substances derived from the maternal intestine. These substances are the products of the micro-organisms originating in fæcally contaminated soil, which are conveyed to man and animals by infected food and water.

DESCRIPTION OF PLATE.

Fig. 1.—Stillborn severely-goitred kid, offspring of a goitrous mother (Class A) which consumed cultures from the fæces of goitrous individuals during pregnancy. Developmental defects are shown in the complete absence of hair and in the condition of the horny hoof. This case is typical of the 10 fœtuses of this class (A).

Fig. 2.—Stillborn goitred kid, offspring of an unmuzzled mother (Class B) which developed goitre during pregnancy. The kid is covered with a thick coat of healthy hair, and the hoofs are normal in appearance.

Fig. 3.—Fœtus of muzzled non-goitrous mother (Class B) showing no goitre.

The Ultra-Violet Absorption Spectra of Blood Sera.

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(Communicated by Sir William Ramsay, K.C.B. Received March 18, 1916.)

At the end of the year 1913 there was introduced a new kind of sector spectrophotometer designed especially for investigation of the ultra-violet spectrum. The possibility of applying the new instrument to the furtherance of medical and physiological science was soon appreciated by the author, and in conference with Dr. C. E. Wheeler it was decided that a study of the ultra-violet absorption spectra of blood sera might lead to results which would be both valuable to science and applicable to clinical practice. The proposal was placed before the Beit Research Fund Committee, the trustees of a fund which had been placed at the disposal of the British Homœopathic Association by Mr. Otto Beit for purposes of scientific research. The necessary support was liberally given by the Association, and still further funds are allotted for continuing the work.

The absorption spectra of blood have engaged the attention of many able and distinguished workers, but the investigation has usually had reference to the visible spectra of hæmoglobin and other colourings and to derivatives of these. The work now to be described has for its object the investigation of the absorption spectra of blood sera in the ultra-violet region. The serum is freed as much as possible from corpuscles by the centrifuge, and the clear pale yellow liquid itself is studied with a view

to determining the various characteristics of the absorption bands and to finding how these may be accounted for.

The subject may conveniently be treated in three divisions:—

(1) Methods and Equipment.
(2) Absorption Spectra of Blood Sera.
(3) Review.

Methods and Equipment.

Most of the older methods of studying ultra-violet absorption spectra depend on taking a series of absorption spectra, obtained on passing light through layers of the substance varying in thickness or in concentration. The modern method is to employ a sector spectrophotometer in conjunction with a quartz spectrograph, and produce only one absorption spectrum resulting on the passage of light through a suitable layer of the substance, and to compare it with each member of a series of normal spectra differing from one another by known amounts in intensity only. Thus, a normal spectrum of one-third of the original intensity will match the absorption spectrum at those wave-lengths where one-third of the light is transmitted, that is, where two-thirds of the light are absorbed; and similarly for any other proportion.

The apparatus employed were: (*a*) A large size ("size C") quartz spectrograph made by Hilger, 1914 model. (*b*) A sector spectrophotometer. Two of these instruments have been employed in the course of the work. The one referred to in the opening of the paper is that introduced by Messrs. Adam Hilger, Limited. It is described by Lankshear in a paper in the 'Memoirs and Proceedings of the Manchester Literary and Philosophical Society' (vol. 58, No. 15, Part 3, pp. 1–12, 1914). The second instrument is a still later model made by Messrs. Bellingham and Stanley, Limited. It differs from the Hilger in its construction providing for the incident pencils of light being divided and brought together again by reflecting prisms instead of by refracting units, and in the "sector" being so constructed as to present an aperture which has the shape of a goemetrical sector, and so to give a constant exposure instead of an intermittent one. This avoids the necessity for correcting each batch of plates for the error due to intermittency. (*c*) A powerful spark lamp provided with nickel steel electrodes.

The experimental procedure adopted for the serum work is as follows:—

A photograph of the two juxtaposed spectra is taken with both sectors of the photometer fully open, and with nothing in either path save an

empty observation cell in each; or if a solution is to be examined, cells of the solvent are interposed. The two spectra should be identical, and thus show that the instruments are in proper adjustment. This photograph is conveniently called a "test band."

One of the empty cells is then replaced by a similar cell containing a layer of the serum, the thickness of which has been determined as accurately as possible, say to the one-thousandth part of a millimetre. This is a matter of great importance, as the layer is usually less than one-fifth of a millimetre thick. Thus a possible error of one-half per cent. is assumed, and this postulates a similar error in measuring the magnitude of the absorption.

The photographic plate is lowered in the camera to expose a fresh strip of the plate. The sector in the other path of light is set at the aperture 0·9, by which the intensity of the light is reduced to 0·9 of the original intensity, and a fresh photograph is taken. The plate is again lowered, the sector set at the aperture 0·8, and another photograph produced. The process is repeated with as many more sectors as desired, usually not less than eighteen and not more than fifty-four. The plates adapted to the spectrograph camera measure 10 inches by 4 inches and accommodate 18 photographs. Finally another test band is registered to show that the adjustment of the instrument remains unaltered.

The plate having been developed and dried, the points of equal intensity in each pair of spectra are marked by small dots. The wave-length of each of these points is determined by reference to a corrected wave-length scale photographed on the plate, and tabulated along with the corresponding sector aperture, that is, along with the corresponding intensity of the light transmitted. In accordance with the usual practice this is applied to determine the absorptive power of the substance in terms of the quantity $\log (I/I')$, known as the "extinction coefficient," where I = the initial intensity, and I' = the intensity of the unabsorbed light.

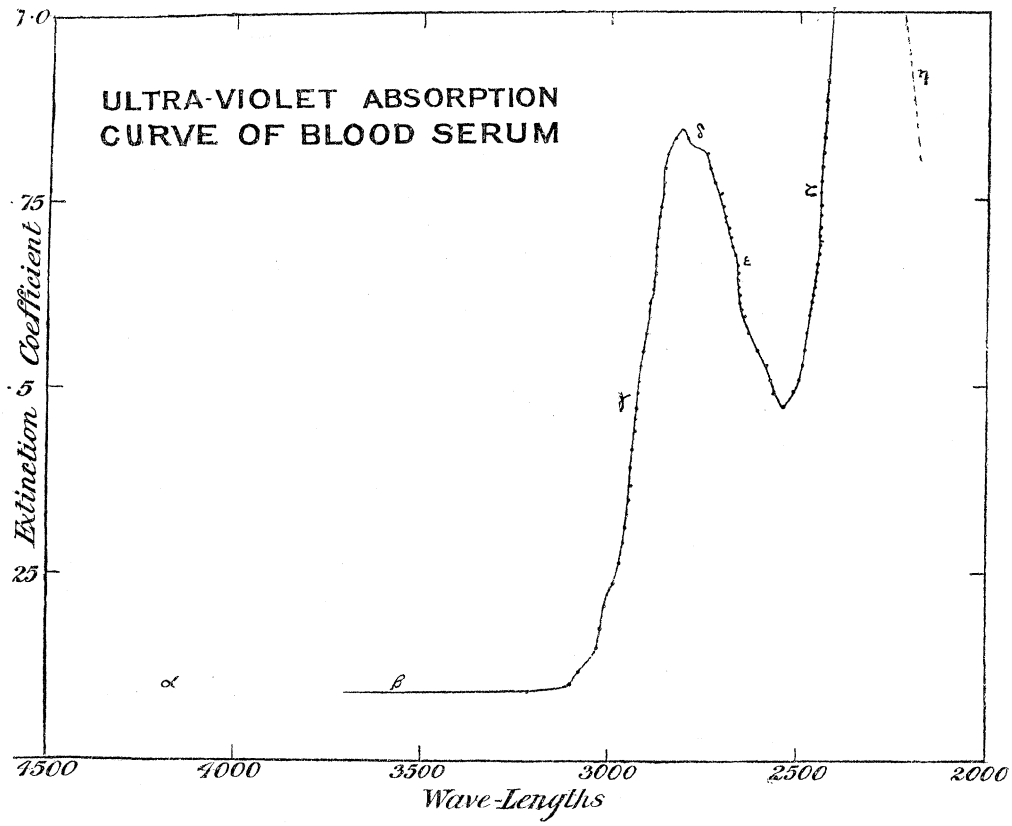
In view of serum being a solution, the composition of which is variable and comparatively unknown, the extinction coefficient must apply to the serum in some form arbitrarily chosen as a standard. The standard adopted is a layer of serum one-tenth of a millimetre thick. When the substance is employed in a thickness or concentration other than that prescribed for the standard, the extinction coefficient must be calculated according to the formula $cd/c'd' \cdot \log (I/I')$, where d is the standard thickness, c the standard concentration, d' the thickness employed, c' the concentration employed.

Since existing work on the spectra of blood is recorded in wave-lengths, it is desirable to continue this in order to correlate the new work with that

of the past. The "absorption curve" is plotted therefore with extinction coefficients as ordinates and wave-lengths as abscissæ.

Absorption Spectra of Blood Sera.

As no investigation of the ultra-violet absorption spectrum of blood serum appears to have been made hitherto, it is desirable, first of all, to study the absorption curve of normal serum in detail.



The varying intensity of the absorption is expressed in the curve shown in the figure. For purposes of reference it is convenient to describe the several well characterised parts of the curve as "sections," and to designate them by the letters of the Greek alphabet. The first section, α, coincides with the visible region of the spectrum. The next section, β, covers the region of least extinction, extending from the borders of the visible to a wave-length of about 3100, where it meets the foot of the steep ascent from this point to the head of the band at about 2800, section γ. The descent

on the other side of the band comprises two sections; section δ describes a short, nearly vertical fall from the head at wave-length about 2800, and an abrupt projection towards the ultra-violet to a point where the curve turns sharply downwards again. Section ϵ extends from this point to the depression at wave-length about 2540, whence section ζ rises steeply to a high value at a wave-length about 2400. A further section, η, has been observed with three or four pathological specimens, and is indicated in the figure by a dotted line.

The peculiarities of the central band call for special attention. Section γ exhibits slight irregularities of form which are found to be constant. Among these, the most evident are slight step-like prominences at about a quarter and half-way down from the top, and one very near the bottom, which is often well developed. Section δ includes with the upper part of section γ a small area of elongated shape, which varies somewhat in altitude. Experience shows that the section ϵ is the part most subject to variation (*vide infra*). Section γ seldom varies much except when, as in some pathological sera, the greater changes in δ or ϵ extend their influence across the band. Section β is subject to some variation; section ζ has rarely been found to alter materially.

Between sixty and seventy specimens of normal blood have been examined, with results which are practically constant. The general character of the curve has never altered. In the course of their study, the various peculiarities of the absorption spectrum have become recognised gradually, and the method of procedure improved from time to time so as to develop the several parts of the curve. The chief conclusions arrived at are recorded in the description already given.

Sex and age have not so far found any well defined expression in the properties of the absorption curve. On the other hand, it cannot be said that there is no general differentiation. The central band appears to be slightly narrower in the female, and to be shallower in the child, but one merges into another so gradually that nothing more precise can be said until a considerable number of specimens have been very carefully compared under the best conditions, taking full advantage of all earlier experience.

Inasmuch as serum is a solution of a mixture of substances in somewhat variable proportions, smooth absorption curves comparable with those obtainable with pure substances cannot be expected. The serum curve is rather the resultant of several superposed curves, each characteristic of some constituent of the fluid. It becomes of interest, therefore, to separate these members and to determine their individual absorptions with

a view to building up the resultant curve from its parts. This problem is one of great importance, for only in such manner can one hope to determine what constituents of the serum are affected in any given disease. It has already been solved in general terms and its precise study is in progress. It has been found that the chief central band is due almost entirely to proteins. The constancy of the form of section ζ points also to the same constituents. This has been ascertained in the following manner:—A portion of serum is weighed and mixed with alcohol, whereby the proteins are precipitated, leaving the non-proteins in solution. The operations are conducted so that after dilution 100 volumes of solution contain the non-proteins from 1 volume of serum. A portion of the solution is examined in a 2 cm. cell; this layer corresponds with the non-proteins in a film of serum 0·2 mm. thick, so that the absorption curve requires but little correction to make it comparable with that of the film of serum usually employed. The effect of the solvent is eliminated in the manner already indicated. The distribution and proportion of that part of the absorption by serum due to non-proteins may then be readily appreciated. It is found to consist essentially of a general absorption which is slight from the visible to a wave-length of about 2100, and then to increase rapidly.

The proteins are washed with 90-per-cent. alcohol, separated in the centrifuge and dissolved in such a quantity of water that 100 volumes of the solution contain the proteins from one volume of serum. The manner of examination is similar to that for the solution of the non-proteins. The form of the resulting curve approximates that for the original serum, showing that the proteins account for most of the absorption observed with serum.

Horse serum (three specimens) has also been the subject of inquiry. Its absorption curve is very similar to that of human serum, but (*a*) the depression is at wave-length 2510 instead of 2540, (*b*) the amplitude from this point to the head of the curve at 2800 is rather greater, and (*c*) the first step-like prominence in section γ is somewhat lower and decidedly more pronounced, so that the curve for the protein band is in general of rather larger dimensions than is the case with human serum.

Egg white (one specimen) was dealt with in similar manner for comparison. The curve of the protein band is more symmetrical in form. The sections ϵ and ζ meet at 2540 as with human serum.

Pathological.—A considerable number of pathological specimens have been examined, with some significant results. As would be expected, abnormality is confined to certain diseases; and again, as must be anticipated, the magnitude of the disturbance is usually small. One cannot look for severe

distortion of the curve, for such would imply great modification of the proteins or other constituents of the serum and point to very serious changes in the condition of the patient. Although about 120 specimens of pathological sera have been studied, it would be premature at this stage to do more than make a few general remarks.

The first observation is a general one and very significant, namely that it is the section ϵ which is most usually disturbed. It is, moreover, the part which shows most variation in normal or so-called normal serum, and it is also here that the most marked differences between human serum and horse serum, and between either of these and egg albumin, are to be found. It appears, therefore, that modification of this part of the absorption band corresponds with some sensitive constituent which varies either in proportion or in constitution in consequence of comparatively slight changes in the condition of the subject.

Some thirty specimens of blood have been examined in connection with typhoid, and the results are very encouraging. The chief effect observed is that the point of least absorption value between the sections ϵ and ζ is shifted from 2540 to 2510, and at the same time raised slightly.

This result has been arrived at in two ways: First, a series of three specimens of blood was taken from each of six soldiers; (*a*) normal, immediately before inoculation against typhoid; (*b*) 41 hours after the first inoculation; (*c*) 20 hours after the second inoculation, 11 days later. The serum was separated and examined in the usual way, and the above-named effect was observed in five out of six cases. Blood from 11 cases of actual or suspected typhoid was examined. In six instances the above effect obtained fully; in two, the displacement was to 2530 only; in two, the position was unchanged. In one, described as clinically a typical case of typhoid and as having failed three times to give the Widal reaction, the movement was in the opposite direction, to 2550.

In most cases there is a reduction in the amplitude of the curve between the depression at 2540 and the head at 2800.

Another modification observable in the inoculation cases is that the step-like prominence at the bottom of section γ is somewhat greater after inoculation than before in five cases out of six. The sixth case is also the exception with regard to the displacement modification mentioned above.

Scarlet fever has proved very interesting. It exhibits a more or less strong disturbance in the protein band in about half of the 33 cases which have been examined. The change is not constant either in quality or quantity, but it is always in the central protein band; usually section ϵ is

most strongly modified, but the whole band may be greatly reduced, with its head thrown towards the extreme ultra-violet.

Tuberculosis has been the subject of inquiry in 27 cases; 10 of them exhibit a band of slightly increased extinction in section β between wave-lengths 3200 and 3500. Another ten show a tendency to some ill-defined absorption in the same regions, or an increase in the step-like prominence at the foot of section γ. The remaining seven were all "mild" cases, and caused no special absorption. However, some others similarly described were not distinguishable from those marked "severe."

In four cases of anæmia the serum presented no abnormality. Miscellaneous cases of rheumatism, rheumatoid arthritis, cirrhosis of the liver, etc., have afforded irregularities in the curve, but confirmation is needed to establish their significance.

Review.

So few natural substances of unknown constitution appear to have been submitted to direct examination by ultra-violet absorption spectrography, that the success attending the present investigation of serum is the more gratifying.

Practically all the properties of the absorption curve of normal serum have proved to be constant and characteristic, while there is enough variation in minutiæ to stimulate a closer investigation, with a view to ascertaining the range and causes of the variations, and the much greater, though still small, changes associated with certain pathological conditions make the inquiry all the more urgent and interesting. The method lends itself to the purposes of clinical practice, for so small a quantity as four or five drops of blood collected in a capillary tube suffice for a complete examination in the ordinary way. Again, no preparation whatever of the specimen is necessary except to separate the serum in the containing tube by means of the centrifuge, and then to transfer it to the observation cell.

It is improbable that any important improvement will be made in the qualitative properties of the absorption curve as described above. The extinction coefficients are, however, only approximately quantitative. The greatest hindrance to reaching the final goal has been the lack of a sufficiently perfect spectrophotometer. It is worthy of emphasis that the work described has been done on the two most modern and most accurate instruments available, which have proved adequate for most academic requirements. But the exacting demands of the serum work reveal the necessity for one still finer. The author has designed a new photometer, which is now under construction, and will, it is hoped, supply the need.

Precision, capacity for truly quantitative measurements, uniformity of adjustment and rapid working, are among the most urgent desiderata. With the new instrument former work will be confirmed and rendered quantitative, while it is hoped to deepen and extend it in such manner as to lay sure the foundations of this new branch of practical science.

An Experimental Investigation into the Rôle of the Blood Fluids in the Intracellular Digestion of Certain Bacteria and Red Blood Corpuscles.

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(From the Inoculation Department, St. Mary's Hospital, Paddington, W.)

Any observer who may have carried out a number of experiments on phagocytosis *in vitro*, especially when some members of the *coli* group, or the gonococcus, have been the microbes under observation, cannot fail to have been struck by the marked intracellular digestion which is seen in the microscopical preparations made in the course of these experiments.

This intracellular digestion is evidenced by many of the organisms ingested by the leucocytes appearing as ill-stained, swollen shadows lying in vacuoles.

Further, several independent workers in this laboratory, when working out the opsonic index in the case of glanders, noticed that those glanders bacilli which had been ingested by the leucocytes after being acted on by the normal human serum showed from their appearance in the opsonic films many more signs of digestion than those which had been acted on by the patient's serum, and this was the case quite independently of the value of the opsonic index of the patient's serum, as is shown by the fact that on occasions when this was especially noted the opsonic indices were 2·6, 2·7, 1·7, 2·3, and 0·96 respectively. Another observation bearing on this subject, and one which many workers may have noticed, is that the gonococci seen in the leucocytes contained in specimens of gonorrhœal pus are sharp cut, taking the stain evenly and deeply, whereas in specimens made for the determination of