in serum are maintained at a constant basic level in starvation.

4. The particles studied consist almost entirely of fat. Blood-fat estimations give curves coinciding with simultaneous particle curves.

5. The particle count rises after a meal, the time curve having two components. The initial rise is essentially due to gut movements and previously ingested fat; the delayed rise is due to actual fat absorbed.

6. The lipæmia following a meal containing fat occurs within 1¼ hours of ingestion, reaches a maximum in 2–3 hours, and the curve returns to the resting level in 4½ hours.

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DESCRIPTION OF PLATE I

Fig. I. Photograph of serum with particles at basic level. Taken with a 1/12 in. objective, × 10 eyepiece, 1/5 sec. exposure, using 35 amp. This field is about twice the size of that used for making particle counts.

Fig. II. Photograph of serum about the peak period after a normal meal. Taken with a 1/7 in. objective, × 20 eyepiece, 1/10 sec. exposure, using 35 amp.

Fig. III. Photograph of whole blood, freshly shed, showing the size of serum particles as compared with red blood corpuscles. Taken with a 1/12 in. objective, × 10 eyepiece, 1/10 sec. exposure, using 35 amp.