A TEXT-BOOK

OF THE

PHYSIOLOGICAL CHEMISTRY

OF THE

ANIMAL BODY.

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OF THE

ANIMAL BODY

INCLUDING AN ACCOUNT OF THE CHEMICAL CHANGES OCCURRING IN DISEASE

BY

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WITH ILLUSTRATIONS.

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TO

SIR ROBERT CHRISTISON, Bart., M.D., LL.D., D.C.L.

EMERITUS PROFESSOR OF MATERIA MEDICA IN THE UNIVERSITY OF EDINBURGH.

Sir,

In the preparation of this work the intention which I had formed from the first of dedicating it to you has always been present with me, and whilst the thought has added pleasure to what has truly been a congenial task, it has incited me to make every effort in my power to render the book worthy of your acceptance.

In offering you this tribute of admiration and regard, I would express my sense of the signal benefits which, by your labours as a teacher and investigator and by your personal example, you have conferred upon the great University of which you were so long an ornament, no less than upon the whole medical profession in this country.

ARTHUR GAMGEE.

Manchester, *July* 19, 1880.

PREFACE.

It has been my desire in the preparation of this work to consider the subject of Physiological Chemistry from the point of view of the biologist and the physician rather than from that of the chemist, and, accordingly, I have adopted a classification of the subject based entirely on morphological or physiological considerations. Whilst I have, however, given special prominence to all those facts which offer at present the greatest interest to the biologist, and have kept in the back ground such as only possess interest to the pure chemist, because involving some doubtful question of constitution, I have, nevertheless, taken care that no chemical fact and even that no chemical speculation should be omitted which appeared likely to throw light upon a biological question.

In the present volume the chemical composition of, and the chemical processes relating to, the elementary tissues of the body are treated of, the blood, lymph, and chyle being included in that classification. This volume forms a complete and independent work, though it is intended that it shall, within twelve months, be followed by a second volume, in which the chemistry of the chief animal functions will be treated of.

Some may be inclined to remark that I have introduced into this work too large a reference to the sciences of

anatomy and physiology and to practical medicine, and that I have not always been consistent in the extent of these references. The attentive reader will however discover, I trust, that I have proceeded with great deliberation, and that if in certain cases I have made greater digressions into the provinces of the cognate sciences than in others, it has been because I considered that I was called upon to do so in the interest of the particular subject, and therefore in the interest of the reader. Thus, in the chapter on the 'Contractile Tissues' the histological descriptions are far more detailed and the general review of known physiological facts much more complete than in the case of the nervous tissues, and the reason is obvious. It would have been unsatisfactory to discuss the chemical processes of muscle without considering, in some cases in considerable detail, the results of the work of the histologist and of the experimental physiologist. On the other hand, in dealing with the scanty facts yet known to us concerning the chemical history of the nervous tissues, only the barest outline of the histology of the nervous system is essential.

Although this volume, in the main, deals with the chemistry of the elementary tissues and not with the processes which are characteristic of the complex organs of the body, for the sake of convenience some exceptions have been made. Thus the chemistry of the organs of sense has been made to follow the chapter on the chemistry of the nervous tissues, because this seemed the most convenient place for introducing a systematic account of any facts relating to them.

It has been a constant object with me to give the reader a very full and, so far as possible, independent account of the state of knowledge on the subjects discussed, and I trust

I may with complete truthfulness say that this work is based upon a study of original memoirs rather than upon a study of text books. In the interest of the student nearly all papers are quoted by their full titles and few have been quoted which have not been read throughout and studied. Whenever quotations have been made at second hand the fact is stated.

Another feature which I have desired to render prominent in this work is the description of the methods which have been followed in important and, to borrow a convenient Germanism, 'epoch-making' researches. It seemed the more important to do this as I desired to write in the interest of the truly scientific student, anxious not merely to learn what has been already acquired to science, but wishful himself to extend her boundaries.

I have, so far as possible, tried all the experimental processes mentioned in this work, and throughout it I have incorporated the results of my own independent researches which in many cases have not yet been published elsewhere.

Thus much as to the plan of this book: I know only too well its deficiencies. I trust, however, that notwithstanding these it may assist the progress of science, and whilst I plead for it the indulgence of my scientific brethren, I would beg of them to aid me by communicating to me any errors which they may discover, or any suggestions for a better exposition of the subjects discussed.

In the discharge of my very arduous work I have been greatly helped by many friends. In the first place I have to express my unbounded acknowledgments to my friend and former pupil Mr John Priestley, who has, with the exception of some comparatively unimportant sections, written the

very important chapter on the 'Contractile Tissues,' and in such a manner as will, I feel sure, attract the good opinion of physiologists. Mr Priestley had, without any intention of writing on the subject, made himself so thoroughly master of all that had been written on the subject of the physiology of muscle, that in the best interests of my readers I asked him to assist me in dealing with this subject. Although any credit which it may merit is due to Mr Priestley for the greater part of this chapter, I must in justice to myself say that every section and almost every sentence in it have been the subjects of discussion between us.

I have been helped by Mr William Dodgson in the reduction of the valuable tables of blood-spectra of Professor Preyer to a scale of wave-lengths, and in the actual drawing of the scale attached to the spectra of haemoglobin and its derivatives. I may here incidentally remark that in the description of spectra of any importance I have referred all measurements to wave-lengths, taking care to check the reduced observations of others by measurements made with the help of one of Herr Zeiss's beautiful spectroscopes furnished with a scale of wave-lengths.

Lastly, I have to express my deep obligations to Dr Alfred Young, to Mr Marcus Hartog, M.A., and to my pupils Messrs Larmuth, Reynolds, and William Thorburn for much useful help. Upon the first of these gentlemen devolved the greater part of the labour of preparing a full and accurate index.

ARTHUR GAMGEE.

Manchester,
July, 1880.

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BOOK I.

THE PROTEIDS.

THE ELEMENTARY TISSUES OF THE ORGANISM.

CHAPTER I.

THE PROTEIDS.

Amongst the organic proximate principles which enter into the composition of the tissues and organs of living beings, those belonging to the class of *proteid* or *albuminous* bodies occupy quite a peculiar place and require an exceptional treatment, for they alone are never absent from the active living cells, which we recognize as the primordial structures of animal and vegetable organisms. In the plant, whilst we recognize the wide distribution of such constituents as cellulose and chlorophyl, and acknowledge their remarkable physiological importance, we at the same time are forced to admit that they occupy altogether a different position from that of the proteids of the protoplasm out of which they were evolved. We may have a plant without chlorophyl and a vegetable cell without a cellulose wall, but our very conception of a living, functionally active, cell, whether vegetable or animal, is necessarily associated with the integrity of its protoplasm, of which the invariable organic constituents are proteids.

In the animal, the proteids claim even more strikingly our attention than in the vegetable, in that they form a very much larger proportion of the whole organism, and of each of its tissues and organs. We may indeed say that the material substratum of the animal organism is proteid, and that it is through the agency of structures essentially proteid in nature that the chemical and mechanical processes of the body are effected. It is true that the proteids are not the only organic constituents of the tissues and organs, and that there are others, present in minute quantities, which probably are almost as widely distributed, such as for instance phosphorus-containing fatty bodies, and glycogen, yet avowedly we can (at the most) only say *probably*, and cannot, in reference to these, affirm that which we may confidently affirm of the proteids—that they are indispensable constituents of every living, active, animal tissue, and indissolubly connected with every manifestation of animal activity.

There are then, it will be admitted, good reasons why a general sketch of the proteid bodies should be the proper introduction to a treatise on physiological chemistry, in which the classification is

intended to be as much as possible one based upon physiological considerations; and the reader will not find it inconsistent that whilst a systematic account of these bodies is given in the first place, apart from any special tissue or organ, in the case of other proximate principles their description and consideration is incorporated in the account of the organ or tissue with which they appear to have the closest connection.

Sec. 1. General Characters of the Proteids.

The bodies included under this category are highly complex, (for the most part) non-crystallizable, compounds of carbon, hydrogen, oxygen, nitrogen and sulphur, occurring in a solid viscous condition, or in solution, in nearly all the solids and liquids of the organism. The different members of the group present differences in physical and, to a certain extent, even in chemical properties; they all possess, however, certain common chemical reactions, and are united by a close genetic relationship.

The following table exhibits the proportions of proteids, or their immediate derivatives, contained in the various liquids and solids of the body (Gorup-Besanez[1]).

A. Liquids.

Cerebro-spinal liquid contains	0·09	per cent.	of Proteids.
Aqueous humour	0·14	,,	,,
Liquor Amnii	0·70	,,	,,
Intestinal juice	0·95	,,	,,
Liquor Pericardii	2·36	,,	,,
Lymph	2·46	,,	,,
Pancreatic juice	3·33	,,	,,
Synovia	3·91	,,	,,
Milk	3·94	,,	,,
Chyle	4·09	,,	,,
Blood	19·56	,,	,,

B. Solid Tissues and Organs.

Spinal Cord contains	7·49	per cent.	of Proteids.
Brain	8·63	,,	,,
Liver	11·74	,,	,,
Thymus (of Calf)	12·29	,,	,,
Muscles	16·18	,,	,,
Tunica media of Arteries	27·33	,,	,,
Crystalline lens	38·30	,,	,,

The proteids of the animal body are all derived, directly or indirectly, from vegetable organisms, which possess the power of constructing them out of the comparatively simple chemical compounds which serve as their food. Such a synthesis never takes place in the

[1] Vide Gorup-Besanez, *Lehrbuch der physiologischen Chemie*, 4te Auflage (1878), p. 128.

animal body, though the latter possesses the power of *converting* any vegetable or animal proteid into the various proteids which are characteristic of its solids and liquids. By the action of certain ferments present in the alimentary juices, all proteids are capable of being converted into closely allied bodies called peptones, which after absorption are capable of reconversion into proteids. In the organism the proteids thus introduced, after forming part of the circulating blood, are partly employed in the reconstruction of slowly wasting proteid tissues and organs; for the most part, however, they are subjected to a rapid series of decompositions, of which presumably the most important take place in the liver, and which finally result in the formation of carbonic acid, water and various imperfectly oxidized organic bodies which contain all the nitrogen originally present in the proteid; of those bodies the most abundant by far is carbamide or urea, $CO\begin{cases}NH_2\\NH_2\end{cases}$.

To the assemblage of chemical processes, or rather to the assemblage of transformations, which a constituent of the organism, such as a proteid, undergoes in its passage through the body, the term *metabolism* has been applied, and we shall frequently employ it in this sense, the processes themselves being designated when convenient *metabolic processes*.

In the processes of *metabolism* to which the proteids are subjected and which result in the formation of CO_2, H_2O and urea, there are formed intermediate bodies, such as glycogen and fats, which play an important part in the economy of the body.

It is further unquestionable that within the animal body certain remarkable synthetic processes occur, by which proteids are built up into bodies of a yet more complex structure, such for instance as the blood colouring matter, Hæmoglobin.

Percentage composition of the Proteids.

The various Proteids differ somewhat in elementary composition, within the limits of the following numbers[1]:

	C	H	N	S	O
From	51·5	6·9	15·2	0·3	20·9
to	54·5	to 7·3	to 17·0	to 2·0	to 23·5.

In addition to these essential constituents, the proteids, however carefully they may have been purified, usually leave when ignited a small quantity of ash, the composition of which varies in different cases, chlorides and phosphates of the alkaline metals being the predominant constituents.

Proteids for the most part soluble.

Certain of the proteids exist in a state of solution in the liquids of the organism; others are present in the same state in the tissues; all may be dissolved by certain reagents, though in some cases not without suffering radical changes.

[1] Hoppe-Seyler, *Handbuch d. phys.- und path.-chem. Analyse*, 4te Aufl. p. 223.

When solutions of the proteids are dried at a gentle heat so as to drive off the water in which they are dissolved, or with which they are combined, they appear as translucent and perfectly amorphous solids, which break with a vitreous fracture, and furnish, when triturated, a yellowish-white or white powder. Unless it has been subjected to a high temperature, the powder thus obtained by evaporating watery solutions of proteids, is found to be again soluble in water. By exposure to too high a temperature the body may be rendered insoluble.

Proteids are Colloids, i.e. non-diffusible.

Solutions of all proteids are found to be non-diffusible through parchment-paper, and this property allows us in certain cases to separate proteids from other matters with which they are mixed, and in some cases even to separate one proteid from another.

Thus the chief proteid constituent of the blood is a body termed serum-albumin. If this body, which is soluble in water, be present in a solution which contains saline ingredients and diffusible organic bodies, such for instance as sugar or urea, we can effect the separation of the albumin by taking advantage of its properties as a *colloid*. If we place the solution in a *dialyser* (Fig. 1 and Fig. 2), *i.e.* in a suitable vessel where it may be in contact with one side of a surface of parchment-paper, the other side of which is immersed in pure water, which is frequently renewed, the diffusible or so-called *crystalloid* constituents, such as the soluble salts, the sugar and the urea, will pass through the parchment-paper into the water, and there will be ultimately left within the dialyser a solution of pure serum-albumin; if there be present in the original solution not only albumin which is soluble *per se* in water, but such a proteid as paraglobulin, which is held in solution by the water in virtue of the salts which may be present, as these diffuse out it is precipitated, so that by the process of dialysis alone we may succeed in separating not only the proteids from diffusible admixtures, but, in certain cases, to separate partially one proteid from another.

The process of dialysis is one which is frequently of great use in physiological chemistry. Various methods of carrying on the process are employed. In some cases the dialyser is made by stretching and tying a sheet of moist parchment-paper over a hoop of gutta percha; the liquid to be dialysed is then placed in this dialyser, which is immersed in a larger vessel containing water (Fig. 1). A convenient form is made of glass of the shape shewn in Fig. 2, the parchment-paper being tied across the wide open mouth of a bell of glass, which is suspended in water by its narrower neck.

Of late, hollow tubes of parchment-paper have been sold for the manufacture of sausages, and these serve admirably as dialysers; the fluid to be dialysed being placed within the tube, which is suspended in water. In this case, as also in using the instruments shewn in Fig. 1 and Fig. 2, it is often advisable to arrange for a constant influx and efflux of water from the vessel in which the dialyser is immersed.

In all experiments on dialysis care has to be taken, before an experiment is commenced, to ascertain that the parchment-paper is quite free

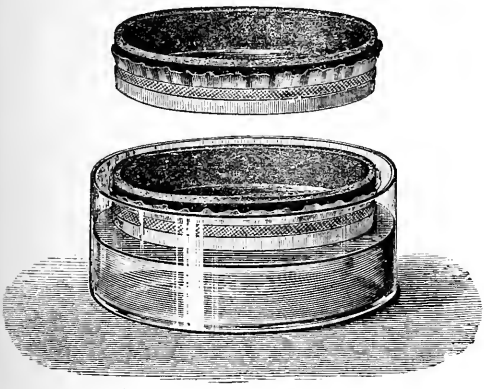


Fig. 1. Hoop Dialyser.

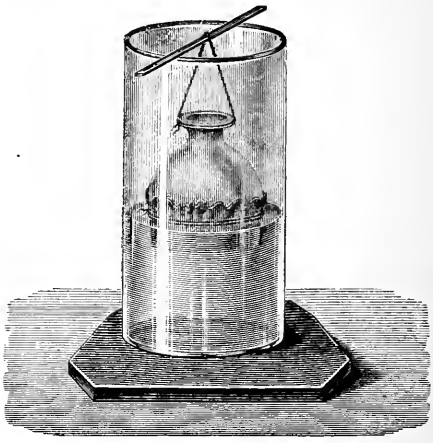


Fig. 2. Bulb Dialyser.

Dialysers described in the text.

from even the minutest holes. These are readily detected if the outer surface of the dialyser (*i.e.* the surface which during the actual experiment is to be immersed in water) be dried and placed upon a sheet of filtering paper, and then water poured into the interior; a leak being evidenced by the appearance of moisture on the outside.

Proteids all rotate the plane of polarized light to the left. Determination of their Specific Rotatory power.

Amongst the organic constituents of the animal body a large number when dissolved possess the power of rotating the plane of polarized light; as for instance the proteids, the sugars, the bile acids, &c. The determination of the fact that a solution of a body rotates the plane of polarized light in a definite direction and to a definite extent is sometimes of great service in aiding its identification, and in enabling its amount to be determined.

As the rotation exerted by an active body dissolved in an inactive liquid is dependent upon the molecules of the active body existing in solution, the degree of rotation will in the case of any particular substance be proportional to the number of active molecules traversed by the light, and therefore proportional to the length of the column of liquid traversed, and to the degree of concentration of the solution. If, for instance, a column of solution of any active substance, say of cane-sugar, of any given length, rotate the plane of polarized light x degrees, then if the column be doubled the rotation will amount to $2x$ degrees; or the double rotation will be observed if instead of doubling the length of the tube the amount of active substance in a given volume of liquid be exactly doubled. It can be shewn that any active body rotates to different degrees the plane of polarization of light of different colours. In determining, therefore, the

rotatory power exerted by different bodies, care must be taken that the nature of the light is the same. The light obtained by volatilizing sodium compounds in a colourless gas flame affords an admirable source of light of one uniform wave-length.

The expression 'specific rotatory power' or 'specific rotation' is used to designate the rotation (expressed in degrees) of the plane of polarized light, produced by 1 gramme of substance dissolved in 1 cubic centimetre of liquid when examined in a column 1 decimetre thick.

Let a be the rotation observed, and p the weight in grammes of the active substance contained in 1 cubic centimetre, and let l be the length of the tube in decimetres, then if we designate by $(\alpha)_D$ the specific rotation for light having a wave-length corresponding to D,

$$(\alpha)_D = \pm \frac{a}{pl}.$$

In this formula the sign + indicates that the substance is *dextrogyrous*, the sign − that it is *lævogyrous*. In some cases the rotation is determined for mean yellow light and not for D, and is expressed by $(\alpha)_j$, the value of which is always somewhat different from that of $(\alpha)_D$.

Various instruments have been devised and much employed in the determination of rotation of the plane of polarization, especially in the estimation of sugar, and are known by the terms Saccharimeters, Polarimeters, and Polaristrobometers. One of the most convenient and most widely employed is the saccharimeter of Soleil, which as modified by Ventke and Hoppe-Seyler, enables the percentage of serum-albumin and of glucose present in a liquid to be directly read from a scale attached to the instrument. In this instrument the rotation is determined for the mean yellow.

The instrument of Soleil[1] has however been of late years surpassed by others, especially by those invented by Wild, Jellett, and Laurent. A description of the latter instrument will alone be given.

Laurent's Polarimètre à Penombres.

This instrument is shewn in Figs. 3 and 4. A V is a Bunsen lamp. A (Fig. 3) is a small spoon of platinum gauze with the tip turned upwards, and in this is placed a small quantity of common salt. The tip of the spoon is placed in the outer flame, and when the salt is volatilized an extremely brilliant sodium flame is produced. At B is a cell containing potassium bichromate, which cuts off all but the yellow rays. To the lever J is attached a double refracting prism which polarizes the light, and at D (Fig. 4.) is a diaphragm of which one half is covered by a plate of quartz. This serves to modify the light in a manner explained in the account of the theory of the instrument. The

[1] For the description of the Soleil-Ventke Saccharimeter, and of Wild's Polaristrobometer, the reader is referred to Hoppe-Seyler's *Handbuch der physiologisch-u. pathologisch-chemischen Analyse*, and for a fuller description of these instruments, as well as for a discussion of the whole subject of rotatory polarization, to Professor Llandolt's recent work entitled *Das optische Drehungsvermögen organischer Substanzen und die practischen Anwendungen desselben.* Braunschweig, Vieweg und Sohn, 1879, p. 237.

eye-piece tube O contains a Nicol's prism as analyser at K (Fig. 3), and the whole tube, with the vernier and reading lens L attached, can

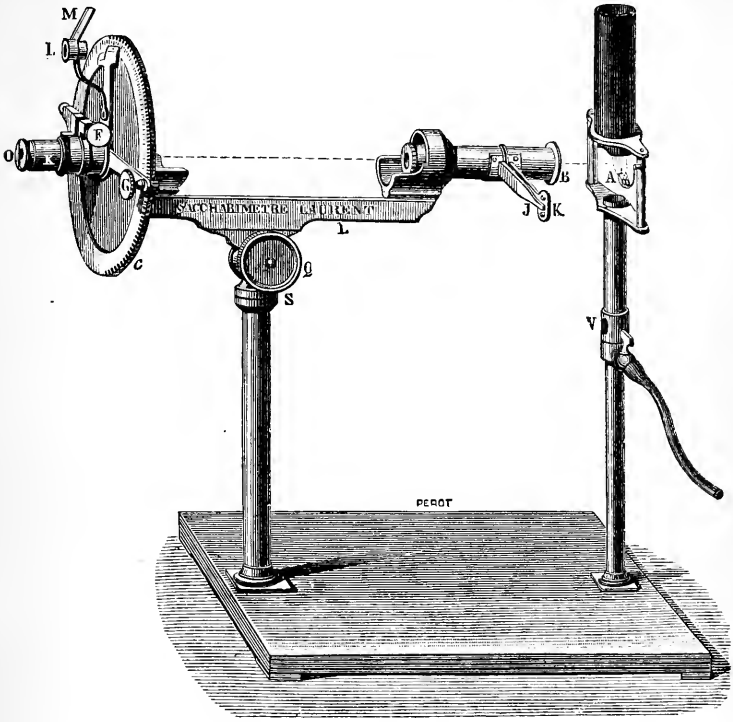


Fig. 3. Laurent's Polarimètre.

be rotated by the screw G, or the eye-piece and vernier remaining fixed the analyser can be rotated independently by the tangent screw F. The vernier moves against the circle c, of which the rim is graduated. When the rotatory power of any substance is to be determined, a tube containing water is first placed in the position T (Fig. 4) and by means of the screw G (Fig. 3) the zero of the vernier is brought to coincide with that of the scale. When the eye-piece has been adjusted so that the line dividing the two halves of the field is perfectly clear and sharply defined, these two halves are brought to the same intensity by means of the screw F, the scale still reading zero. Should the illumination of the field be too faint it may be increased by moving the lever J (Figs. 3 and 6) slightly, though it is preferable to work with the instrument when the lever is in such a position that almost all the light is cut off. The water tube is now replaced by that containing the substance to be tested. If it is active the two halves of the field will at once be seen to be of unequal intensities. The

screw G is then turned till the equality is restored and the reading of the circle at once gives the rotation due to the substance, right- or left-handed, according as the vernier is to the right or left of the zero on the scale. The following is an example of the determination of the rotatory power of a solution of sodium glycocholate in alcohol.

The solution in a tube 2 decimetres long gave a rotation of $+1^\circ\ 40'$ or $1^\circ{\cdot}666$. On evaporation, 10 c.c. of the solution gave $0{\cdot}322$ grm. of dry residue, or 1 c.c. contained $\cdot0322$ grm. of the salt. Now the specific rotation α_D being defined as that due to a column of liquid 1 decimetre long and containing 1 grm. of salt per 1 c.c., we have

$$+1^\circ{\cdot}666 = \alpha_D \times 2 \times \cdot0322$$

or

$$\alpha_D = \frac{1{\cdot}666}{\cdot0644} = +25^\circ{\cdot}77.$$

Theory of Laurent's Polarimètre[1]. The light from the sodium flame A (Fig. 4) is deprived of all traces of blue or violet rays by the potassium bichromate solution in the cell B. It then passes to the doubly refracting prism P, whence half of it emerges polarized in one plane, the other half, polarized in a perpendicular plane, being refracted away from the axis and stopped by a diaphragm.

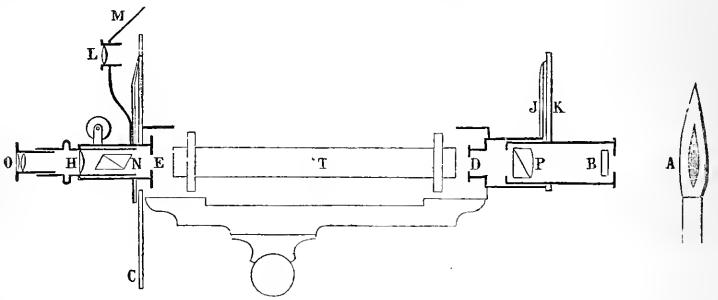


Fig. 4. Diagram of Laurent's Polarimètre.

At D is a diaphragm of which one half is covered by a plate of quartz *cut with the axis in the surface and parallel to the edge*[2]. To understand the effect of this crystal let Fig. 5 (1) represent the diaphragm, the shaded part being the quartz plate. Let OB be the direction of vibration of the light after polarization by the prism. This will still continue to be the direction of vibration of the light which goes through the right half of the diaphragm, but a ray vibrating parallel to OB will on entering the quartz on the left be resolved into two rays, one vibrating parallel to the axis OA, which we represent by Oy, the other perpendicular to the axis, which we represent by Ox. These two rays will travel at different rates through

[1] For this account of the theory of Laurent's Polarimètre, I am indebted to my friend Mr J. H. Poynting, M.A., Fellow of Trinity College, Cambridge.

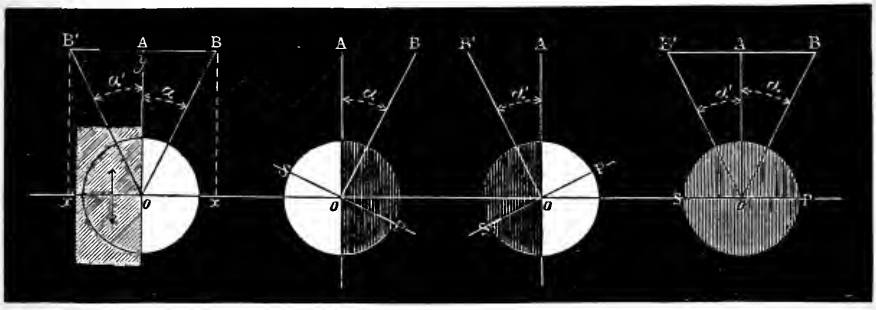
[2] When cut in this manner quartz has no rotatory power but behaves just as any other uniaxal crystal.

the crystal, which is cut of such a thickness that one ray is retarded in its passage just half a wave-length of sodium light behind the other, or what amounts to the same, executes half a vibration more than the other while in the crystal. On emergence then, while one vibration is from O to y the other instead of being from O to x is from O to x' in the opposite direction, and the two now unite to form a resultant vibration OB' equal to OB but at an angle AOB' equal to AOB on the other side of OA.

Now if the tube T (Fig. 4) only contain water or some non-rotating liquid, the two rays will pass through it with their directions of vibration OB, OB' unaltered to the analysing Nicol's prism N. This will only allow rays to pass through it which vibrate parallel to a particular direction. If the prism be turned so that this direction SP (Fig. 5, (2)) is perpendicular to OB, the right-hand ray having no component parallel to SP is extinguished, while the left-hand ray will have a more or less considerable component in that direction and the left-hand side of the diaphragm D will alone be visible in the telescope OH (Fig. 4).

So if the prism be turned round till SP is perpendicular to OB' (as in Fig. 5, (3)) only the right-hand side of the diaphragm is visible.

But if SP be turned so as to be perpendicular to OA, vibrations parallel to OB, OB' have equal components parallel to SP, and the two



(1) (2) (3) (4)

Fig. 5.

halves of the diaphragm appear equally illuminated (as in Fig. 5, (4)). In this position of the analyser the instrument should read 0°.

It is possible to adjust SP perpendicular to OA with very great accuracy, for when OB, OB' make small angles with OA a very small rotation of the analyser makes a great difference in the relative illumination of the two halves of the field[1].

When SP is thus adjusted perpendicular to OA and the instrument reads 0°, let a liquid possessing the power of rotating the plane of polarization be

[1] This will be seen at once from the mathematical expression for the intensity of the component parallel to SP.

Let AOB Fig. 5 (2) $=\alpha$, $BOP=90-\theta$, where $\theta=\alpha$ in the position of equality of illumination. Let the intensity of the resolved part of the ray OB parallel to $SP=I$.

Then $I=OB^2\cos^2 BOP=OB^2\sin^2\theta$, and $\frac{dI}{d\theta}=2OB^2\sin\theta\cos\theta$.

Therefore $\frac{1}{I}\frac{dI}{d\theta}=2\cot\theta$.

This, which expresses the proportion between the change of intensity and the original intensity, is greatest when θ is least, and therefore α should be as small as possible.

placed in the tube *T*. Both the directions of vibration *OB*, *OB'* will be turned through equal angles in the same direction, and their components along *SP* will be no longer equal, and one half of the field will appear brighter

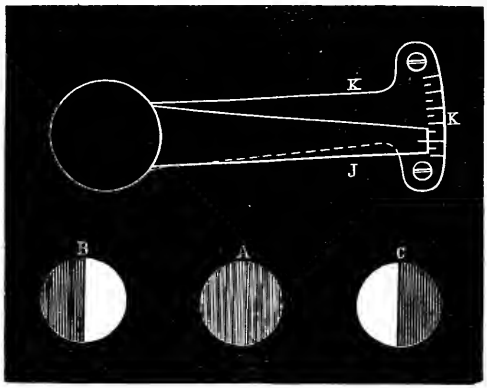


Fig. 6.

than the other (Fig. 6, B or C). If the prism be now turned round by means of the screw *G* till we again have equal illuminations in the two halves of the field (Fig. 6, A), *SP* has evidently been turned through the same angle and in the same direction as that through which the liquid has rotated the planes of polarization *OB*, *OB'*, and the reading of the instrument in its new position at once gives us the angle of rotation.

TABLE EXHIBITING THE SPECIFIC ROTATION OF SOME OF THE CHIEF PROTEID BODIES FOR THE YELLOW LINE *D*. (COMPILED FROM THE OBSERVATIONS OF HOPPE-SEYLER[1] AND HAAS[2].)

Proteid.	Observer.	Value of $(\alpha)_D$
Serum-albumin.	Hoppe-Seyler.	-56°
Egg-albumin.	Hoppe-Seyler.	$-33^{\circ}{\cdot}5$
	Haas.	$-38^{\circ}{\cdot}08$
Paraglobulin obtained from ascitic fluid by dilution of CO_2.	Haas.	$-59^{\circ}{\cdot}75$
Sodium-albuminate prepared from pure egg-albumin.	Haas.	$-62^{\circ}{\cdot}20$
Acid-albuminate (Syntonin) prepared from pure egg-albumin by action of acetic acid.	Haas.	$-63^{\circ}{\cdot}12$
Syntonin prepared from myosin by solution of that body in very dilute hydrochloric acid.	Hoppe-Seyler.	-72°
Casein, dissolved in solution of magnesium sulphate.	Hoppe-Seyler.	-80°

[1] Hoppe-Seyler, *Zeitschrift f. Chem. u. Pharm.* 1864, p. 737.

[2] Haas, "Ueber das optische und chemische Verhalten einiger Eiweisssubstanzen, insbesondere der dialysirten Albumine." Pflüger's *Archiv*, vol. XII. pp. 378–410.

Sec. 2. Chemical reactions characteristic of the Proteids[1].

Only certain of the proteids are soluble in water; they are all soluble however, especially with the aid of heat, in concentrated acetic acid, and in solutions of the caustic alkalies; they are insoluble in cold absolute alcohol and in ether.

Solutions of the proteids are precipitated by the following reagents:—

1. By strong mineral acids added in sufficient quantities.
2. By acetic acid and potassium ferrocyanide.
3. By acetic acid and a large addition of concentrated solutions of neutral salts of the alkalies or alkaline earths.
4. By basic lead acetate.
5. By mercuric chloride.
6. By tannic acid.
7. By powdered potassium carbonate added to the solution until it is nearly saturated.
8. The majority of the proteids are completely precipitated from their solutions by alcohol, though in the presence of free alkali they are slightly soluble in hot alcohol.

Detection of Proteids in solution.

When proteids are present in a solution the following reactions are employed in their detection:—

1. The liquid is boiled and nitric acid added, so as to produce a strong acid reaction. The occurrence of a precipitate on boiling, which is undissolved by nitric acid, and the immediate production of a precipitate by nitric acid indicates the presence of a proteid, to be confirmed by other tests.

2. The liquid is rendered strongly acid with acetic acid, and solution of potassium ferrocyanide added; all proteids are thrown down in the form of a white flocculent precipitate.

3. The liquid is rendered strongly acid with acetic acid, and is boiled with its own volume of a saturated solution of sodium sulphate, which will precipitate any proteid present.

The above tests are very satisfactory except in the case of only slight traces of proteids being present; under any circumstances it is desirable to obtain confirmatory evidence; the following methods are then useful:—

4. Millon's reaction. When a strongly acid solution of mercuric nitrate, made according to the directions to be afterwards given, is

[1] In preparing a part of this section the author has availed himself greatly and followed very closely, in some sentences almost literally, §§ 135 and 136 of Professor Hoppe-Seyler's *Handbuch der physiologisch- und pathologisch-chemischen Analyse.* 3rd Edit. 1870.

added to a solution containing even a trace of a proteid, and the mixture heated, the liquid assumes a purple-red colour. This reaction is common to all the proteids and to their immediate derivatives.

Millon's reagent is made by dissolving 1 part by weight of mercury in 2 parts of nitric acid of specific gravity 1·42 and after complete solution diluting each volume of liquid with two volumes of water.

5. Xanthoproteic reaction. The liquid supposed to contain a proteid is boiled for some time with concentrated nitric acid. If a proteid be present the liquid assumes a yellow colour, which changes to an amber-red when an excess of alkali is added to it.

Methods of completely separating proteids from solutions containing them.

It is often of great importance to remove all the proteids which a liquid contains, so as to proceed to the search for other substances. The following methods are available :—

1. The liquid is treated with several times its volume of absolute alcohol, and acetic acid added until the reaction is acid. After 24 hours the fluid is filtered; the proteids are contained in the insoluble matters on the filter.

2. To the liquid rendered faintly acid and heated to boiling, and from which all the proteids separable by mere boiling have been removed, a solution of ferric acetate, made by saturating acetic acid with recently precipitated ferric hydrate, is added. After boiling for a few minutes a solution is obtained which contains neither proteids nor iron.

3. In some cases when soluble proteids precipitable by boiling are present, by merely boiling the liquid they are entirely separated; such is usually the case with albuminous urine. If the liquid have an alkaline reaction, a little acetic acid should be added, in quantity just sufficient to neutralize the free alkali. If the quantity of acid be either too scanty or too great the separation is incomplete; under these circumstances the addition of a few drops of the solution of ferric acetate mentioned in the last paragraph brings about the complete precipitation and separation.

Determination of the temperature at which the proteids coagulate.

As will be shewn in the sequel, two groups of proteid bodies (the *albumins* and the *globulins*) are precipitated from their solutions when they are heated, and the temperature at which coagulation occurs is in some cases an important characteristic.

The method of determining the temperature of coagulation is illustrated by Fig. 7. A glass beaker containing water is placed within a second larger beaker also containing water, the two being separated by a ring of cork. Into the water contained in the inner beaker there is immersed a test-tube, in which is fixed an accurately graduated thermometer, provided with a long narrow bulb. The solution of proteid of which the temperature of coagulation is to be determined

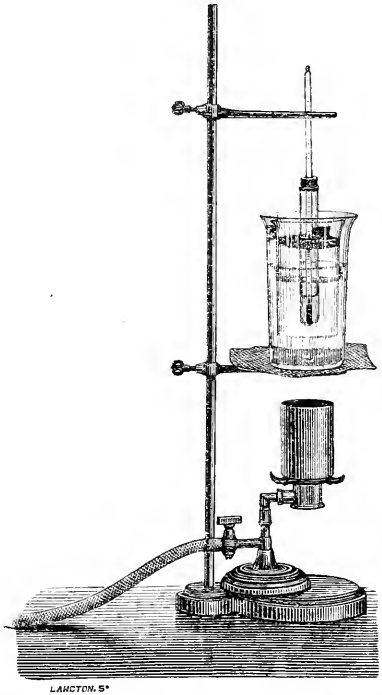


Fig. 7. Apparatus employed in determining the temperature of coagulation.

is placed in the test-tube, the quantity being just sufficient to cover the thermometer bulb.

The whole apparatus is then gradually heated. With the arrangement described the rise in temperature of the contents of the test-tube takes place very slowly and equably throughout. Care being taken to have as good an illumination as possible (the best plan being to place the apparatus between the operator and a well lighted window) the experimenter notes the temperature at which the liquid first shows signs of opalescence; he afterwards notes again the temperature at which a distinct separation of flocculent matter occurs.

TABLE EXHIBITING THE TEMPERATURE AT WHICH SOLUTIONS OF VARIOUS PROTEIDS, BELONGING TO THE GROUP OF ALBUMINS AND GLOBULINS, COAGULATE.

I.

Name of Proteid.	Character of the Solution.	Observers.	Temperature at which opalescence first occurs.	Temperature of Coagulation.
Serum-Albumin.	Dissolved in blood-serum, hydrocele fluid, &c.	Hoppe-Seyler[1].	60°—65°	72°—73°
Egg-Albumin.	Dissolved in water.	Hoppe-Seyler[1].		72°—73°

II.

Vitellin.	Dissolved in a weak solution of NaCl.	Weyl[2].	70°	75°
Myosin.	Dissolved in a weak solution of NaCl.	Kühne[3]. Weyl[2].		55°—60°
Fibrinogen.	Dissolved in the liquor sanguinis.	Frederique[4].		56° (55°–57°)
Paraglobulin.	Dissolved in solution of NaCl.	Hammarsten[5].	68°	75°

Sec. 3. Synopsis of the chief Proteid bodies.

The various proteid bodies occurring in the animal body will be described in connection with the tissues of which each is most characteristic; it will be convenient, however, to give a synopsis exhibiting the principles upon which they have been classified.

Soluble in pure water.

Class I. *Albumins:* proteid bodies which are soluble in water and which are not precipitated by alkaline carbonates, by sodium chloride, or by very dilute acids. If dried at a temperature below 40°, they present the appearance of yellow transparent bodies, breaking with a vitreous fracture, which are soluble in water.

Their solutions are coagulated when heated to temperatures varying between 65° and 73°.

(1) Serum-albumin. Specific rotation $(\alpha)_D = -56°$. Not precipitated from its solutions when these are agitated with ether.

[1] Hoppe-Seyler, *Handbuch d. phys.- u. path.-chem. Analyse.*

[2] Weyl, "Beiträge zur Kenntniss thierischer und pflanzlicher Eiweisskörper." *Zeitschrift f. physiol. Chem.*, vol. I, p. 72.

[3] Kühne, *Untersuchungen über das Protoplasma und die Contractilität*, Leipzig, 1864, p. 317.

[4] Frederique, "De l'existence dans le plasma sanguin d'une substance albuminoïde se coagulant à + 56°." *Annales de la Société de Médecine de Gand*, 1877.

Frederique, *Recherches sur la constitution du Plasma sanguin*, Gand, 1878, p. 25.

[5] Hammarsten, "Ueber das Paraglobulin. Zweiter Abschnitt," Pflüger's *Archiv*, 1878, vol. XVIII. p. 67. According to the amount of salt present, and the greater or less

Soluble in pure water.

(2) Egg-albumin. Specific rotation $(\alpha)_D = -35°{\cdot}5$. Precipitated from its solutions when these are agitated with ether.

Class II. *Peptones:* proteid bodies exceedingly soluble in water. Solutions not coagulated by heat; not precipitated by sodium chloride, nor by acids or alkalies. Precipitated by a large excess of absolute alcohol and by tannic acid. In the presence of much caustic potash or soda, a trace of solution of copper sulphate produces a beautiful rose colour.

Insoluble in pure water, but soluble in weak solutions of common salt.

Class III. *Globulins:* proteid substances which are insoluble in pure water, but soluble in dilute solutions of sodium chloride; *their solutions are coagulated by heat;* they are soluble in very dilute hydrochloric acid, being converted into acid-albumins; they are also readily converted by alkalies into alkali-albumins.

(1) Vitellin, not precipitated from its solutions when these are saturated with common salt. Solutions coagulate at 70°—75° C.

(2) Myosin, precipitated from its solutions in weak common salt when these are saturated with sodium chloride. Solutions coagulate at 55°—60° C. Solutions in common salt not coagulated by solution of fibrin-ferment.

(3) Fibrinogen, soluble in weak solutions of sodium chloride. Precipitated from them completely by the addition of sodium chloride, when this amounts to 12 or 16 per cent. Solutions coagulate on the addition of fibrin ferment. Temperature of coagulation 56° C.

(4) Paraglobulin, soluble in weak solutions of sodium chloride. From very weakly alkaline solutions paraglobulin is precipitated by the addition of a very small quantity of common salt; a further addition of this body leads to re-solution of the precipitate, which is thrown down again when the amount of sodium chloride in solution exceeds 20 p.c. The precipitation of paraglobulin by sodium chloride is never complete. Paraglobulin is completely precipitated when its solutions are saturated with magnesium sulphate. Solutions not coagulated by addition of fibrin-ferment. Temperature of coagulation varies (according to amount of salts present and mode of heating) between 68°—80° C.; on an average 75° C.

Class IV. *Derived Albumins*[1]*:* proteid bodies insoluble in pure water and in solutions of common salt, but readily soluble in dilute hydrochloric acid and in dilute alkalies. Solutions not coagulated by heat.

(1) Acid-albumins: obtained by the action of dilute acids (preferably dilute hydrochloric acid) upon solutions of proteids, by action of strong acids upon the solid proteids, and as first products in the action of gastric juice upon proteids. On neutralizing solutions of acid-albumins, they are precipitated *even in the presence of alkaline phosphates.* NaCl, added to saturation, also precipitates them.

(2) *α.* Alkali-albumins or alkaline albuminates: obtained by the action of dilute alkalies upon the proteids. Possess the properties of subclass 1, with the exception that in the presence of alkaline phosphates

rapidity of heating, the coagulation temperature varies, according to Hammarsten, between 68° and 80°.

[1] This convenient designation I borrow from Dr Michael Foster. See *Text-book of Physiology*, Appendix.

the solutions are not precipitated by neutralization. When heated with strong solution of caustic potash potassium sulphide is *not* formed.

β. Casein, the chief proteid constituent of milk. Same properties as α, but when heated with strong solution of caustic potash, potassium sulphide is formed. In milk is coagulated by *rennet*.

CLASS V. *Fibrin:* Insoluble in water and in weak solutions of sodium chloride. White elastic solid, usually exhibiting fibrillation when examined under a high magnifying power; swells up in cold hydrochloric acid of ·1 per cent., but does not dissolve; when thus swollen dissolves with ease when a solution of *pepsin* is poured over it. When heated for a great many hours at 40° in dilute hydrochloric acid, it dissolves and the solution contains acid-albumin.

CLASS VI. *Coagulated Proteids:* Insoluble in water, dilute acids and alkalies. Give Millon's reaction. Are dissolved when digested at 35°—40°, in artificial gastric or pancreatic juice, giving rise to peptones.

CLASS VII. *Lardacein*, so-called *amyloid substance:* Insoluble in water, in dilute acids, in alkaline carbonates; not dissolved by gastric juice at the temperature of the body. Coloured brownish-red or violet by iodine.

SEC. 4. PRODUCTS OF DECOMPOSITION OF PROTEIDS.

The methods which the chemist follows in arriving at a knowledge of the constitution of a body are various; his chief information is derived from a careful study of the way in which the body is decomposed under various circumstances, and of the structure and amounts of the various products thus obtained; subsidiary information is derived from a consideration of physical properties, which sometimes suggest analogies which otherwise would pass undetected. The correctness of any view as to the structure of a body will be tested by its being able, or not, to account for all known reactions, and it will receive singular confirmation if it enable the experimenter to effect the synthesis of the subject of speculation.

Great though the progress of organic chemistry has been, and remarkable the development of our knowledge of the constitution of bodies, we are yet far from being able to unravel the constitution of such complex bodies as the proteids. We can therefore merely record the results of laborious experiments which shew the products, or rather the classes of products, yielded by the proteids, and scarcely venture to surmise what the exact constitution of the proteids may be.

In the animal body, the proteids are ultimately subjected to processes of oxidation of which the chief ultimate results are water, carbon dioxide and urea; what all the intermediate substances may be we do not exactly know, though it is certain that glycine, leucine, tyrosine and some other bodies are formed; moreover it is certain that substances destitute of nitrogen, such as carbohydrates, and also fats, may take their origin in the decomposition of the proteids. Can

these processes be imitated in the laboratory? Only in part, indeed, for, in spite of certain statements to the contrary, no one has given valid proof of having, by an artificial oxidation, obtained urea.

The following are the chief facts which have been discovered in reference to the decomposition of proteids; after quoting these we shall refer to some of the theoretical views to which they have given rise.

1. *Action of water.* When heated with water in sealed tubes at a temperature of 100° C., the proteids are in part dissolved, the solution afterwards undergoes decomposition, it being found to contain sulphuretted hydrogen, and a number of complex bodies of which some are soluble in alcohol and ether (Gautier).

2. *Action of heat.* When subjected to dry distillation, the proteids furnish the oily liquid long known as *Dippel's oil*, which contains (1) ammoniacal salts of the fatty acids, as *ammonium butyrate*, *valerate* and *caproate;* (2) amines, derived from the monatomic alcohols, *viz. methylamine*, *propylamine*, *butylamine;* (3) aromatic compounds, as *benzine*, *aniline*, *phenol;* (4) *picoline* and *lutidine*, which are bases which combine with the iodides of alcohol radicals to form compound ammonium iodides.

3. *Action of putrefaction.* When exposed to the combined influences of air and moisture, especially at a high temperature, the proteids yield *ammonia, ammonium sulphide, carbon dioxide, volatile fatty acids, lactic acid, amines, leucine and tyrosine.* Under certain circumstances *indol* may be formed.

4. *Action of strong mineral acids and of caustic alkalies.* Prolonged boiling with sulphuric and hydrochloric acid and fusion with caustic alkalies gives rise to products of which the chief are the same in the two cases, *viz. leucine, tyrosine, aspartic acid* and *glutamic acid.*

When proteid bodies are treated with dilute acids they undergo *hydrolytic decomposition*, and certain definite compounds may be extracted from the resulting mass. Their proportion is however small in comparison with the by-products which we have no means of investigating.

The term "*hydrolytic decompositions*" has been applied by Hermann[1] to designate decompositions in which a body splits up after combining with the elements of water; thus under various circumstances the neutral fats combine with the elements of water and decompose into a fatty acid and glycerin, as shewn in the case of stearin by the following equation:

$$\underbrace{C_{57}H_{110}O_6}_{\text{Stearin.}} + \underbrace{3H_2O}_{\text{Water.}} = \underbrace{3C_{18}H_{36}O_2}_{\text{Stearic acid.}} + \underbrace{C_3H_8O_3}_{\text{Glycerin.}}.$$

Nasse first observed that the nitrogen in proteids appears to exist in two conditions, as evidenced by the fact that a certain fraction of it is much more unstable, apparently more feebly combined, than the rest. Schützenberger has fully confirmed these observations. He heated proteids with caustic baryta, in aqueous solution, up to 100° and collected the ammonia given off in sulphuric acid. There separated a good deal of granular matter, which increased as the reaction proceeded and which was found to consist of carbonate, a little sulphate, oxalate, and phosphate, of barium.

[1] Hermann, *Elements of Human Physiology*, 2nd English ed., p. 2. Smith, Elder and Co., 1878.

The ammonia evolved, as well as the CO_2 in combination with barium, were estimated and found to be in the same ratio as would result if urea were treated in a similar manner. Although the boiling was continued for some time, the decomposition still progressed and only terminated when the liquid was heated in sealed tubes up to 150° C. The relative amounts of CO_2 and NH_3 still remained the same.

The substance thus treated did not give out any more ammonia, even when heated to 200°. Nearly the whole of the resulting mass could be got into a crystalline form, and Schützenberger was able to identify the following substances[1]:—the elements of *urea*, (CO_2 and NH_3): traces of CO_2, H_2S, *oxalic* and *acetic* acid, *tyrosine: amido-acids* of the series $SH_{2n} + NO_{2n}$ corresponding to the fatty series $C_nH_{2n}O_2$, from *amido-aenanthylic* to *amido-propionic acid: leucine, butalanine,* and *amido-butyric acid* predominated. There were also obtained one or two acids nearly allied to *aspartic* and *glutamic* acid, and one or two related to Ritthausen's *leguminic* acid; furthermore a small quantity of a substance resembling dextrin.

5. *Action of hydrochloric acid and stannous chloride.* When heated with these reagents there are formed *ammonia, aspartic acid, glutamic acid, leucine* and *tyrosine.*

6. *Action of various oxidizing agents.* *a.* When oxidized by means of manganese dioxide and sulphuric acid, or potassium bichromate and sulphuric acid, the proteids furnish bodies belonging to the aromatic and fatty groups. Amongst others the following: *benzoic aldehyde, propionic aldehyde, propyl cyanide, benzoic acid, valerianic aldehyde, butyl cyanide, hydrocyanic acid, acetic acid, propionic acid, valerianic acid.*

b. By the action of nitric acid, there is first produced a yellow insoluble body (*xanthoproteic acid*) which dissolves on further action, *paroxybenzoic* and *oxybenzoic* acids being ultimately formed.

c. When oxidized by means of chlorine, the proteids yield, amongst other products, *fumaric acid, oxalic acid* and *chlorazol.*

d. When heated with bromine and water, under pressure, there are formed *carbon dioxide, oxalic acid, aspartic acid, leucine, leucimide, bromacetic acid, bromoform, bromanil* and *amidotribromobenzoic acid.*

Sec. 5. Theoretical views as to the constitution of the Proteids.

"Under the most diverse influences:—action of water and strong acids, action of bases, oxidations, putrefactions...&c.—the proteid bodies when decomposed yield: firstly amides, such as glycocine and leucine, containing radicals derived from fatty acids, or from the homologues of lactic acid, as well as more complex amides, such as aspartic acid, $C_4H_7NO_4$, the amide of malic acid, and glutamic acid,

[1] Schützenberger, *Bulletin de la Soc. Chimique.* 15 Février, 5 Mars, et 15 Mars, 1875.

$C_5 H_9 NO_4$, which is a homologue of the preceding; secondly, amides having aromatic nuclei, such as tyrosine; thirdly, amides containing sulphur, such as cystine: fourthly, acids and aldehydes corresponding to the radicals of the amides before mentioned. The proteid substances behave as amides containing both radicals of the higher homologues of lactic and tartaric acids and residues of aromatic acids. Hence it follows that when the proteids are oxidized there is simultaneous production of fatty acids, of aromatic compounds and doubtless also of bodies analogous to urea.

"Though all the proteids when they are decomposed or oxidized nearly always furnish the same products, they yet do not furnish them in the same proportions. It must therefore follow that the different radicals which they contain differ, not only in their arrangements, but in their relative proportion, and in some cases even in their nature[1]."

The views of Schützenberger[2].

It is beyond the scope of this work to discuss hypotheses as to the constitution of bodies unless these appear to possess a legitimate interest to the biologist or the physician. The speculations of Schützenberger can therefore only be summarized in a few words. From the products obtained by the action of caustic baryta upon the proteids (see pp. 19 and 20), this author looks upon the proteids as complex *ureids*, *i.e.* as resulting from combination in different proportions of urea with amido-acids, some of which belong to the *leucine* series, others to the *aspartic* series, whilst the more complex products of decomposition allied to leguminic acid must be considered as resulting from complete decomposition. Tyrosine represents the aromatic group, and is the source of benzoic acid found amongst the products of the putrefactive decomposition of proteids. When decomposed by means of caustic baryta, he assumes that the molecule of albumin, which he represents by the empirical formula $C_{72} H_{112} N_{18} O_{22} S$, yields, in addition to urea, acetic acid, and some sulphur-containing body, a substance to which he ascribes the formula $C_{68} H_{132} N_{14} O_{34}$ and which he admits may be split up in various ways. Useful, nay indispensable as are such hypotheses as suggesting lines of research to the actual chemical worker, they possess no interest as yet to the biologist.

Pflüger's[3] views relative to the constitution of the Proteids.

It is in the cells of the organism that the processes take place whose results are the external activities which it manifests; it is within the cells that the oxidation processes of the economy have their seat.

There is nothing more striking than the wide contrast which exists between the non-living proteid matter, say that of

[1] Gautier, *Chimie appliquée à la Physiologie, à la Pathologie et à l'Hygiène*, tome premier, p. 251.

[2] Schützenberger, "Recherches sur l'Albumine et les matières albuminoïdes," *Bulletin de la Soc. Chimique*, v. 23 and 24.

[3] Pflüger, "Ueber die physiologische Verbrennung in den lebendigen Organismen," Pflüger's *Archiv*, Vol. x., p. 251.

white of egg, and that which forms part of the living cell. The former may be kept for years, the latter is continually decomposing without any influence from without being necessarily exerted upon it.

The proteids which we consume as food are indifferent to neutral oxygen; so soon as they are taken up by organized cells they change their character, by changing the structure of their molecules, and are now subject to the influence of oxygen. The molecule of albumin begins to live by breathing oxygen.

How thoroughly independent of an *immediate* supply of oxygen very complex animal processes may be, which are essentially associated with the metabolism of cell protoplasm, is, however, shewn by certain remarkable experiments in which Pflüger introduced living frogs into chambers containing no oxygen, and the temperature of which was kept low, and observed that for many hours *all* the processes of the organism continued to be performed.

How can we explain the immensely increased instability of the living protoplasmic proteid matter as contrasted with non-living proteid matter?

The assimilation of proteid matter is looked upon by Pflüger as due to the formation of ether-like combinations between the proteid of the cell protoplasm, and the proteid which serves as its food, water being eliminated. In this process a living proteid molecule may bind to itself a non-living, but isomeric, proteid molecule, and this process of polymerism may be conceived to go on almost indefinitely, so that a large and heavy mass may be produced out of, and yet continue to exist as, a simple molecule[1].

Pflüger inclines to the belief that in this process of assimilation by the cell, proteid matter undergoes a change in its constitution, the nitrogen passing from the state in which it exists in amides to the more unstable condition in which it exists in cyanogen and its compounds. In this way Pflüger explains why it is that in uric acid, as in many other products of proteid metabolism—creatine, guanine, &c.—cyanogen radicals are contained, whilst none of these decomposition products are to be obtained from non-living proteids.

Bodies (so called Albuminoid) **related to the proteids.**

In concluding this sketch of the proteids, it must be mentioned that there occur in the epithelial and connective tissues of the organism certain bodies which have somewhat close relationship to the proteids, but which are nevertheless distinct from them; these are chondrin, collagen and gelatin, mucin, elastin, keratin. They will be considered in detail in future sections of this work.

[1] The Author understands Pflüger to say that the same constituent atoms or groups of atoms (*radicals*) must be present in different proteids: that the difference is caused either by the final molecule being a different multiple of the same group or groups of atoms (*polymerism*) or by the oxygen or nitrogen occupying different relative positions with respect to groups of atoms which they serve to link together (*metamerism*), or by differences in the relative position of groups of atoms or their constituent parts with respect to one another (*general isomerism*).

CHAPTER II.

THE BLOOD.

Sec. 1. The Physical Characters of the Blood.

Physical Characters.

The blood as it circulates in the vessels of man and vertebrates generally is a viscous, and to the naked eye homogeneous liquid of red colour: the blood of the pulmonary veins, of the left side of the heart, and of the systemic arteries being normally of a bright scarlet hue, and the blood of the right side of the heart, of the systemic veins, and of the pulmonary artery being of a brownish-red colour. On exposure to air or to oxygen the brown-red colour of venous blood soon changes to scarlet, and this change takes place most rapidly when the blood and gas are shaken up together.

In order to collect for purposes of analysis or demonstration pure arterial or venous blood, or both, so as to avoid contact with air, the following apparatus or some modification of it may be employed:—

A and *B* are two glass tubes of about 100 c.c. capacity, which at their lower extremities are connected by means of elastic tubing with a forked tube *C*, to which is attached the elastic tube *D*, which at its other end is connected with the glass bulb *R*, having a capacity of about 250 c.c. At their upper extremities, *A* and *B* have connected with them two glass stop-cocks, the tubes leading from which are of narrow diameter; it is convenient that these tubes should be of such a size that india-rubber tubing of narrow diameter can easily be attached to them. The tubes are fixed in two separate iron clamps such as are shewn in the drawing, attached to a firm upright rod of iron with a firm stand. The reservoir *R* is also held by a similar clamp, which can easily be attached either to the top or to the bottom part of the upright rod, so as to place it above or below any given level in relation to the tubes *A* and *B*.

The reservoir being, say, in the lower position indicated in the figure, mercury is poured into it so as to fill it. It is next unclamped and raised

so that its lower part is above the level of the stop-cocks of *A* and *B*. These are now opened, mercury rises into the tubes, driving the air which they previously contained before it; when the tubes are filled and a stream of mercury is issuing from them, the stop-cocks are closed. In order to determine whether the stop-cocks do not leak, the reservoir *R* may now be held in the hands of the experimenter at thirty-five or forty inches below the stop-cocks of *A* and *B*. The mercury in these tubes will naturally fall at first and then remain steady: on raising the reservoir cautiously the metal should however rise and fill the tubes completely.

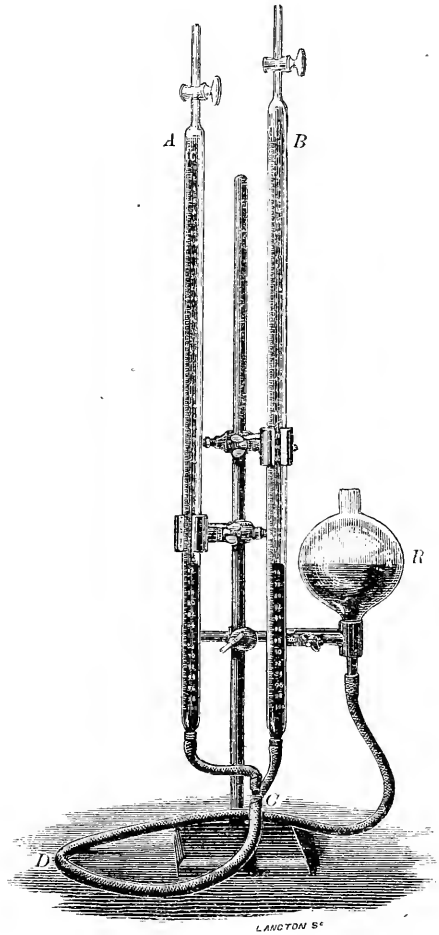


FIG. 8. APPARATUS FOR COLLECTING BLOOD OVER MERCURY WITHOUT ALLOWING IT TO COME IN CONTACT WITH AIR.

In order to collect apart arterial and venous blood, glass cannulae, to which are attached narrow elastic tubes of considerable length, are in-

serted into the artery and vein of the animals to be experimented upon[1], which should be deeply anaesthetized[2]. The clips which control the entrance of blood from the cardiac side of the arterial and the distal side of the venous cannula having been removed or opened, blood is allowed completely to fill the elastic tubes attached to the cannulae, which are held at a fairly high level so as to allow the blood to rise and expel the air before it. The instant the tubes are filled their open ends are slipped over the ends of *A* and *B*.

The reservoir *R* having been placed in its lower position, the stop-cocks of *A* and *B* are opened; blood will then flow from the artery and vein into the respective tubes. As soon as enough has been obtained the stop-cocks are closed, and the tubes are simultaneously shaken by assistants so as to defibrinate their contents. On placing the two tubes side by side the contrast between the colour of arterial and venous blood will appear most striking.

A detailed description of this procedure has been given as, *mutatis mutandis*, it illustrates the method in which blood can be obtained from blood-vessels without being brought in contact with air, not only for purposes of class demonstration, but also in researches on the gases of the blood.

Where it is required to keep the blood for some hours, as for example in order to make repeated analyses, one or both tubes may be taken out of their respective clamps and laid in troughs containing broken ice. In some cases it is desirable to obtain two separate samples of the same blood; in such cases the free upper ends of *A* and *B* have attached to them a T tube, to which is connected the elastic tube leading to the artery or vein. The blood-stream will then divide itself equally between the two tubes.

Although to the naked eye the blood appears to be a homogeneous red liquid, it is found on microscopic examination to consist of a colourless fluid—the so-called *liquor sanguinis*, or *plasma* of the blood—holding in suspension large numbers of solid bodies, the coloured and colourless corpuscles of the blood. It is the former of these which preponderate very greatly over the latter, and which by the colouring matter, *haemoglobin*, of which they mainly consist, confer upon the blood its red colour; the shade of this at any time depends, as will be shewn in the sequel, chiefly

[1] *Handbook for Physiological Laboratory*, p. 212.

[2] The Author would very strongly recommend all experimenters who have occasion to perform experiments upon the lower animals, and especially dogs, to employ as the chief means of producing insensibility to pain, subcutaneous injections of morphia. Solutions of bimeconate of morphia may be obtained which contain as much as two grains in half a drachm. As large a dose as two grains of the bimeconate may with perfect safety be injected under the skin of a dog of medium size; the injection is followed in about half an hour by salivation and by a staggering gait, and then by deep somnolence. In this state the animal is quite passive, and may without a struggle and without any fear being evinced on its part, be properly fixed, and then rendered completely insensitive to pain by the administration of ether or chloroform; as was pointed out by Claude Bernard, under these circumstances chloroform anaesthesia is induced with remarkable ease, and persists for a long time. This method not only abolishes the fear which often must constitute the most important part of the pain inflicted by a physiological experiment, but in those rare cases where the animal must be allowed to recover after the experimental proceeding has been carried out, the long period of narcotism which succeeds it secures the absolute and beneficial rest of the animal.

upon the chemical relations of the colouring matter to oxygen, though in part also upon the shape of the coloured blood corpuscles, which is subject to various physical influences.

The specific gravity of the living blood cannot for obvious reasons be ascertained; that of defibrinated human blood drawn from healthy subjects has been found to vary between 1045 and 1062[1], the average being 1055; greater variations than are indicated by the above numbers are however consistent with health, the widest limits being probably indicated by the numbers 1045—1075.

The mean specific gravity of the blood of the dog was found by Pflüger to be 1060[2], and by Nasse to be 1059[3]; that of the blood of the rabbit was found by Gscheidlen to vary in three cases between 1042 and 1052.

As blood is drawn from a vessel it is found to vary slightly in density, that drawn first having a somewhat higher specific gravity than that which follows, owing to the quantity of water of the blood increasing as a result of haemorrhage[4].

Reaction. Blood always possesses a feebly alkaline reaction, which rapidly diminishes from the time of its being shed to the time of its coagulation.

The red colouring matter of the blood interferes with the ready determination of the reaction as by simply immersing ordinary test-papers into the fluid, and therefore one or other of the three following methods may be employed, of which the second and third, *b* and *c*, are to be preferred.

(*a*) *Kühne's Method*[5] consists in placing a drop of blood in a specially constructed tiny dialyzer of parchment-paper; this is then immersed in a drop of water contained in a watch-glass. After a short interval the reaction of the water is determined by means of litmus paper.

(*b*) *Liebreich's Method*[6]. Plaster of Paris absolutely free from alkaline reaction is cast into thin slabs, which are then dried, and afterwards coloured by dropping upon them a perfectly neutral solution of litmus. When a droplet of blood is allowed to fall upon the coloured slab, the fluid of the drop is soon absorbed by the porous gypsum whilst the corpuscles are left. On placing the spot under a stream of water, the corpuscles are washed away and the colour of the slab at the site of the blood spot is found to be a more or less deep blue.

(*c*) *Zuntz's Method*[7]. This method rests upon the fact that the

[1] Becquerel et Rodier, *Recherches sur les altérations du sang*. Paris, 1844.

[2] Pflüger, "Ueber die Ursache der Athembewegungen, sowie der Dyspnoe und Apnoe." *Archiv d. gesammten Physiologie*. Bd. I. (1868) p. 75.

[3] Nasse, *Haematologische Mittheilungen*. Quoted by Gscheidlen, *Physiologische Methodik*, p. 328.

[4] Becquerel et Rodier, *Traité de Chimie Pathologique, appliquée à la Médecine pratique*. Paris, 1854, p. 41 et seq.

[5] Kühne, "Ein einfaches Verfahren, die Reaction hämoglobinhaltiger Flüssigkeiten zu prüfen." Virchow's *Archiv*, vol. XXXIII. (1865), p. 95.

[6] Liebreich, "Eine Methode zur Prüfung der Reaction thierischer Gewebe." *Berichte d. deutschen chem. Gesellsch. zu Berlin*, 1868, p. 48.

[7] Zuntz, *Centralblatt*, 1867, No. 34. See also Adam Schulte, *Ueber den Einfluss des Chinin auf einen Oxydationsprocess im Blute*. Inaugural Dissertation. Bonn, 1870. p. 9 et seq.

blood colouring matter does not diffuse out of the blood corpuscles into solutions of common salt of considerable strength. Litmus paper is moistened with a strong solution of salt and a drop of the blood to be tested is placed upon it; after a few seconds a drop of the same salt solution is placed over the drop of blood; the liquid is then sucked up by means of filter paper. By following this method the blood can be so removed from the test-paper that the colour of the latter may be readily observed. The litmus paper to be used for this purpose must be highly glazed and the tincture of litmus used in its preparation must have been neutralized with acid until its colour is violet.

By adding standard solutions of acids to blood, and employing the above method for ascertaining when the reaction became faintly acid, Zuntz determined the previously mentioned diminution of alkalinity of blood removed from the body.

The Phenomena of Coagulation.

As it circulates in the blood-vessels of the living body, the blood consists, as we have said, of a liquid, the so-called *liquor s. plasma sanguinis* (often designated *blood-plasma*, or more shortly *the plasma*), holding in suspension the blood corpuscles. Within a short time of its being shed—usually between two and six minutes—the process known as coagulation commences—a process in which the blood passes first into the state of a soft red jelly, which gradually acquires greater consistence, and which, by a contraction of one of its constituents, expresses a fluid—*the serum*, which surrounds the clot, and in which the latter often ultimately floats.

If we desired to ascertain the exact time when this coagulation commenced in a sample of blood, we should collect it in a watch-glass and at very short intervals pass a needle through the liquid; as soon as coagulation had set in the needle would, in its passage through the fluid, entangle itself in the newly formed jelly, which would then be apparent on drawing the needle out.

When blood coagulates, the process usually commences on the surface of the liquid and then near the sides of the vessel which contains it, the newly formed coagulum having in the former case the appearance of a pellicle. Very rapidly, however, the process invades the whole mass of the blood, which then presents the appearance of a soft, easily broken, jelly. Soon this acquires greater consistence, so that the blood has, as it were, taken a cast of the vessel which contained it, adhering closely to its sides and permitting of the vessel being inverted without any escape of the contents; at the same time drops of serum begin to transude from the clot. This transudation of serum is brought about by the contraction of the clot and continues for a time varying between ten and forty-eight hours, at the end of which the clot is found to be surrounded by serum. According to Nasse, the first stage of coagulation (characterized by the formation of a pellicle) commences in the blood of men in about 3 minutes 45 seconds, in that of women in 2 minutes 50 seconds; the second stage, in which not only the surface but the portions of blood next to

the walls of the vessel have become gelatinized, occurs on an average in the blood of men in 5 minutes 52 seconds, and in that of women in 5 minutes 12 seconds; the third stage, in which the blood has been converted throughout into a soft jelly, is usually developed in the blood of men in 9 minutes 5 seconds, and in that of women in 7 minutes 40 seconds; the fourth stage, of complete solidification with obvious commencement of transudation of serum from the clot, occurs in the blood of men in about 11 minutes 45 seconds, and in that of women in 9 minutes 5 seconds[1].

This process of coagulation is due to the separation from the plasma of a body called Fibrin, which entangles in its meshes the corpuscles of the blood, the mechanical interlocking of the corpuscles by the threads of fibrin giving rise to the *crassamentum* or blood clot.

The blood of certain animals coagulates more rapidly than that of others: we might with fair accuracy arrange the blood of various common domestic animals in the following order, according to the rapidity of coagulation, the first-named coagulating most rapidly—rabbit, sheep, dog, ox, horse; in the latter animal coagulation commences usually between five and ten minutes after the blood is shed. If human blood were included in the above list it would immediately precede that of the ox.

When the commencement of coagulation is delayed for several minutes—as it normally is in horse's blood, and as it usually is in the blood of men and other animals when suffering from inflammatory diseases—the blood corpuscles, being specifically heavier than the plasma, have time to subside partially before coagulation commences, so that the uppermost layers of such blood if undisturbed are nearly free from coloured corpuscles; subsequently when the blood coagulates, the clot exhibits the phenomenon of the *buffy-coat*, 'inflammatory crust,' or *crusta phlogistica, i.e.* the upper part of the clot is of a yellowish colour; in the lower strata of the buffy-coat are found large numbers of colourless corpuscles, which being specifically lighter than the red have not time to sink as far as the latter before coagulation occurs. The formation of the buffy-coat, though in part due to slow coagulation, is dependent greatly upon the blood corpuscles aggregating so as to form little clumps, which more readily overcome the resistance offered by the fluid and therefore sink more readily than individual corpuscles.

If instead of allowing blood to coagulate undisturbed, it be stirred or whipped with twigs immediately after it is shed, the process of coagulation is modified. The fibrin generators unite to form *fibrin*, but this does not entangle the blood corpuscles; it separates as a stringy mass, which adheres to the instruments which have been used to stir the blood, whilst the blood corpuscles remain suspended in the serum, the mixture being designated defibrinated blood. Defibrinated blood differs from the living blood which has yielded it,

[1] Nasse, Article *Blut*, Wagner's *Handwörterbuch d. Physiologie*, Vol. I. pp. 102, 103.

merely in having lost the fibrin-generators, which have united to form fibrin.

Circumstances which hasten Coagulation.

The following circumstances hasten or promote coagulation:

a. Exposure to a temperature higher than that of the living body (Hewson[1], Hunter[2], Thackrah[3], Scudamore[4], Davy[5], Gulliver[6]), but probably not exceeding 52° C. or 54° C.

b. Contact with foreign matter: thus the time of coagulation will be affected by the shape of the vessel in which blood is collected, the process occurring sooner where a large surface of blood is in contact with the vessel, as for example when it is allowed to flow into a wide shallow vessel. The influence of foreign matter in promoting coagulation will be again referred to.

c. Closely connected with *b.* is the effect of agitation, which, as Hewson[7] and John Hunter[8] shewed, and as has been fully confirmed, hastens coagulation.

d. The dilution of blood with not more than twice its volume of water (J. Hunter[9], Prater[10]).

e. The addition of *minute* quantities of sodium chloride, sodium sulphate or other neutral salt (Ancell[11]).

Conditions which retard or suspend Coagulation.

The following circumstances hinder or suspend coagulation:—

a. Exposure to a low temperature.

Blood which is rapidly reduced to the temperature of melting ice does not coagulate (Davy once kept blood fluid for one hour at 0° C.): it may be frozen and remain in a frozen condition for hours without losing its power of coagulating when thawed (Hunter[12], Hewson[13], Davy[14]). It may be frozen and thawed several times in succession without coagulating or losing its property of coagulating (Davy).

[1] Hewson, *Properties of the Blood*, p. 3. The Works of William Hewson, F.R.S. edited with an introduction and notes by George Gulliver, F.R.S. London, printed for the Sydenham Society, 1846.

[2] *Works*, edited by Palmer, iii. 26, 110.

[3] Thackrah *On the Blood*, ed. 1834. Exp. 44, 45, 50, 51, 52, 56.

[4] Scudamore *On the Blood*, p. 20. 8vo. London, 1824.

[5] Davy, *Researches, Physiological and Anatomical*. London, 1859, Vol. 2, p. 78.

[6] Gulliver, *Hewson's Works*, p. 4. Note III.

[7] Hewson, *op. cit.*, p. 15.

[8] Hunter, *Works*, ed. by Palmer, Vol. III. 31.

[9] John Hunter, *General Principles of the Blood*, at p. 135 of Vol. III. of Palmer's edition of *The Works of John Hunter*.

[10] Prater, *Experimental Inquiries in Chemical Physiology*, p. 81. Part I. 'On the Blood.' London, 1832.

[11] Ancell, *Course of Lectures on the Physiology and Pathology of the Blood*, &c. Lecture VII. *Lancet*, 1839-40, p. 522.

[12] Hunter, *Works of*, by Palmer, Vol. III., p. 67.

[13] Hewson, *op. cit.*, p. 17.

[14] Dr John Davy, *op. cit.*, Vol. II., p. 75.

The following is the best method of exhibiting this fact for purposes of class demonstration:

A small platinum crucible, or still better, as permitting more easily of an examination of its contents, a small platinum basin is immersed in a vessel containing a mixture of ice and salt; a frog is then decapitated, and the blood is allowed to flow into the frozen vessel, where it instantly congeals. The platinum vessel can be taken out of the ice and held up so as to shew the hard frozen drops of blood. The experimenter then places the vessel on the palm of his hand, the heat of which almost instantly thaws the blood, which can then be dropped into a watch-glass. The platinum vessel is again placed on the ice and the thawed blood transferred to it, to be frozen a second time. This freezing, thawing, and transference from one vessel to another may be repeated several times; at last the blood is allowed to remain in the watch-glass, when after a few minutes it sets into a firm jelly.

b. Contact with the living tissues.

If a vein be exposed and ligatures be applied to it so as to confine a quantity of blood within it, and it be then cut out of the body, it will be found that on opening the vein after an hour the blood will still be fluid, though after contact with foreign matter it will coagulate in a few minutes (Hunter[1], Hewson[2]). For some hours after somatic death the blood remains fluid in all vessels except the heart and principal trunks, *provided that the vessels have been previously healthy.* Blood will remain fluid for hours in a vein after being exposed with the utmost freedom to the air by being poured in a thin stream from one vein to another (Lister[3]).

c. The addition of a sufficient quantity of sodium chloride, sodium sulphate, potassium nitrate or some other neutral salts (Hewson[4], Davy[5]), will prevent coagulation, which will however occur subsequently if a sufficient quantity of water be added.

Thus to quote Hewson's own words, "if six ounces of human blood are received from a vein upon half an ounce of true Glauber's salt reduced to a powder, and the mixture agitated so as to cause the salt to be dissolved, that blood will not coagulate on being exposed to the air, as it would have done without the salt; but if to this mixture about twice its quantity of water be added, in a short time the whole will be jellied or coagulated, and on shaking the jelly, the coagulum will be broken, and the part so coagulated can now be separated as it falls to the bottom and proves to be lymph" (*i.e.* fibrin).

[1] Hunter's *Works*, by Palmer, Vol. III., p. 29.
[2] Hewson's *Works*, p. 22.
[3] Lister, "On the Coagulation of the Blood;" the Croonian Lecture for 1863. *Proceedings of the Royal Society*, Vol. XII. p. 580.
[4] Hewson, *op. cit.*, p. 11 et seq.
[5] Davy, *Researches*, Vol. II. 101-2.

Sec. 2. The Liquor Sanguinis. Fibrin and its supposed precursors.

The Liquor Sanguinis.

Methods of obtaining Liquor Sanguinis.

It has already been stated that in the living blood the corpuscles float in a fluid termed the *liquor sanguinis* or *plasma*, and that when blood coagulates it does so in consequence of the separation from the plasma of a proteid substance termed *fibrin*. We have now to describe the mode of obtaining liquor sanguinis, to describe fibrin, to examine the bodies which the plasma contains, and to examine the facts which relate to the separation from it of fibrin.

Almost as soon as the liquor sanguinis is withdrawn from the living vessels, it undergoes that change which results in the separation of fibrin and serum. The change may however be hindered by various methods, which may be employed to furnish us with plasma for examination.

1. In order to obtain plasma in a state of great purity, blood must be rapidly cooled to a temperature approaching that of melting ice, at which temperature its coagulation is, as has been already stated, deferred.

The blood of most animals coagulates so rapidly that it is difficult to cool any considerable quantity of blood to a temperature at which coagulation would be long deferred, before the process has actually occurred. The blood of the horse or donkey, however, usually coagulates so slowly that with the aid of suitable contrivances considerable quantities may be cooled to near 0° C. before coagulation has had time to occur; and once at that temperature the process of coagulation may be long postponed.

Under these circumstances the corpuscles sink pretty rapidly, tending to form a sediment at the bottom of the vessel in which the blood was received, and leaving an upper stratum of liquor sanguinis perfectly free from red colour. The liquor sanguinis, decanted from the corpuscles and exposed to a temperature favourable to coagulation, exhibits the phenomena which have been described as characterizing the coagulation of the blood, save that the coagulum is colourless. If the fluid be stirred with twigs there will separate from it stringy fibrin exactly similar to that obtained by similar treatment from blood, save in the absence of colour derived from entangled blood corpuscles.

A convenient contrivance for collecting considerable quantities of plasma from the blood of the horse is shewn in the annexed figure, and was suggested by Dr Burdon Sanderson[1]. The apparatus consists of a vessel with

[1] *Handbook for the Physiological Laboratory*, p. 168.

three concentric compartments. Into the central and external of these are placed small lumps of ice, whilst into the intermediate compartment blood is received as it issues from the vessels of the animal. The middle compartment being very narrow (its width not exceeding half an inch) the whole of the liquid, which it contains, is rapidly reduced to the temperature of melting ice. In the course of about two hours the corpuscles have subsided to the lower part of the partition containing the blood, and considerable quantities of pure plasma may be drawn off, with the aid of a syphon or pipette.

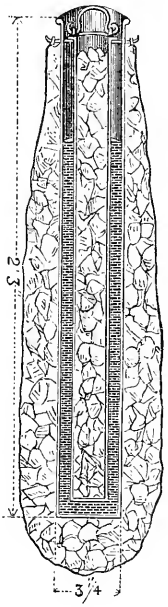


FIG. 9. DR SANDERSON'S APPARATUS FOR COLLECTING LIQUOR SANGUINIS. (*Handbook for the Physiological Laboratory.*)

2. Plasma may be much more easily obtained, though mixed with water and saline matters, by mixing blood, immediately on its being shed, with solutions of certain neutral salts of sodium, potassium or magnesium, or by dissolving suitable quantities of such salts in the blood before coagulation has occurred. From such mixtures of blood and neutral salts the corpuscles separate by subsidence, and the plasma may be obtained by decantation or filtration. The following are the proportions in which sodium sulphate and magnesium sulphate, which are the salts chiefly employed, should be added to blood in order to prevent coagulation and lead to the separation of the liquor sanguinis.

a. One part of finely powdered sodium sulphate is added to 12 parts of blood and the powder is gently stirred with the blood to hasten its solution. Instead of employing the solid salt in the

above proportions (Hewson's method[1]), it is more usual to mix the blood with a saturated solution of the salt; the blood is received directly into a vessel, which contains $\frac{1}{7}$th of its volume of a saturated solution of sodium sulphate[2], and the two liquids are gently mixed.

b. Magnesium sulphate, as has been shewn by Schmidt[3], Semmer[4] and by Hammarsten, is decidedly preferable to sodium sulphate for hindering the coagulation of the blood and for yielding a plasma suitable for experimental researches on the formation of fibrin. According to Semmer four parts of blood are mixed with one part of a solution of magnesium sulphate containing 25 p.c. of the salt. According to Hammarsten[5] the blood is mixed in the same proportion with a *saturated* solution of magnesium sulphate[6].

In addition to the substances, which, when added in suitable proportions, prevent the coagulation of the blood, there are others which merely postpone its occurrence and facilitate the separation of blood corpuscles from the plasma. Thus when frog's blood is mixed with its own volume of a $\frac{1}{2}$ p.c. solution of cane-sugar, the corpuscles may be separated from it by filtration, and there passes through the filter-paper a clear fluid which consists of plasma diluted with solution of sugar, which coagulates after a short interval. This method of separating the blood corpuscles from the plasma was suggested by Johannes Müller[7].

In relation to the action of neutral salts in hindering the coagulation of the plasma it must be remembered that these substances only exert their action when present in certain proportions; if added in too small quantities to blood, coagulation occurs, and if sufficient water be added to blood or plasma which has been kept from coagulating, the process sets in. Thus, as Hewson shewed, if to blood which has been maintained in a fluid state by the addition of solid sodium sulphate in the proportions previously mentioned, there be added twice its volume of water, in a short time the whole will coagulate.

Properties of Liquor Sanguinis.

Plasma, obtained by subjecting blood to a low temperature, is a viscous liquid possessing the same colour as the serum which separates from the blood of the same animal after coagulation; if kept at a temperature below 5° C. it may be filtered from any colourless corpuscles floating in it[8].

[1] Hewson's *Works*, p. 11.

[2] Denis, *Mémoire sur le sang*, 1859, p. 31.

[3] A. Schmidt, *Haematologische Studien*. Dorpat, 1865, p. 44.

[4] Semmer, quoted by Gscheidlen, *Physiologische Methodik*, p. 342.

[5] Hammarsten, "Zur Lehre von der Faserstoffgerinnung," Pflüger's *Archiv*, Vol. XIV. (1877) p. 220.

[6] Many other neutral salts may be employed instead of those previously mentioned, as was shewn by Hewson, Gulliver and Davy. The reader will find much valuable information on this subject in Gulliver's edition of Hewson's *Works*, p. 12, and in Davy's *Researches*, Vol. II., p. 101.

[7] Joh. Müller, "Beobachtungen zur Analyse der Lymphe, des Blutes und des Chylus." Poggendorff's *Annalen*, Vol. XXV. (1832) p. 540.

[8] Alex. Schmidt, Pflüger's *Archiv*, Vol. XI. (1875) p. 318.

The specific gravity of plasma doubtless differs imperceptibly from that of the serum which separates from it, and which in the case of man varies between 1026 and 1029. It is stated by Gautier that the density of human plasma varies between 1027 and 1028, though no authority for the statement is given[1].

The reaction of the plasma is, like that of the blood, and of the serum which separates from it after coagulation, alkaline.

The coagulation of the liquor sanguinis, which may be readily watched by allowing the temperature of the fluid separated from horse's blood at 0° C. to rise slightly, follows exactly the same course as the coagulation of the blood. The process commences on the surface and sides of the liquid and then extends throughout the whole mass, which assumes the appearance of a colourless trembling jelly; the surface of this jelly is from the first seen to be somewhat depressed, and from it there exude droplets of clear serum. After some hours the coagulum is found to have contracted and floats in serum exactly as does a blood clot under similar circumstances; in the case of the coagulation of plasma, however, the coagulum, as it does not entangle blood corpuscles, is colourless and comparatively small.

The serum is found to be more alkaline than the plasma from which it has separated.

The plasma, it has already been remarked, differs from the serum in its containing the body or bodies which, separating from it, form fibrin. It will be convenient therefore to examine first of all the properties of fibrin and then to consider the facts which relate to the assumed precursors of fibrin in the plasma.

Fibrin.

Microscopic observations on the character and arrangement of Fibrin in blood clot.

When a drop of freshly drawn blood is examined under the microscope in the usual way, filaments are often observed to stretch across the preparation; these are usually only seen under tolerably high powers and by careful focusing; the filaments consist of the newly formed fibrin. If a pretty thick stratum of frog's blood be mounted for microscopic examination in the usual way, the edges of the preparation being touched with paraffin to prevent evaporation, after some hours the coloured corpuscles are seen to have arranged themselves into patches, the corpuscles in each patch appearing to radiate from a centre, at which are seen minute granulations. Under a sufficiently high power each individual blood corpuscle is seen to have assumed a pear shape. The appearances alluded to, which have been admirably described by Ranvier[2], are due to the contraction of filaments of fibrin, which have the aforementioned granulations for their centre. The actual arrangement of

[1] Gautier, *Chimie appliquée à la Physiologie*, 1874, Vol. I., p. 489.
[2] Ranvier, *Traité technique d'Histologie*, p. 214 et seq.

fibrin in the clot of human blood can be admirably and easily shewn by following the method also described by Ranvier. A pretty large drop of human blood (obtained by pricking the finger) is treated as was mentioned in the case of frog's blood. After some hours, the paraffin is scraped off, the cover-glass is lifted, and the coagulum of blood which adheres to the slide or cover-glass, or to both, is subjected to the action of a gentle stream of water. Ranvier allows the water to flow out of a pipette, but the Author finds that a very small stream at very low pressure from a water tap is even preferable. After all the red colour has disappeared, a drop of a strong solution of magenta is placed upon the site of the former blood clot; this is then covered with a covering glass and examined. The preparation is then seen to be covered by reticula, each of which appears to radiate from a central granulation. The granulations as well as the fibres are stained by magenta and by solution of iodine, but not by carmine or picrocarmine. These granulations will be further referred to in connection with the part which the formed elements of the blood play in its coagulation.

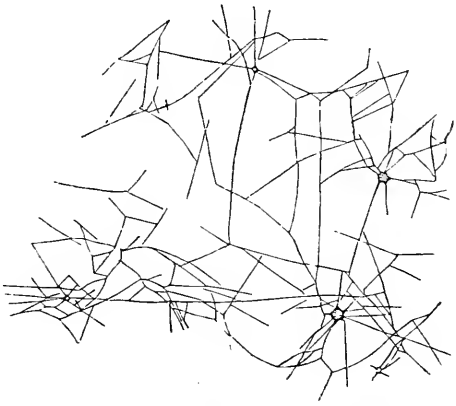


FIG. 10. RETICULUM OF FIBRIN FROM THE BLOOD OF MAN. 500 diam. (Ranvier.)

Mode of separating Fibrin for chemical examination.

Fibrin may be obtained either from blood or from liquor sanguinis, either by allowing these fluids to coagulate at rest, or by stirring them with twigs, or by agitating them with small pieces of metal or glass. The fibrin, obtained by stirring blood, adheres to the instrument employed; it is at first deeply stained with blood, but by washing in a stream of water it gradually loses its red colour and presents the appearance of a white, stringy, elastic body.

When obtained by the first method from plasma, the coagulum at first presents a gelatinous appearance; if, however, the coagulum be placed in a cloth and be kneaded with water, as the serum is squeezed out, there is left fibrin in the form of a white stringy solid.

It is in the latter form that fibrin always separates from blood when it is stirred or shaken with foreign matters. When dried, fibrin presents the appearance of a greyish white solid. In order to purify fibrin it is carefully dried at a temperature not exceeding 110°C., and is then reduced to powder; the powder is successively and repeatedly treated with water holding hydrochloric acid in solution, with alcohol and with ether. However carefully the process of purification may be carried out, fibrin always retains a small quantity of inorganic salts amounting to about 0·9 in 100 parts.

Properties of Fibrin. Fresh fibrin is an elastic substance, as evidenced by the way in which serum is squeezed out of the clot which forms in plasma or blood.

Fibrin belongs to the group of *proteid* or *albuminous* substances, from the majority of which it differs in that once formed it is insoluble in pure water, though it has not been subjected to the action of heat or acids or metallic salts.

Fibrin has the following elementary composition; C, 52·6 : H, 7·0 : N, 17·4 : S, 1·2 : O, 21·8.

Freshly prepared moist fibrin is soluble in a 6 per cent. solution of potassium nitrate, if digested with it for some time at a temperature of 30° or 40°. It is similarly soluble in solutions of sodium chloride, and in a 10 per cent. solution of magnesium sulphate. The solutions of fibrin in the neutral salts are coagulated by heating to 60° or 65°, by the addition of acids and of alcohol, and by the addition of powdered magnesium sulphate.

Denis asserted that fibrin obtained from arterial blood is not soluble in 10 per cent. solutions of the neutral salts, whilst that obtained by stirring venous blood is soluble in the same solutions.

When placed in water containing about 5 parts of hydrochloric acid in 1000, moist fibrin swells into a transparent jelly, which does not dissolve. In water containing 1 part of hydrochloric acid per 1000, fibrin dissolves in a few hours, at a temperature of 40° C. The fibrin is in this process converted into so-called acid-albumin or syntonin. Solutions of syntonin are not precipitated when they are boiled; when they are carefully neutralized, the proteid which had been dissolved is thrown down in the form of gelatinous flakes which are insoluble in water, but are readily soluble in dilute solutions of acids, of alkalies and alkaline carbonates. Acetic and phosphoric acids exert a similar action to hydrochloric acid. From the acetic solution of fibrin, potassium ferrocyanide throws down a white precipitate.

When digested at the temperature of the animal body in dilute solutions of ammonia, or of potassium or sodium hydrate, fibrin dissolves, and the solutions are not coagulated by heat, but are precipitated by mercuric chloride, lead acetate, and copper sulphate.

Fibrin possesses the power of decomposing solutions of hydric peroxide, H_2O_2, which enter into effervescence, owing to the liberation of oxygen; if it be first immersed in a tincture of guaiacum and afterwards in a solution of hydric peroxide or in a mixture of

the two reagents, it assumes an intensely blue colour. This is due to the oxidation of the resin of guaiacum by the oxygen which the fibrin has liberated from the peroxide.

Quantity of fibrin in the blood. Human venous blood in health yields from 2·2 to 2·8 parts of fibrin per 1000, and it is said that arterial yields somewhat more than venous blood.

The Assumed Precursors of Fibrin in the Liquor Sanguinis.

1. *Serum-Globulin or Paraglobulin.* (Schmidt's *fibrinoplastic substance.*)

Schmidt's methods of obtaining Paraglobulin. When plasma is diluted with ten or fifteen times its volume of ice-cold water and subjected to the action of a stream of carbon dioxide[1], or when it is carefully neutralized with acetic acid, the liquid soon becomes turbid, and deposits after some time a proteid substance to which the above terms have been applied, of which the first indicates its resemblance to a proteid contained in the crystalline lens to which the name of globulin was long ago ascribed; and the second the property which has been ascribed to it of inducing, under certain circumstances, the separation of fibrin from solutions containing fibrinogen.

The quantity of dilute acetic acid (25 per cent.) to be added is 4 drops for every 10 c.c. of serum diluted with 150 c.c. of H_2O.

As the body which is precipitated under these circumstances is not only contained in the plasma but also exists in the serum, the latter much more readily available fluid may be employed for its preparation.

The same substance it is[2], which is precipitated when blood serum is subjected to dialysis (see p. 6), a process which may be employed for the quantitative estimation of paraglobulin. With this object a known weight or volume of serum is dialysed for 24—36 hours; at the end of this time the contents of the dialyser have become turbid, and they are subjected to a current of CO_2; the precipitate produced is collected on a filter, washed with water and alcohol and dried. Following this method Schmidt found that 100 c.c. of the serum of ox's blood yielded on an average 0·887 grammes of dry paraglobulin.

Hammarsten's method of separating Serum-globulin. It has however been shewn by Hammarsten[3] that neither by acetic acid, nor by dialysis and carbonic acid, is paraglobulin fully precipitated: indeed these reagents only throw down a small fraction of the paraglobulin contained in the serum or plasma. Having discovered

[1] A. Schmidt, "Weiteres über den Faserstoff und die Ursachen seiner Gerinnung. 1. Die fibrinoplastische Substanz." *Archiv f. Anatomie u. Phys.*, 1862, p. 429 et seq.

[2] A. Schmidt, "Untersuchung des Eiereiweisses und des Blutserum durch Dialyse." *Beiträge zur Anatomie und Physiologie, als Festgabe Carl Ludwig gewidmet.* Leipzig, 1875. Part I., p. 101.

[3] Hammarsten, "Ueber das Paraglobulin," Erster Abschnitt. Pflüger's *Archiv*, Vol. xvii. (1878) p. 447 et seq.

that magnesium sulphate, added to complete saturation, precipitates every trace of paraglobulin present in a solution, whilst it has no action on serum-albumin, Hammarsten has by its aid determined how much paraglobulin the blood serum contains. His determinations would appear to leave no doubt that paraglobulin is in many cases the chief proteid of the serum, as can be seen by studying the accompanying table :—

Variety of Serum.	Total Solids in 100 pts.	Total Proteids in 100 pts.	Serum-globulin in 100 pts.	Serum-albumin in 100 pts.	Lecithin, fat, salts, &c. in 100 pts.	$\frac{\text{Serum-globulin.}}{\text{Serum-albumin.}}$
From blood of horse	8·597	7·257	4·565	2·677	1·340	$\frac{1}{0·591}$
„ „ „ ox	8·965	7·499	4·169	3·329	1·466	$\frac{1}{0·842}$
„ „ „ man	9·207	7·619	3·103	4·516	1·587	$\frac{1}{1·511}$
„ „ „ rabbit	7·525	6·225	1·788	4·436	1·299	$\frac{1}{2·5}$

Properties of Serum-globulin.

Serum-globulin precipitated by any of the methods described is found to be soluble in water holding CO_2 in solution, in water holding oxygen in solution, in very weak aqueous solutions of the alkalies, in lime water, in weak solutions of neutral alkaline salts, in solution of sodium phosphate and of the carbonates of the alkalies.

When considerable quantities of serum-globulin are dissolved in very weak solutions of the alkalies, perfectly neutral solutions are obtained which are not coagulated by heat, but which are so when very cautiously treated with acetic, hydrochloric, nitric, or sulphuric acids, the precipitate being readily dissolved by an excess of the reagent; such weak alkaline solutions are precipitated by the addition of a large quantity of alcohol.

Serum-globulin is in great part, though by no means completely, precipitated when sodium chloride is dissolved to saturation in its solution; the precipitated serum-globulin is found to be soluble in weak solutions of sodium chloride.

It was stated by A. Schmidt that paraglobulin is completely precipitated by the addition of powdered NaCl to its solutions, but Eichwald and Hammarsten, and especially the latter, have shewn conclusively that Schmidt was in error. On the other hand, the body to be next described, viz. Fibrinogen, is completely precipitated when treated in the same manner by NaCl.

According to Hammarsten[1], if a very small quantity of common

[1] Hammarsten, "Ueber das Paraglobulin," Zweiter Abschnitt. Pflüger's *Archiv*, Vol. XVIII. (1878) p. 39 et seq.

salt (from 0·03 to 0·5 or 0·7 p. c.) be added to a very feebly alkaline solution of paraglobulin this body is precipitated, but on a further addition of salt the precipitate re-dissolves, only to be again precipitated when the amount of sodium chloride exceeds about 20 p. c.

Solutions of paraglobulin (as for example in NaCl) coagulate at temperatures varying between 68° and 80° C., most commonly at 75° C., the variations being due to the amount of sodium chloride present, to the duration of the process of heating, and perhaps to other circumstances. (Weyl[1], Hammarsten[2], Frederique[3].)

Moderately concentrated solutions of paraglobulin are not precipitated by the addition to them of 16—20 p. c. of NaCl (Hammarsten[4]).

Serum-globulin is said to diffuse with considerable ease through animal membranes. On the other hand, it is absolutely unable to pass through parchment paper.

The term *paraglobulin* sufficiently indicates that this body belongs to that group of proteids of which the first well-known member was the proteid constituent of the crystalline lens to which the name of Globulin was given.

Because of its assumed co-operation in the formation of fibrin, the term *fibrinoplastic substance* was ascribed to it by A. Schmidt, but, as will be shewn in the section on coagulation, there are no longer grounds for ascribing this function to pure paraglobulin.

Paraglobulin is not only found in the plasma and in the serum, but it is a constituent of the colourless and coloured (?) blood-corpuscles, of the lymph, chyle, &c.

According to A. Schmidt's more recent views, the paraglobulin of the serum is derived from the colourless corpuscles of the liquor sanguinis, which in breaking down liberate this constituent, and the body known as the fibrin-ferment. Hammarsten, whilst not denying that a portion of the paraglobulin of serum may be derived from the colourless corpuscles, does not believe that it all takes its origin in this manner, for he has found the plasma to contain large quantities of paraglobulin; he is moreover inclined to think that some portion of the globulin found in serum may be derived from the decomposition of fibrinogen. The Swedish observer has found that when a solution of pure fibrinogen coagulates, besides fibrin, there is formed a soluble proteid which belongs to the group of globulins, and which therefore, if present in the serum, would be reckoned as paraglobulin.

The view has been held by Brücke and Heynsius, that para-

[1] Weyl, "Beiträge zur Kenntniss thierischer und pflanzlicher Eiweisskörper," Pflüger's *Archiv*, Vol. XII. p. 635—638.

[2] Hammarsten, *loc. cit.*, p. 64.

[3] Frederique, *Recherches sur la constitution du Plasma Sanguin.* Gand, 1878.

[4] Hammarsten, "Zur Lehre von der Faserstoffgerinnung," Pflüger's *Archiv*, Vol XIV. (1877) p. 224.

globulin is an alkaline albuminate[1]. According to Hammarsten paraglobulin would be a proteid having the characters of a weak acid[2].

2. *Fibrinogen.*

Schmidt's method of obtaining Fibrinogen.

When plasma which has been diluted with ten or fifteen times its volume of ice-cold water, and has been freed from paraglobulin by the action of a long-continued stream of CO_2, is still further diluted, and again subjected to CO_2, there separates a second precipitate which is found to consist of a body very closely resembling paraglobulin, but yet possessing certain marked distinctions. This body is denominated *fibrinogen*, a term which sufficiently indicates that it is presumed to be one, at least, of the precursors of fibrin.

Unlike paraglobulin, fibrinogen does not exist in the serum which separates from blood clot, but it is present in the liquid found in many serous cavities, as in the pericardium, the peritoneum, the pleurae; also in the liquid of hydrocele.

From all these liquids fibrinogen may be separated by the method previously referred to, viz. by dilution with water, and the subsequent action of CO_2—or instead of passing CO_2, the liquids may be cautiously neutralized with acetic acid. Fibrinogen may also be precipitated from liquids which hold it in solution by adding common salt.

Like paraglobulin, fibrinogen is insoluble in pure water, but soluble in water which holds oxygen in solution; it is soluble in weak solutions of the alkalies, and in solutions of many neutral salts, as in weak solutions of sodium chloride.

Hammarsten's method of separating Fibrinogen.

The behaviour of fibrinogen to solutions of common salt has been studied with care by Eichwald and Hammarsten[3], and is so important as to deserve careful consideration, for upon it is based a method of separating this substance from paraglobulin, and obtaining it in a pure condition from the fluids which contain it.

Both fibrinogen and paraglobulin are soluble in solutions of sodium chloride which contain 5—8 per cent. of the salt. When however the quantity of salt attains 12—16 p.c., fibrinogen is precipitated whilst paraglobulin remains in solution; the quantity of salt must amount to more than 20 p.c. before any appreciable quantity of paraglobulin is thrown down.

In order to obtain pure fibrinogen Hammarsten proceeds as follows:—

The blood of the horse is mixed on its issue from the blood-

[1] Heynsius, "Ueber die Eiweissverbindungen des Blutserums und des Hühnereiweisses," Pflüger's *Archiv*, Vol. IX. 514—552.

[2] Hammarsten, "Ueber das Paraglobulin," Erster Abschnitt. Pflüger's *Archiv*, Vol. XVII. (1878) p. 466.

[3] Hammarsten, "Untersuchungen über die Faserstoffgerinnung. § 5, Ueber eine neue Methode zur Reindarstellung des Fibrinogens aus dem Blutplasma". *Nova Acta Regiae Societatis Scientiarum Upsalensis.* Ser. III., Vol. X. 1, p. 31, Separatabdruck.

vessels with one-third of its volume of a saturated solution of magnesium sulphate. The mixture is then subjected to filtration in order to obtain salted plasma free from corpuscles. As filtration is, however, often very difficult from clogging of the filter, and at all times very slow, I have, in repeating Hammarsten's experiments, subjected the mixture of blood and magnesium sulphate to the action of the centrifugal machine (see p. 58); in this way, in about half an hour, perfectly clear salted plasma may be obtained in considerable quantities.

To the salted plasma there is now added an equal volume of a saturated solution of common salt; the fluid instantly becomes turbid, and in two or three minutes an abundant flaky precipitate forms.

From this point the process may be conveniently modified as follows:—The liquid with the suspended precipitate is carefully stirred, whereby the precipitate usually floats to the surface and forms a thick dense layer on the top of the liquid, which is then syphoned off. The precipitate is now well mixed with a solution made by diluting saturated solution of common salt with an equal volume of water, the quantity of the half saturated solution of salt being equal to that of $MgSO_4$ plasma which was employed in the process. The precipitate floats up to the surface, the NaCl solution is syphoned off, and a fresh quantity of the same added; the process of washing and syphoning being repeated not less often than six times. The fibrinogen is then collected on a separate funnel, pressed between folds of filtering paper, suspended in water and the solution filtered. The whole process can be completed in from $2\frac{1}{2}$—3 hours[1].

This is separated by filtration, and may be washed with saturated solutions of NaCl. The precipitate is freed from much adhering moisture by pressing between folds of blotting paper, and is then mixed with a solution of common salt containing 6—8 p.c. of the salt, and in this it soon dissolves. The solution is filtered, and to it is added an equal volume of saturated solution of NaCl, which again throws down fibrinogen, but in a purer condition than at first, in the form of gelatinous flakes. The precipitate may be again dissolved in the weak solution of sodium chloride and precipitated a third time. It may then be assumed to be pure; it is at least free from the minutest traces of paraglobulin and of serum-albumin. In consequence of the common salt which adheres to it, the precipitate is found to be soluble in pure water. A solution of fibrinogen thus obtained is found not to be spontaneously coagulable, but to yield fibrin when mixed with serum or other solutions possessing the peculiar ferment action to be subsequently referred to when speaking of *Theories of coagulation*.

Solutions of fibrinogen containing 1—5 p.c. of NaCl coagulate at 52° C.—55° C. (Hammarsten, Frederique).

Solutions of fibrinogen coagulate at 56° C. according to Frederique,

[1] Hammarsten, "Ueber das Fibrinogen." Pflüger's *Archiv*, Vol. XIX. (1879) p. 563, et seq.

with whose observations agree those made on the same subject by Weyl and Hammarsten. Frederique has shewn that if an excised jugular vein of the horse, tied so as to confine blood within it, be heated to 56° C., a proteid matter separates, and the plasma is thereafter found to be uncoagulable. No better proof than this could be given to shew that fibrinogen is really contained *as such* within the living blood. Frederique has made use of the low temperature at which fibrinogen coagulates to separate this body from paraglobulin and determine its amount. He thus determined 100 grammes of the plasma of the horse (in one experiment) to contain 0·4299 of fibrinogen and to yield 0·375 grms. of fibrin.

Theories of Coagulation.

The views of the Ancients.

The first step in the accurate study of the nature of the coagulation of the blood was made when it was positively determined that coagulation is due to the separation of a solid constituent from the liquor sanguinis, and this fact was assuredly first determined with certainty by Hewson. It is true that, as previously mentioned, Borelli had expressed himself with correctness in the same sense; still he did not adduce evidence which can be considered to furnish full proof of his position[1].

Even before the discovery of this fact the cause of the coagulation had been sought for, and various views had been expressed, none of which, as even Hewson shewed, were at all capable of accounting for the phenomenon. Thus it had been assumed by some that the blood is maintained in a liquid condition in the living body by the continual movement to which it is subjected (Borelli[2], Lower[3]); by others, that coagulation was due to the action of air upon the blood; by a third set, that coagulation was due to the cooling of the blood on its withdrawal from the vessels; by a fourth, that the coagulation of the blood was an act of life and connected with the vitality of the blood (Hunter). The first of these views is contradicted by the

[1] See Borelli, *De Motu Animalium.* Opus posth., pars altera, 4to. Romae, 1681. Under the heading "Analysis sanguinis in suas partes integrales, et forma compositionis ejus inquiritur" (Prop. cxxxii.) Borelli says, "Deinde sicut in lacte adest succus concrescibilis in caseum, sic in sanguine reperitur succus viscosus, et glutinosus, qui postmodum facta concretione, abit in fibras, vel membranas reticulares; quodque tales fibrae sic condensatae non praeextiterint intra vasa animalis viventis, facile suadetur ex eo, quod tales fibrae, et membranae albae sanguineae microscopio inspectae, crassiores sunt vasis sanguineis capillaribus, et ideo neque excipi, neque effluere in iis possint, cum saltem longitudine filamentorum, et latitudine membranarum, vias illas angustissimas obstruerent. Ideo fatendum est, gluten album sanguineum lubricam et fluidam consistentiam retinere dum in animali viventi movetur."

[2] Borelli, *op. cit.*, Vol. II. p. 266.

[3] Lower. The only passage in Lower's works which appears to the Author to indicate that he entertained this opinion (which has been attributed to him) is the following: "In cordis systole, qua liquor sanguinis conquassatur usque et ad ventriculi latera et vasorum parietes alliditur, paululum diutius elanguescat: succus ejus nutritius in partes secedere, grumescere, et gelatinae in modum incrassari, tandemque intra fibras cordis hinc inde pendentes implicari, et ipsis ventriculorum parietibus accrescere, et a cordis aestu indurari incipit, &c." Lower, *De Motu Cordis.*

fact that the blood retains its fluidity within the healthy and yet living blood-vessels even though the circulation have ceased; the second is disproved by the fact that blood retained in vessels which contain no air and are shut off from air, coagulates with readiness; the third is summarily and conclusively disproved by the facts that whilst a low temperature hinders coagulation instead of hastening it, a temperature such as that of the body of warm-blooded animals is specially favourable to its occurrence. The fourth view is set aside by the fact that the coagulation of the blood can be postponed almost indefinitely by exposure to a sufficiently low temperature or by the addition to it of certain salts, and that after long periods have passed, the experimenter may, by altering the conditions, induce the previously inhibited coagulation, as for instance by suitably diluting blood of which the coagulation has been prevented by the addition of large quantities of neutral salts.

If coagulation were a vital act, the results of the above experiments would, as Gulliver remarked[1], be equivalent to a demonstration that we can pickle the life of the blood, that it is preserved after repeated freezing and thawing, and that the blood may remain alive many hours after the death of the body, when the muscular fibre has lost its irritability, the limbs have stiffened, and even partial decomposition has begun.

In considering the progress of research and the succession of doctrines relating to coagulation, it is well to remember that the following facts amongst many others were demonstrated by Hewson, and were published by him in the year 1772: Firstly, that the coagulation of the blood is due to the coagulation of the liquor sanguinis, a fact which he proved (*a*) by skimming off the liquor sanguinis of the slowly coagulating blood of inflammatory diseases after the corpuscles had subsided, and determining that it coagulated, (*b*) by ligaturing a vein so as to include fluid blood within it, and opening it after the corpuscles had subsided, and drawing off the clear liquor sanguinis, which then coagulated. Secondly, that the coagulation of the blood drawn from the body cannot be explained as due to loss of heat, to arrest of motion, or exposure to air. Thirdly, that coagulation may be restrained by cold and by the addition of neutral salts to blood, the process setting in when the conditions are modified. Fourthly, that the walls of the living blood-vessels exert a remarkable influence in restraining coagulation.

Discoveries of Buchanan.

The serous sacs of the body, even in health, contain small quantities of liquid which at first sight appears closely to resemble the serum of blood, but which is similar to that found in the lymphatic vessels, viz. *lymph*. Of such serous sacs the pericardium is the one which invariably contains after death more or less liquid, which has received the name of *liquor pericardii*. In disease, the fluid contents of the serous sacs may however increase

[1] Hewson's *Works*, note 12, p. 21.

very materially, and sacs which normally contain no appreciable quantity of liquid may contain large amounts; this is, for instance, true of the *tunica vaginalis testis*, the serous sac which envelopes the testis, which is liable to become distended with liquid, the condition being denominated *hydrocele*.

The liquor pericardii of man after it has remained for some hours after death in the pericardium, and the liquid of hydrocele, if removed without any admixture of blood, do not coagulate spontaneously, and they differ in that respect from the liquor sanguinis[1].

It was however shewn by Dr Andrew Buchanan of Glasgow in 1831[2], that on adding to ascitic fluid, to serum from the chest, and to hydrocele fluid the liquid obtained by pressing a blood clot in linen cloth, there was produced a coagulum similar to that which separates spontaneously from blood.

At first Dr Buchanan believed that the blood-colouring matter was the agent present in the squeezed clot, which conferred upon these transudations the property of coagulating. On mixing, however, some peritoneal fluid with the serum of blood, a coagulum was obtained. On subsequently mixing perfectly clear blood-serum with peritoneal fluid and with the fluid of hydrocele, removed after death from the body of the same man, a beautiful pellucid and pretty firm coagulum was obtained. Dr Buchanan remarked, "I repeated the experiment very frequently with serum obtained from the serous cavities of the testis, from the peritoneum, from the cavities of the pleura, and from the pericardium. The result has generally been as I have just described, but not always so."

These observations of Dr Buchanan on the coagulation of the fluids of serous cavities with other most interesting facts and generalizations were published in 1845[3].

"The opinions commonly entertained by physiologists and chemists to which allusion has just been made, are that fibrin has a spontaneous tendency to coagulate; that this spontaneous coagulability is a characteristic property of fibrin, by which it is distinguished from albumin and casein; and that the coagulation of the blood and of various animal fluids depends on the spontaneous coagulation of the fibrin which they contain. My experiments, on the other hand, shew that fibrin has not the least tendency to deposit itself spontaneously in the form of a coagulum: that, like albumin and casein, fibrin often coagulates under the influence of suitable

[1] The liquor pericardii of the dog and of the horse does not coagulate spontaneously; that of the rabbit coagulates, however, with readiness.

[2] "Contributions to the Physiology and Pathology of the Animal Fluids, containing Experiments and Observations on the effects of certain substances upon the blood; on the coagulation of the blood; on the difference between membranous and sanguineous serum; on the formation of the buffy or inflammatory crust; on the formation of pus; and on the process of sanguification, by Andrew Buchanan, M.D., Junior, Surgeon to the Glasgow Infirmary." *London Medical Gazette*, vol. XVIII. (2nd vol. for session 1835—36), p. 50.

[3] "On the Coagulation of the Blood and other fibriniferous liquids," *London Medical Gazette*, 1845, Vol. I. (New Series) p. 617. (Communicated to the Glasgow Philosophical Society, Feb. 19, 1845.) Reprinted in the *Journal of Physiology*, 1879.

reagents: and that the blood and most other liquids of the body which appear to coagulate spontaneously, only do so in consequence of their containing at once fibrin and substances capable of reacting upon it and so occasioning coagulation."

Dr Buchanan then announced that he had found that the addition of that which he designated washed blood clot was most efficient in inducing the coagulation of such liquids as do not coagulate spontaneously, but do so on the addition of blood. The 'washed blood clot' he obtained by mixing one part of liquid blood with from six to ten parts of water, and stirring carefully for five minutes. After the mixture had stood for twelve or twenty-four hours, it was filtered through a coarse linen cloth, and the substance left in the cloth washed with water.

When a small portion of this washed clot was reduced to fragments and diffused through the liquid of hydrocele, coagulation ensued, in many cases as rapidly as in the blood itself. The washed coagulum retains, according to Buchanan, its coagulating power for a long period, and with the addition of a little spirit of wine may be kept for many months with its activity unimpaired.

"The power," Buchanan remarked, "which the washed clot has of coagulating fibrin, is not less remarkable than that of rennet in coagulating milk, to which indeed it may be aptly compared."

The 'washed clot' of Buchanan is a mechanical mixture of fibrin with colourless corpuscles. Upon which of these constituents did its coagulant power depend? Buchanan concluded, from many considerations, that this was seated in the colourless corpuscles. He found that the buffy-coat of the blood of the horse, which is exceedingly rich in colourless corpuscles, possessed a much greater power of inducing coagulation, and preserved that power after being kept for months and pulverized (from which statement we must conclude that the substance was dried). Moreover that the upper layers of red clot which are comparatively rich in colourless corpuscles have a stronger coagulating power than the lower layers. Furthermore Buchanan found that many tissues of the body, muscle, connective tissue and central nerve-organs possess, though in a much less degree, the coagulant power, and he leant to the opinion that their influence is seated in their cellular elements ('primary cells or vesicles').

To recapitulate:—Buchanan held that the coagulation of the blood is due to the conversion of a soluble constituent of the liquor sanguinis into fibrin by an action exerted probably by the colourless corpuscles and comparable to the action which rennet exerts in effecting the coagulation of milk. Furthermore, that the liquid which accumulates in certain serous sacs may be made to yield a coagulum of fibrin when subjected to the action of liquids or solids rich in the cellular elements with which the coagulant action appeared to be associated.

Although not altogether forgotten by a few individuals in England,

these most interesting results of Professor Buchanan have not formed part of the common stock of scientific knowledge, and are generally known only as re-discovered and greatly added to by Professor Alexander Schmidt of Dorpat.

Denis' 'Plasmine' and its relation to Fibrin.

Although Buchanan believed in the existence of fibrin in solution in the liquor sanguinis he had no idea of separating the dissolved substance. Denis in 1859 announced[1] the separation from the plasma of a proteid body to which he gave the name of Plasmine and which yields fibrin as a product of decomposition.

Denis commences by mixing uncoagulated blood with one-seventh its volume of a saturated solution of sodium sulphate. After the corpuscles have subsided, the supernatant mixture of liquor sanguinis and solution of sodium sulphate is decanted and sodium chloride is added little by little as long as it is dissolved. The solution becomes turbid and soon acquires a creamy consistence, from the separation of a bulky flocculent precipitate. The fluid is thrown upon a filter and washed with a saturated solution of sodium chloride. The matter which remains undissolved is the *plasmine* of Denis. Of this plasmine Denis obtained 14·59 grammes from 1000 grammes of human blood.

If plasmine, thus precipitated through the agency of sodium chloride, be placed in water, the solution, in the course of a few minutes, undergoes spontaneous coagulation; the coagulum consists of fibrin similar to that obtained directly from blood, and the amount yielded by the plasmine also corresponds with that which would have been obtained directly from blood. In addition, however, to the insoluble fibrin which separates, there is found to be present in the solution a proteid substance to which Denis gives the name of 'fibrine soluble' to distinguish it from the first 'fibrine concrète' or 'fibrine ordinaire.'

Denis therefore believed that the precursor of fibrin in the blood is a complex body, *plasmine,* which at the moment of coagulation splits up into two proteids, of which the one separates in the form of the insoluble fibrin and the other dissolves in the serum. These views of Denis will be again referred to when speaking of the more recent investigations of Hammarsten.

The discoveries and hypotheses of A. Schmidt.

The fundamental fact discovered by A. Schmidt was the very same which it has been shewn was clearly described long before him by Dr Andrew Buchanan, viz. that there occur animal fluids from which fibrin does not separate spontaneously but only after the addition of blood or of blood-serum, or certain of their constituents[2].

[1] Denis, *Mémoire sur le sang,* 1859, p. 32.

[2] A. Schmidt, "Ueber den Faserstoff und die Ursachen seiner Gerinnung." *Archiv f. Anat. u. Physiolog.,* 1861, p. 545.

Schmidt however soon proceeded a step further[1]. He studied the effect of dilution upon, and the passage of carbon dioxide through, liquor sanguinis and serum, and shewed how to obtain in this way, though certainly not in a state of purity, the bodies which have been described as paraglobulin and fibrinogen. He discovered that when these bodies in a separate condition exist in solution and the solutions are mixed, *if circumstances be favourable,* coagulation occurs sooner or later.

These facts he explained by supposing that the formation of fibrin is due to the inter-action of the two closely allied proteids, of which the one, *fibrinogen,* is often present without the other, *paraglobulin;* and to designate the property which the latter possesses of leading to the formation of fibrin from fibrinogen, Schmidt applied to it the name of the *fibrinoplastic substance.*

Schmidt at first supposed that the plasma contained both fibrin-generators in solution, there being, however, an excess of the fibrino-plastic substance. When blood or plasma coagulates, he supposed the whole of the fibrinogen to be used up, whilst the paraglobulin over and above the quantity which had taken part in the formation of fibrin, remained in solution in the serum, whence it could be separated by dilution and neutralizing either with CO_2 or acetic acid. Fluids which, like hydrocele, do not coagulate spontaneously, but only after the addition of paraglobulin, he supposed to be wanting in this body, which he regarded as one of the two essential fibrin-generators.

There are many ways of repeating Schmidt's observations on the coagulating influence of paraglobulin on fibrinogen. One of the most convenient is the following: the serum of blood is diluted, precipitated by dilute acetic acid (10 c.c. of serum being diluted with 150 c.c. of water and treated with four drops of 25 p. c. acetic acid). The precipitate is washed with water. Fibrinogen is then precipitated (in an impure condition) by saturating any fluid which contains it, *e.g.* hydrocele fluid, with sodium chloride. The precipitate is collected on a filter, and after the filtrate has passed through, the filter is filled up with water, which dissolves the precipitated fibrinogen, in virtue of the sodium chloride adhering to it. To this solution of impure fibrinogen the previously precipitated paraglobulin is added, when coagulation *sometimes* occurs.

Amongst the facts which were adduced by Schmidt and which appeared to give great support to his views was this one: that if from diluted plasma, the paraglobulin is precipitated by dilution of water and passage of a stream of carbon dioxide, the power of spontaneous coagulability is unquestionably destroyed, whilst it may be *occasionally* restored by the restoration of the removed paraglobulin to the fibrinogenous liquid.

It is to be remarked that Schmidt never committed himself to a

[1] A. Schmidt, "Weiteres über den Faserstoff und die Ursachen seiner Gerinnung." *Archiv f. Anat. u. Phys.*, 1862, pp. 428—469 and 533—564.

statement of the way in which the two bodies which he believed to be fibrin-generators, associated themselves in the formation of fibrin. He however believed that he had proved the actual co-operation of paraglobulin in the formation of fibrin by shewing that the amount of fibrin which separates from a solution containing paraglobulin is to a certain extent influenced by the amount of paraglobulin added to that fluid.

The Fibrin-ferment.

Such were the principal facts published by A. Schmidt anterior to 1872, and the views which he based upon them. It will be seen how widely these views differed from those of Buchanan and of Denis, each of whom was acquainted with many of the most important facts independently discovered by the Dorpat professor. But in their turn the views of Schmidt soon received from their author most important modifications.

Schmidt's theory of coagulation postulated that when a fluid containing fibrinogen did not coagulate spontaneously, this was due to an absence of the fibrinoplastic substance. But he discovered that the two fibrin-generators may be present in the same fluid and yet coagulation not occur. Hydrocele fluid is for instance by no means free from paraglobulin and may sometimes contain considerable quantities of that body, without coagulating spontaneously, though the addition of blood or of blood serum will lead to its coagulation. Does blood or blood serum then contain some constituent other than paraglobulin which exerts a fibrinoplastic action?

It appears so, and this body Schmidt believes to be of the nature of a ferment which is liberated after the blood is removed from the blood-vessels, and which in an impure condition he prepares as follows[1]:

Schmidt's method of preparing solution of Fibrin-ferment.

Blood or, still better, serum separated from the clot of coagulated blood, is treated with twenty times its volume of alcohol and the mixture set aside in a stoppered bottle for at least a fortnight, but preferably for a period of three months. The alcohol coagulates the proteid matters of the plasma and corpuscles as well as the haemoglobin contained in the latter, and by the prolonged action of alcohol these various matters are *for the most part* rendered insoluble in water. The insoluble matter is then collected on a filter and dried over sulphuric acid, and, when dry, finely pulverized. The powder is treated with water; the aqueous solution is found to contain the so-called fibrin-ferment.

Such a solution when added to a liquid which contains fibrinogen and paraglobulin but which does not coagulate spontaneously, often rapidly gives rise to a coagulum. The amount of fibrin which separates is, according to Schmidt, in no respect influenced by the

[1] A. Schmidt, "Neue Untersuchungen über die Faserstoffgerinnung." Pflüger's *Archiv*, Vol. VI. (1872) p. 445.

amount of the ferment, but the rapidity of coagulation is so influenced. The influence of solutions of the fibrin-ferment may be well seen by adding it to dilute solutions of salted plasma. It has been said that blood or plasma which has been prevented from coagulating by the addition of a neutral salt, such as sodium or magnesium sulphate, will coagulate if a sufficient quantity of water be added. The coagulation is, however, under the circumstances not an immediate one. But if to a slowly coagulating mixture of plasma, water, and neutral salt, there be added some of Schmidt's solution of fibrin-ferment the process may be remarkably hastened.

The Author's Method of preparing a solution of Fibrin-ferment[1].

In narrating the discoveries of Dr Andrew Buchanan attention was called to the action of the so-called '*washed blood clot*' of that author, in bringing about the coagulation of certain fluids; washed blood clot being really fibrin obtained by washing the coagulum which separates from blood when, at the time of being shed, that fluid is mixed with about 10 times its volume of water. As Buchanan pointed out, such fibrin possesses remarkable coagulant power, and, if preserved in weak spirit, will retain that power for many months.

By digesting Buchanan's washed blood clot in an 8 p.c. solution of common salt, a solution is obtained which possesses in a very intense degree the properties of Schmidt's solution of fibrin-ferment. This solution contains a proteid in solution which possesses all the reactions of a globulin; it is rendered inactive by exposure to temperature of 56°—58° C., and when it is saturated with powdered magnesium sulphate.

The origin of the Fibrin-ferment.

After the discovery of the so-called fibrin-ferment, Schmidt's views might be stated as follows, though not in his words:—*In cases where a fluid coagulates spontaneously with the formation of fibrin there must be present the two fibrin-generators and a yet unknown body, the fibrin-ferment, whose presence is, however, essential in order that the two bodies shall associate themselves.*

Where again a liquid does not coagulate spontaneously but does so on the addition of blood or of serum, the absence of coagulation may be due to the absence of ferment, the two fibrin-factors being present; or it may be, and sometimes is, due to the absence of paraglobulin. In the first case coagulation will be induced by the addition of fibrin-ferment alone, in the latter not until the previous addition of paraglobulin. The interaction of the fibrin-factors necessitates, however, the presence of certain quantities of salts, and especially of sodium chloride.

According to Schmidt, then, the formation of fibrin is due to the interaction of two bodies under the influence of a ferment.

[1] A. Gamgee, "Some old and new experiments on the Fibrin-ferment." *Journal of Physiology*, 1879. No. II.

But whence comes the ferment? Schmidt received the blood as it flowed from the blood-vessels of a living animal directly into absolute alcohol and then subjected the product to the process followed in the separation of the fibrin-ferment, and found that the solution obtained under these circumstances was free from any ferment action, and he therefore concluded that the ferment is generated in the blood after it is withdrawn from the blood-vessels. But how generated? Many facts conspired to connect the formation of ferment with the colourless corpuscles of the blood.

Schmidt found that liquids coagulate more or less rapidly, very much according as they contain many or few colourless corpuscles; he found that horse plasma, diluted with ice-cold water and filtered from all corpuscles, coagulates not only much more slowly but also much more feebly than the same plasma unfiltered; that in cooled horse plasma from which the corpuscles have subsided, the upper layers, most free from corpuscles, coagulate more imperfectly, yielding actually less fibrin than the lower, richer in corpuscles, and that such plasma free from corpuscles, when subjected to the process for separating fibrin-ferment, yields a solution comparatively inactive, when compared with a solution prepared from plasma rich in corpuscles. Moreover Schmidt found that by adding paraglobulin to the above plasma the yield of fibrin was increased.

Furthermore Schmidt thinks he has proved that in the short interval which, at ordinary temperatures, intervenes between the shedding and coagulation of the blood there is a rapid breaking down of colourless cells and of cells which appear in some way intermediate between the colourless and coloured cells, which are nucleated like colourless cells, but whose protoplasm is tinged with hæmoglobin. He therefore has come to the conclusion that the coagulation of the blood is due to the union of fibrinogen, which exists preformed in the plasma, with paraglobulin derived from the colourless corpuscles—a union which takes place under the influence of a ferment-like body which also arises in the same cells, and which like paraglobulin is derived from them in the short interval which elapses before coagulation.

In their latest developments the views of Schmidt approach much more closely to those of the man whose facts and theories have both been buried in oblivion, Dr Buchanan. Both observers look upon coagulation as due to a ferment-like action, exerted upon a constituent of the plasma, which is, in the living body, dissolved in that fluid; both connect that ferment action with the colourless cells of the blood, and Schmidt adds definiteness to the older views of Buchanan by connecting the ferment action with the actual breaking down of those bodies.

The chief point of divergence—the one element in Schmidt's theory which had no place in Buchanan's—relates to the accessory body *paraglobulin*, whose existence he did not even surmise, much less consider to be essential to the formation of fibrin. But is it essential?

The Researches of Hammarsten[1].

In describing paraglobulin and fibrinogen it has been stated that the researches of Eichwald and Hammarsten, and especially of the latter, have shewn that the behaviour of the two bodies which, according to Schmidt, are the fibrin-factors, in respect to sodium chloride is exceedingly diverse. Both bodies are precipitated from their solutions when these are saturated with sodium chloride, though fibrinogen alone is completely precipitated. Fibrinogen is precipitated from its solutions when these contain 13 p. c. of sodium chloride or more, whilst paraglobulin only becomes insoluble when the solution contains about 20 p. c. or more of sodium chloride.

Making use of these reactions and following the method which has been described when speaking of fibrinogen, Hammarsten has separated fibrinogen which is free from all traces of serum-albumin and of paraglobulin, and has found that such fibrinogen dissolved in weak solutions of sodium chloride may be kept indefinitely without undergoing coagulation. When, however, there is added to it serum of blood, or a solution of fibrin-firment prepared according to the directions of Schmidt or by improved methods, coagulation occurs with great rapidity.

Fibrinogen the one precursor of fibrin in plasma.

According to Hammarsten, then, the coagulation of the blood depends upon the production of fibrin from *one* body, *fibrinogen*, existing in solution in the liquor sanguinis, under the influence of that yet non-isolated body, the fibrin-ferment. Although provisionally employing the term *fibrin-ferment*, Hammarsten, like Schmidt, does not commit himself to the view that this body is really of the nature of a ferment.

The grounds upon which Hammarsten has come to the conclusion that paraglobulin is not *indispensable* to the formation of fibrin are the following:—1st. The fibrinoplastic action is not a specific property of paraglobulin, but is exerted by some other substances, such as calcium chloride and impure casein. 2nd. The fibrinoplastic activity does not belong to pure paraglobulin, but only to that substance when precipitated from serum and certain other fluids. In accordance with this statement Hammarsten has obtained from hydrocele fluids, which were quite free from ferment, a pure paraglobulin, which possessed all the typical properties of that body, but exerted no fibrinoplastic activity. 3rd. The chief proof in support of Schmidt's hypothesis is based upon the surmise that those hydrocele fluids which do not coagulate when treated with ferment alone, but only after the addition of paraglobulin, either do not

[1] Hammarsten: "Untersuchungen über die Faserstoffgerinnung." *Nov. Acta Reg. Soc. Scientiar. Upsal.*, Ser. x. Vol. x. Separatabdruck, Upsala, 1878.—"Zur Lehre von der Faserstoffgerinnung." Pflüger's *Archiv*, Vol. xiv. (1877) pp. 211—274.—"Ueber das Paraglobulin." Pflüger's *Archiv*, Vol. xvii. pp. 413—468.—"Ueber das Paraglobulin, zweiter Abschnitt." Pflüger's *Archiv*, Vol. xviii. pp. 38—116.—"Ueber das Fibrinogen." Pflüger's *Archiv*, Vol. xix. pp. 563—622.

contain paraglobulin or at most mere traces of it. This surmise is however thoroughly incorrect, as Hammarsten's quantitative analyses have shewn that such fluids contain, on the contrary, very considerable quantities of paraglobulin. This paraglobulin possesses, however, no fibrinoplastic activity, affording another proof that the fibrinoplastic property is to be ascribed to some contaminating substance. 4th. The most weighty fact in opposition to Schmidt's hypothesis is however the possibility of obtaining solutions of fibrinogen which are free from paraglobulin, and which, when treated with ferment solutions which are free from paraglobulin, yield typical fibrin.

The observations of Hammarsten corroborate those of Schmidt in reference to the living plasma containing less paraglobulin than serum, and he believes with Schmidt that some of the paraglobulin is derived from the colourless corpuscles; he does not however, as has been said in speaking of paraglobulin, ascribe the origin of this body entirely to this source; much is doubtless present in solution in the living liquor sanguinis, and some may perhaps originate as a product in the decomposition which gives rise to fibrin, for even Hammarsten was at first inclined to view coagulation very much as Denis did, viz. as being a process in which a complex body decomposes with the formation of simpler products, of which fibrin is one.

Hammarsten corroborates Schmidt also in his statement that the addition of paraglobulin to scantily coagulating plasma or to a transudation which will not coagulate in the presence of ferment, may in the first case lead to an increase of the fibrin produced and in the second to the production of a coagulum. But Hammarsten shews that many substances besides paraglobulin will under the same circumstances exert the same fibrinoplastic influence. The addition for example of calcium chloride, $CaCl_2$, to some specimens of hydrocele fluids, which will not coagulate on the addition of Schmidt's fibrin-ferment, produces the same effect as the addition of paraglobulin.

If paraglobulin were specifically one of the fibrin-factors, it would not, presumedly, be replaceable by any other proteid substance. Hammarsten having, however, by a process for which the original must be consulted, prepared casein which was readily soluble in solutions of sodium chloride, found that the addition of its solution to transudations led not merely to an acceleration of the process of coagulation, but to a remarkable increase in the amount of fibrin formed.

In other experiments he found that the mere neutralization of a transudation, which does not coagulate spontaneously, will often lead to coagulation setting in. Furthermore, Hammarsten has found that from some hydrocele fluids, which will not coagulate on the addition of fibrin-ferment, it is possible to separate, by his process, fibrinogen, which when dissolved and treated with the same fibrin-ferment, will yield a coagulum of fibrin.

It is obvious, then, that in a fluid there may exist substances which either hinder the formation of fibrin, or prevent its precipitation when formed. We know, for instance, that such substances

as free alkalies or their carbonates, and some salts, will actually exert such an influence. The addition of any body which will combine with, or neutralize, the substances which prevent the separation of fibrin will naturally lead to its formation. If, for example, a hydrocele fluid, which contains but a small quantity of fibrinogen and which will not coagulate on the addition of ferment, be treated with solution of calcium chloride, coagulation will sometimes occur. Now it is conceivable that in this case the chloride acts by decomposing the alkaline carbonate which hinders the precipitation of the fibrin, for were sodium carbonate and calcium chloride to come in contact the reaction would be as follows: $Na_2CO_3 + CaCl_2 = 2NaCl + CaCO_3$. Paraglobulin may, perhaps, act in a similar manner, by combining with substances which hinder the precipitation of fibrin.

Schmidt had discovered that the addition of serum which has been freed from paraglobulin (by dilution with water, passage of CO_2 and concentration *in vacuo*) to hydrocele fluid does not cause the latter to coagulate, and Hammarsten confirms the statement in reference to hydrocele fluid or to solutions which are as poor in fibrinogen as that fluid. If, however, such serum, free from paraglobulin, be added to a strong solution of fibrinogen, the latter will coagulate well. Hammarsten's explanation is the following:—A solution of fibrinogen, prepared according to the method of Schmidt, contains more free alkali than the original fluid did whilst it contained paraglobulin. The former contains therefore a larger proportion of substances capable of dissolving fibrin, and when it is mixed with a liquid containing only a small quantity of fibrinogen, a larger portion of the resulting fibrin, or it may be the whole of it, may be held in solution. When, however, the same solution is added to a fluid containing an abundance of fibrinogen, the substances capable of dissolving fibrin are no longer capable of holding in solution all the fibrin which is formed; and in this case coagulation occurs.

Reviewing all the facts which have been recorded in the preceding pages, it would appear that, on the whole, the evidence is decidedly in favour of the view that the coagulation of the blood is dependent upon the presence in the plasma of a proteid body, *fibrinogen*, which under favourable circumstances undergoes conversion or perhaps decomposition into *fibrin*. The conversion of fibrinogen into fibrin outside of the body appears to be connected with the action of a ferment produced in the colourless cells of the blood and probably only set free when these cells break down.

The influence of salts on coagulation.

In the course of his researches Schmidt discovered that salts exert a remarkable action in furthering the spontaneous coagulation of liquids containing the various fibrin-factors.

If from two liquids which yield when mixed a coagulum of fibrin, the salts be separated by dialysis, and the proteids which are precipitated

in the process (the supposed fibrin-generators) be dissolved in weak solutions of sodium hydrate and mixed, no coagulation will occur, unless there be added to the mixture the dialysate from the two operations, reduced by evaporation to a small volume, or unless sodium chloride be added until it amount to 1 per cent. of the mixture; then, however, coagulation does occur. The quantity of salt which is needed to bring about coagulation increases with the volume of the solution of the fibrin-factors, a circumstance which fully explains why by largely diluting a spontaneously coagulating fluid, a slowing of the process of coagulation, and a diminution in the quantity of fibrin produced, are always brought about.

Non-coagulation of the blood within living blood-vessels.

Any theory of the coagulation of the blood which would lay claim to truth or completeness should be adequate to explain the remarkable circumstances that the blood does not coagulate as long as it is contained within the living uninjured vessels, but that it does coagulate when the vessel is injured or dies.

Let us examine the facts which we at present possess in reference to this matter.

(1) So long as the vessels are uninjured and alive, the blood which circulates within them does not coagulate. When a foreign body is however introduced into the vessels, as when a silver needle is made to transfix an artery, a coagulum of fibrin forms around the metal, although it be in the stream of living blood.

(2) If, however, the coats of an artery be diseased or injured in such a way that the endothelial coat which lines it ceases to be intact, coagulation will occur, giving rise to a solid plug or 'thrombus,' the latter term being applied specifically to the coagulation which occurs in a vessel during life. Perhaps the most common example of a thrombus is that which is occasioned by the application of a ligature to an artery; in this case both the middle and internal coats are usually severely injured, the continuity of the endothelial lining of the internal coat being certainly affected, and, almost immediately, there results coagulation.

Another common example of the production of thrombus during life is afforded by the occurrence of the process in aneurisms, in which, amongst other lesions of the arterial walls, a direct breach in the continuity of the endothelium certainly often exists.

(3) But not only does thrombosis occur where a direct break in the continuity of endothelium can be distinctly proved to exist, but also where an injury of any kind is inflicted upon an artery. The process has been studied with great minuteness by Zahn in the case of the arteries of the frog and deserves particular attention in reference to the doctrines of coagulation.

Zahn[1] has observed that when a crystal of sodium chloride is

[1] Zahn, Virchow's *Archiv*, Vol. LXII. p. 81. See Cohnheim, *Vorlesungen über allgemeine Pathologie*, 1877, Vol. I. p. 150 *et seq.*

thrust deeply into the tissue of the tongue or mesentery of the frog so as to be in close proximity to an artery or a vein, the inner wall of the vessel at the point which corresponds to the crystal becomes covered by colourless blood corpuscles, whose number continually increases; soon there are three or four layers of colourless corpuscles closely pressed against the wall, whilst the heap grows ever larger and larger as the blood which flows past continually brings fresh white corpuscles to add to it. Soon the vessel becomes completely plugged by this agglomeration of colourless corpuscles. The subsequent progress of such a thrombus may be various. In some cases however the following process may distinctly be observed:—The whole mass of cells undergo a fine granulation and the contours of individual cells become less distinct. Then the contours of the cells become lost altogether, and a feebly refracting finely granulated mass results, which is said to be not unlike a mass of fibrin.

(4) When blood is occluded by ligatures within a living vein, it will be found to remain uncoagulated for many hours, providing the vitality of the vein persist. This remarkable experiment was first performed by Hewson, and was subjected to a careful study by Professor Lister, and more lately by Frederique. If, however, the vitality of the vein be destroyed by the application of caustic ammonia to its exterior, coagulation will soon result (Lister). The experiment is best performed with the jugular veins of horses. The animal having, as is usually done in slaughtering horses, been struck down insensible by a blow on the head, the jugular vein or veins are exposed, and two ligatures are applied to the vein at a distance of several inches apart, so as to include the blood contained within this portion of the vein in a tube with venous walls. The vein may then be dissected out without allowing its contents to escape. Such a vein may be kept for many hours, and on being opened the blood will be found still fluid within it, coagulating however when allowed to flow into any ordinary vessel. After an interval of many hours, however, the vitality of the vein being destroyed, the blood coagulates. This experiment we owe to Hewson. Reasoning from it, it might be surmised that the cause of the coagulation was the opening of the vein and the exposure of its contents to air; that such an explanation is entirely erroneous was shewn by Professor Lister, who determined that blood would remain fluid for hours in a vein after being exposed with the utmost freedom to the air by being poured in thin streams from one venous capsule to another.

The observation of Lister might lead one to the conclusion which Professor Brücke arrived at from his experiments. That eminent observer, extending the observations of Hewson, shewed that blood injected into the separated, but yet living, contracting, heart of a turtle, would preserve its fluidity for days, and came to the conclusion that the walls of the vascular system possess a power of restraining coagulation—a view which was assuredly shared by Hewson, but which in this case appeared to find its most striking

proof. On returning, however, to other observations of Lister we are warned to pause before we draw the above conclusion. The author quotes Professor Lister's description of one of his experiments conveyed to him in a private communication:

"The manner in which I did succeed in my experiments long ago on the coagulation of the blood in maintaining its fluidity outside the living body seems to me, if I may venture to say so, deserving of more attention than I think it has received. Having ascertained that the blood remains fluid for many hours after somatic death in all vessels except the heart and principal trunks provided that the vessels have been previously healthy, I removed a portion of the jugular vein of an ox, after tying it in two places to retain the blood; and then, holding the portion of vein vertical and opening the upper end with scrupulous care that the instruments employed should not touch the blood, I slipped down with the utmost steadiness a piece of glass tube nearly as large in calibre as the vein, the lower end of the tube being of full width and smooth while the upper end was drawn out and connected by an india-rubber tube with a stop-cock for closing it. The blood having filled the large part of the tube and passed on into the narrow part till it escaped at the stop-cock, the stop-cock was turned to close it, after which the whole apparatus was rapidly inverted so that the blood was now in the glass vessel with its mouth covered with the vein as a cap. The vein was next carefully withdrawn and a cap of gutta-percha tissue was tied over the mouth of the tube to prevent evaporation. The blood was now in a vessel composed entirely of ordinary solid matter, as distinguished from living tissue, but with the peculiarity as compared with blood shed into a basin that only the circumferential parts of the mass of blood had been exposed to the influence of the ordinary solid. The result was that after 24 hours, or in one experiment 48 hours, the blood was found still fluid except a crust of clot in contact with the containing vessel, the fluid blood coagulating at once on being poured upon a plate. I had previously ascertained that blood would remain fluid for hours in a vein after being exposed with the utmost freedom to the air by being poured in thin streams from one venous capsule (if I may so speak) to another; while, on the contrary, want of steadiness in pushing down the glass tube into the vein and consequent admixture of the circumferential parts which had touched the glass with the rest would, like a stir with a stirring rod, have made the whole coagulate.

"Thus by this simple experiment was demonstrated incontrovertibly the fact that healthy blood has no spontaneous tendency to coagulate and therefore that Brücke's idea of the fluidity of the blood being due to an action of the walls of the vessels upon it was erroneous. At the same time was illustrated the truth, which, indeed, ought to have been apparent enough from the results of every vivisection wound, that a perfectly undisturbing coagulum resembles healthy living tissue in failing to induce coagulation in its vicinity."

The difference between Lister's and Brücke's explanation of the above facts will be perhaps more apparent by the following categorical statement. Brücke explains the non-coagulation of the blood contained in the uninjured and yet living jugular vein by sup-

posing that blood does possess a strong tendency to coagulate and that the tendency which the blood has to coagulate is inhibited by a peculiar influence exerted by the living vascular walls. Lister on the contrary maintains that blood possesses no spontaneous tendency to coagulate and only does so when brought in contact with any foreign body; it coagulates within a blood-vessel when the latter dies because then its walls become as all other extraneous matter, but not because there is any cessation of an action previously exerted.

After all, there appears to be less difference between the views of Lister and Brücke than would at first appear to be the case. Let us examine however which of their views appears most probable in the light afforded by recent discoveries.

Of all the facts which have, thanks to the labours of Buchanan, Schmidt, and Hammarsten, been collected, in reference to the exact mode of origin and nature of coagulation, none appear to be so consistent and satisfactory as those which connect the colourless cells of the blood with the developement of a ferment-like body which, once liberated, soon converts soluble into insoluble proteid matter; the developement of ferment being apparently connected with a disintegration of certain of the colourless cells.

As this disintegration has a tendency to occur whenever the blood removed from the living blood-vessels is kept at temperature above 0° C., we can scarcely agree in the proposition of Professor Lister that the blood has of itself no tendency to coagulate, and we should rather be inclined to say that inasmuch as it contains colourless corpuscles within it, it does contain the elements for its future coagulation.

The remarkable phenomena of the non-coagulation of blood within the yet living venous walls is probably connected with a persistence in an intact condition of the colourless cells, or rather of those cells in which the fibrin-ferment originates, and not as might have been supposed, upon the destruction of the fibrin-ferment by the vascular walls at the moment of its liberation. But it is yet impossible to conceive why the colourless corpuscles should not break down under the circumstances of Lister's experiments.

Sec. 3. The Serum and the constituents of the Liquor Sanguinis which remain in it.

Modes of obtaining serum.

The serum is the liquor sanguinis from which fibrin has separated; it differs from that fluid in having lost its fibrinogen and perhaps in having gained some paraglobulin.

In order to obtain perfectly pure serum when horse's blood is available, liquor sanguinis may be first separated by subjecting the blood to a lower temperature in the apparatus described at page 32, and the plasma allowed to coagulate.

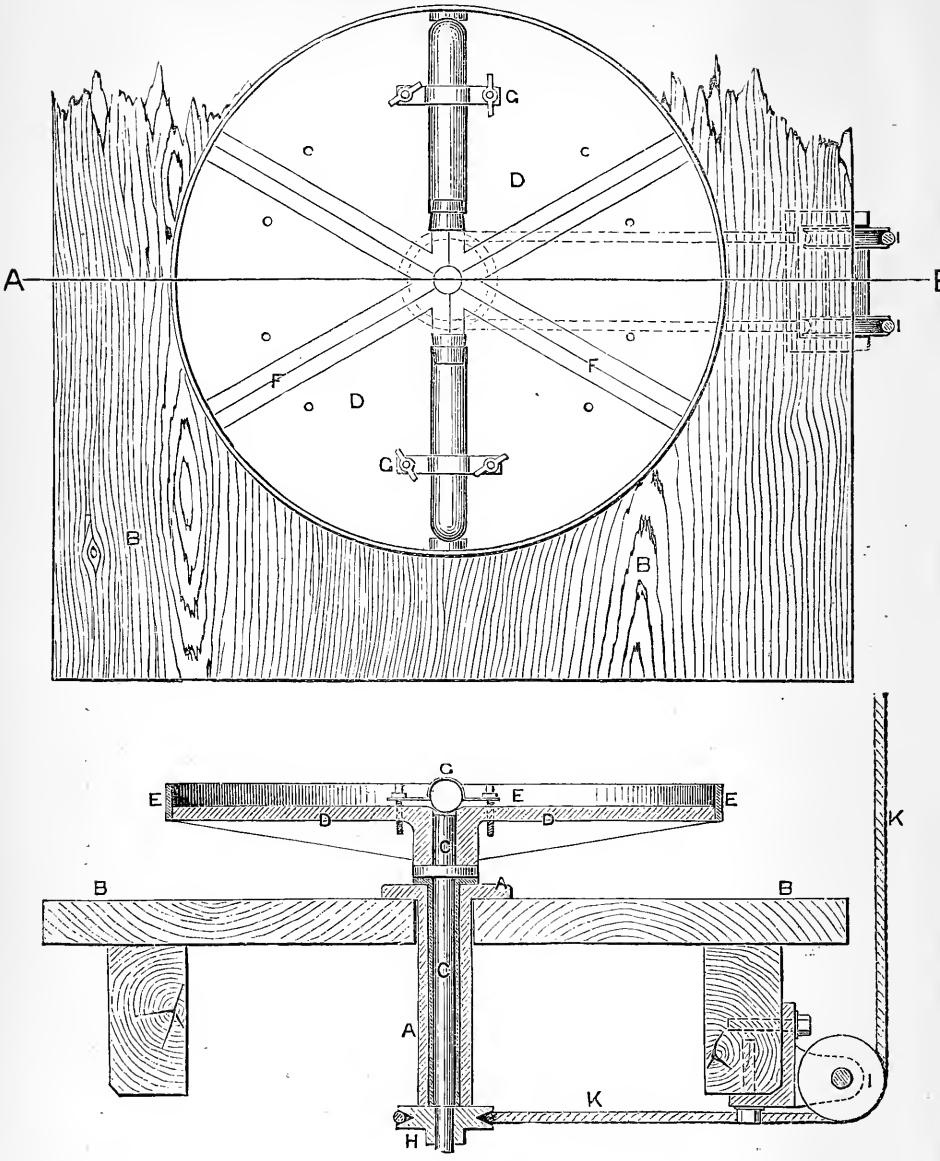


FIG. 11. PLAN AND SECTION OF THE CENTRIFUGAL MACHINE IN THE PHYSIOLOGICAL LABORATORY OF OWENS COLLEGE.

A. An iron socket secured to top of table B.
C. A steel spindle carrying the turn-table D, and turning freely in A.
E. A flange round turn-table D.
F. F. Shallow grooves on face of D, in which the test-tubes are fixed by clamps G. G.
H. A pulley fixed to end of spindle C and turned by the cord K.
I. I. Are two guide pulleys for cord K.

It is more usual however to obtain serum by allowing blood (preferably arterial blood) to coagulate, when after some hours serum will separate and can be decanted.

The process of separation of serum is immensely facilitated and the resulting serum is obtained most completely free from suspended blood cells by subjecting recently coagulated blood to the action of a centrifugal machine, such as is represented in the accompanying figure.

The blood as it flows from the blood-vessel is collected in stout test-tubes provided with india-rubber stoppers. When the blood has coagulated the tubes are fixed to the turn-table so that the stoppers are directed centrally. The turn-table is then made to revolve with great velocity for about half an hour, after which time the clot is found to have retracted itself to the peripheral end of the tube, leaving a large quantity of clear serum occupying the ends of the tube directed towards the centre of the rotating disk.

The centrifugal machine enables us to obtain in a short time considerable quantities of perfectly clear serum, which is thus obtained before any putrefactive change can have affected its composition. When serum has been merely decanted from the clot it is generally more or less reddish from the presence of suspended corpuscles. From such reddish serum, serum quite free from corpuscles can be obtained by subjecting it to rotation in the centrifugal machine for about half an hour.

Description of physical characters of serum.

The serum which separates from the blood of a healthy man, whilst fasting, is a liquid of a transparent yellow colour like light sherry wine, varying in depth of colour but always perfectly clear. In the lower animals the colour of the serum differs somewhat, being colourless in the rabbit, amber coloured in the horse, of a very red amber tint in the ox, and in the dog somewhat yellowish, nearly identical with that of man. After a full meal the serum ceases to be transparent and becomes more or less milky in appearance; this phenomenon is usually described as occurring only after an abundant fatty diet, but although seen to greatest advantage after such a diet it constantly occurs after a full meal of meat.

The observations of Dr Andrew Buchanan[1] on this matter are of great interest, and two of them are quoted as illustrating the above statement:—

"A vigorous man of about 35 years of age, after fasting 19 hours, had for dinner, twenty ounces of beef-steak, sixteen liquid ounces of brown soup and eight ounces of bread. He was bled immediately before his meal and three times after it, two ounces of blood being taken away each time. The serum obtained from the first bleeding before the meal was perfectly limpid; the serum from the second bleeding, three hours and

[1] Buchanan, "On the white or opaque serum of blood," *Proceedings of the Philosophical Society of Glasgow*, Vol. I. (1841—4), p. 226.

fifteen minutes after the meal, was turbid; the serum from the third bleeding, eight hours and fifteen minutes after the meal, was still thicker; while that from the last bleeding eighteen hours after the meal, was again limpid, although some supper had been eaten in the interval.

"The young man first mentioned, after fasting eighteen hours, dined upon sixteen ounces of brown soup, four ounces of bread, eight ounces of potatoes, twenty ounces of beef-steak, and sixteen ounces of London porter, and fasted eighteen hours after the meal. He had blood taken from his arm four times to the extent of two ounces each time. The serum of the blood first taken, immediately before the meal, was of an amber yellow and quite transparent; the serum from the second bleeding, two hours and ten minutes after the meal, was turbid; the serum from the third bleeding, eight hours after the meal, was exactly of the colour of water gruel and quite opaque; the serum of the blood last taken, eighteen hours after the meal, was still turbid, its limpidity not having been, as after his usual fare, restored by an eighteen hours fast."

The milkiness of such blood is due to finely divided fat which often may be observed to float to the surface and presents the appearance of oil globules or drops.

The specific gravity of the serum obtained from human blood varies between 1027 and 1032, but is on an average 1028. Its reaction is alkaline, and its alkalinity is greater than that of the plasma.

1000 grammes of blood yield between 440 and 525 grammes of serum (Gautier).

Serum contains roughly about 10 per cent. of solid matters in solution; of these the most abundant are proteid in nature, the chief being serum-albumin; in addition to the proteids, the serum holds in solution small quantities of nitrogenous matters soluble in alcohol, which are technically grouped under the term *extractives* or *extractive matters*, fats, sugar, inorganic salts and certain gases. These various constituents will now be discussed in detail.

The Proteids of the Serum.

I. *Serum-globulin or Paraglobulin.*

This constituent has been already discussed at considerable length in relation to the subject of coagulation, and the reader is referred to page 37 for the method of obtaining it from serum, as well as for a discussion of the views which have been held as to its origin.

It was formerly held that serum-globulin was present in much smaller quantities in the serum than serum-albumin. According to Hammarsten, however, the older methods employed in the separation of this substance were insufficient. He has discovered that magnesium sulphate effects the complete precipitation of serum-globulin, and therefore admits of the accurate determination of its amount. In the following table are shewn the results of analyses

in which he determined the total quantity of proteids in the serum and also the amount of serum-globulin; the quantity of serum-albumin being found by subtracting the second result from the first. It will be seen that according to Hammarsten the proportion of serum-globulin to serum-albumin varies remarkably, in some cases (horse and ox) the former being the more abundant constituent, in others (dog and rabbit) the latter.

TABLE SHEWING THE AMOUNT OF SOLIDS, PROTEIDS, AND ALSO THE RELATIVE PROPORTIONS OF SERUM-GLOBULIN AND SERUM-ALBUMIN IN 100 PARTS OF THE SERUM OBTAINED FROM THE BLOOD OF VARIOUS ANIMALS[1].

	Solids.	Total Proteids.	Serum-globulin.	Serum-albumin.	Serum-globulin / Serum-albumin
Serum from horse	8·597	7·257	4·565	2·677	$\frac{1}{0·591}$
Serum from ox	8·965	7·499	4·169	3·329	$\frac{1}{0·842}$
Serum from man	9·207	7·619	3·103	4·516	$\frac{1}{1·511}$
Serum from rabbit	7·525	6·225	1·788	4·436	$\frac{1}{2·5}$

By the term *serum-casein* some authors have designated the proteid matter which is obtained from serum by adding a small quantity of acetic acid to it after paraglobulin has been precipitated by diluting and subjecting to a stream of CO_2. We now know, however, that dilution with water and the action of CO_2 are not sufficient to precipitate all the serum-globulin contained in the serum, and we cannot doubt that Panum's serum-casein is merely serum-globulin which has escaped precipitation by CO_2.[2]

2. *Serum-albumin.*

Having separated from the serum the serum-globulin which it contains, there still remains in solution the most important and usually the most abundant of its constituents, viz. serum-albumin.

In consequence of the serum-albumin which it contains, when serum is heated to about 60° C. it becomes slightly opaque, full coagulation occurring at 75°, the separation of the albumin being accompanied by an increase in the alkaline reaction of the liquid.

[1] Hammarsten, "Ueber das Paraglobulin." Pflüger's *Archiv*, 1878.

[2] The reader who wishes to acquaint himself with the older statements as to serum-casein is referred to Kühne, *Lehrbuch*, p. 175, and to Gorup-Besanez, *Lehrbuch d. physiolog. Chemie*, 1878, p. 119.

When alcohol is added to serum in considerable excess, as in the proportion of two volumes of absolute alcohol to one of serum, the albumin is precipitated: at first the precipitate can be redissolved in distilled water; by prolonged contact with the alcohol it becomes almost absolutely insoluble. In order to secure the latter result as perfectly as possible, as for instance in the preparation of solutions of fibrin-ferment, the quantity of alcohol added must be much larger than that indicated above, even 15 or 20 times as much alcohol as serum being used, and the action of the former upon the latter being continued for about three months.

The albumin recently precipitated by alcohol from serum, when it is redissolved in water, yields a faintly opalescent liquid.

Preparation of Serum-albumin. Various methods have been suggested for the preparation of pure serum-albumin; they all yield a substance, which can only be regarded as approximately pure, inasmuch as they fail in separating inorganic salts which, more or less, always continue to adhere to the substance and to modify its physical properties.

Hoppe-Seyler's method.

Blood serum is treated with dilute acetic acid, as for the preparation of paraglobulin, and the clear liquid is filtered from the latter body. The liquid is then concentrated by evaporation in shallow basins at a temperature which must not exceed 40° C.

The concentrated liquid is neutralized with sodium carbonate and is then placed in a dialyser suspended in distilled water, which must be very frequently renewed. The dialysate is tested from time to time with solution of silver nitrate; when this reagent no longer produces a marked opalescence it is concluded that all diffusible impurities, of which sodium chloride is the chief, have been removed; the contents of the dialyser are then emptied into a flat capsule and evaporated at a temperature not exceeding 40° C.

Prepared by this process, serum-albumin still contains from 0·5 to 1·0 per cent. of salt, and is obtained in the form of a transparent, yellowish, brittle solid, which breaks with a glassy fracture, and which furnishes, when pulverized, a yellowish white powder. It is soluble in distilled water, the solutions being slightly opalescent and, when concentrated, viscous. Solutions of serum-albumin deviate the plane of polarization to the left; $(A)j = -56°$. The specific rotatory power is remarkably little affected by the presence of salts or by the degree of dilution.

When dry, solid, soluble albumin, prepared by the previously described method, is heated to 100° C. it is, after a considerable time has elapsed, rendered insoluble in water.

Solutions of serum-albumin are not precipitated by carbon-dioxide, by acetic or by orthophosphoric acid. They are precipi-

tated by mineral acids, and especially by nitric acid; they are likewise precipitated by tannic acid and by metaphosphoric acid.

When heated to 60°C. solutions of serum-albumin usually become opalescent, and at temperatures between 72°—75° the albumin separates in a flocculent form. Solutions of albumin which have been long dialysed, but are not free from salts, are exceptions to these statements. (See Schmidt's and Aronstein's pure albumin.)

Most metallic salts, such as mercuric chloride, copper sulphate, lead acetate, at once precipitate ordinary serum-albumin.

Ether does not precipitate serum-albumin, whilst it does precipitate egg-albumin.

Schmidt's and Aronstein's pure serum-albumin and its reactions.

It was asserted by Graham[1] that by mixing egg-albumin with acetic acid and placing the mixture in a dialyser, all the acid, together with the alkaline and earthy salts, diffused out, leaving the albumin pure, so that the dried substance, on being ignited, left no ash. This albumin was found by Graham to have a slightly acid reaction. Kühne and Hoppe-Seyler were unable to confirm the statement of Graham.

Aronstein[2], working under the direction of A. Schmidt, asserted that if serum be subjected to long continued dialysis, the whole of the paraglobulin is precipitated and the whole of the salts are removed from the albumin, which when burned leaves no ash. He asserted that such albumin when dissolved in water is not coagulated by boiling, and is not precipitated by alcohol. The addition of small quantities of common salt leads, according to Aronstein, to the albumin being again coagulable by heat and by alcohol. These observations of Aronstein received the full confirmation of A. Schmidt[3], who, in addition, asserted that dilute solutions of pure albumin, obtained by dialysis, gave no precipitate with copper and zinc sulphate, with neutral lead acetate, with mercuric chloride and many other salts which precipitate ordinary albumin. Platinum tetrachloride, nitric acid, tannic acid, ferrocyanide of potassium and acetic acid, were stated to be the reagents which most easily precipitate pure albumin from its solutions.

The observations of Aronstein and Schmidt have not however been confirmed.

Heynsius[4] found it impossible to obtain any serum-albumin (by Aronstein and Schmidt's process) free from ash, and attributed the non-coagulation in Aronstein's experiments to the presence of a slight alkaline residue. Similarly Winogradoff[5] repeating Aronstein and Schmidt's

[1] Graham, "Liquid diffusion applied to analysis." *Philosoph. Transact.*, 1861.

[2] Aronstein, "Ueber die Darstellung salzfreier Albuminlösungen vermittelst der Diffusion." Pflüger's *Archiv*, 1873, Vol. VIII. p. 75.

[3] A. Schmidt: "Untersuchung des Eiereiweisses und Blutserums durch Dialyse." Ludwig's *Festgabe*, 1874, pp. 94—115. "Weitere Untersuchungen des Blutserums, Eiereiweisses und der Milch durch Dialyse mittelst geleimten Papiers," Pflüger's *Archiv*, Vol. II. pp. 1—52.

[4] Heynsius, "Ueber die Eiweissverbindungen des Blutserums und des Hühnereiweisses." Pflüger's *Archiv*, Vol. IX. pp. 514—552.

[5] Winogradoff, "Darstellung und Eigenschaften salzfreier Eiweisslösungen." Pflüger's *Archiv*, Vol. II. p. 605.

experiments, under the direction of Salkowski, failed to obtain albumin free from ash. Huizinga[1] by continued dialysis found that serum-albumin contained from 0·36 to 0·56 p. c. of ash. Haas[2] was able from the fluid of ascites to obtain serum-albumin containing only 0·3 p. c. of ash; he found that its solutions were precipitated by alcohol and by ether; when boiled, the solutions always became opalescent, and often yielded precipitates.

Are peptones present in the serum?

It has been surmised that peptones, &c. which are formed in such large quantities in the alimentary canal, and which are doubtless absorbed into the blood, may be present as peptones in that fluid. The most recent research, of Drosdoff, shews that the presence of peptones cannot be demonstrated with certainty even in the blood of the portal vein taken whilst absorption is progressing[3].

The Extractive Matters of the Plasma and Serum.

By the term extractive matters, physiological chemists formerly designated organic substances present in very small quantities in the various solids and liquids of the body, and extracted from them by various liquids, especially by alcohol, but which could not be obtained in a sufficiently pure condition to admit of their identification as definite proximate principles.

The progress of research has, to a very great extent, enabled us to resolve the group of 'extractive matters,' obtained from most liquids and solids, into its components; still the term remains as a convenient one for the purpose of grouping the organic constituents present in small quantities, and capable of extraction by various liquids.

The extractive matters present in the plasma all pass into the serum. These bodies, although present in small quantities in these fluids, are yet possessed of the highest physiological importance. It is more in accordance with the plan of the present work to consider at length the individual extractive matters of the blood in connection with the functions of the body with which they are most closely related, and the author therefore limits himself in this place to little more than an enumeration. As indicating the method of treatment which has been adopted, the reader is informed that the fats of the serum will be considered under digestion and in connection with the chemistry of the nervous organs, sugar when discussing the functions of the liver, urea and uric acid in connection with the secretion of urine, creatine and creatinine when treating of muscle.

[1] Huizinga, "Zur Darstellung des dialysirten Eiweisses." Pflüger's *Archiv*, II. pp. 392—402.

[2] Haas: "Ueber das optische und chemische Verhalten einiger Eiweisssubstanzen, insbesondere der dialysirten Albumine." Pflüger's *Archiv*, Vol. XII. pp. 378—410. "Ueber die Eigenschaften des salzarmen Albumins," *Prager medic. Wochenschrift*, 1876, Nos. 34—36; also Maly's *Jahresbericht*, Vol. VI. (1877), p. 5.

[3] Drosdoff, "Resorption der Peptone, des Rohrzuckers und der Indigoschwefelsäure vom Darmcanal aus und ihr Nachweis im Blute der Vena portae." *Zeitschrift f. physiol. Chem.*, I. 216—232.

Neutral Fats, Lecithin and Cholesterin.

When serum is evaporated to dryness and the residue is powdered and boiled with ether, this liquid extracts all the above bodies. Especially is this the case when the serum presents, as it does some hours after food, the milky appearance which has already been described. The serum of fasting animals contains on an average about 0·2 per cent. of fats and cholesterin, that of animals in digestion may contain from 0·4 to 0·6 p.c. The fats present in the serum are triolein, tristearin, and tripalmitin. It was formerly believed that in addition to these fats, soaps, *i.e.* alkaline salts of the fatty acids, were present in the blood. The incorrectness of this surmise has been demonstrated by Röhrig[1], who has shewn that soluble soaps cannot exist in the blood.

Cholesterin, which will be studied in connection with the chemistry of the nervous organs, may constitute 10 p.c. of the ether extract of blood. It has been found by Hoppe-Seyler[2] to vary in the serum of the blood of fattened geese from 0·019 to 0·314 per cent. Besides the fats and cholesterin the serum of blood always contains, according to Hoppe-Seyler, some lecithin; this substance will be treated of under 'brain.'

Sugar.

Glucose is a normal constituent of the blood, and is contained in the serum which separates from it after coagulation. Its amount appears, according to the most recent researches (Abeles[3], Pavy[4], v. Mehring[5]), to be nearly the same in the blood of all the vessels with the one exception of the blood of the portal vein, which contains an excess of sugar after the ingestion of a saccharine diet.

The quantity of glucose present in the blood of the dog was found by Pavy to vary between 0·81 and 1·231 parts per 1000.

In the experiments of v. Mehring, the amount of sugar in the serum of dogs was found to vary between 0·125 and 0·330 p.c. This matter will be fully treated of under 'liver' and 'nutrition' (see also Chapter IV.).

Urea, Uric acid, Creatine, &c.

Creatine, creatinine, urea, carbamic acid, xanthine, hypoxanthine, uric acid and hippuric acid are found in the serum. The amount of urea present in the normal blood of man varies between 0·02 and 0·04 p.c.

A yellow pigment is found dissolved in the serum of the blood of man and most animals, although certain animals (*e.g.* the rabbit) have colourless serum.

[1] Röhrig, "Ueber die Zusammensetzung und das Schicksal der in das Blut eingetretenen Nährfette." Ludwig's *Arbeiten*, 1874.

[2] Hoppe-Seyler, "Ueber das Vorkommen von Cholesterin und Protagon und ihre Betheiligung bei der Bildung des Stroma der rothen Blutkörperchen." *Med. Chem. Untersuchungen*, p. 145.

[3] Abeles, "Der physiologische Zuckergehalt des Blutes." *Med. Jahrbücher*, Heft III., 1875; also Maly's *Jahresbericht*, Vol. VI. p. 95.

[4] Pavy, "The Croonian Lectures on certain points connected with Diabetes." London, 1878.

[5] v. Mehring, "Ueber die Abzugswege des Zuckers aus der Darmhöhle." *Archiv f. Anat. u. Physiol.* 1877, pp. 380—415.

The Salts of the Plasma and Serum.

When an organic liquid such as the plasma, the serum, or the blood, is evaporated to dryness, and the dry residue is exposed to a red heat in a crucible, the organic matters are oxidized and the products of oxidation escape, leaving the inorganic or mineral matters behind. We cannot, however, suppose that even the whole of the inorganic matters originally present in the liquid can be obtained in this way, for, however carefully we may proceed, there will always be more or less volatilization of certain saline constituents as, for instance, of sodium chloride. Still less are we justified in concluding from the inorganic compounds left in the ash after the most cautious ignition, that the same compounds were originally present in the liquid, for they may only have been produced under the action of heat, and at the expense of some constituents of organic bodies. We must bear these considerations in mind in our judgment of the results of analyses of the ashes of plasma and serum.

Much more reliable, however, is the information furnished us by the direct precipitation of the inorganic constituents of the serum by the addition of certain reagents to it. Under the direction of Ludwig, Pribram[1] and Gerlach[2] have *directly* determined the amount of calcium, magnesium, and phosphoric acid in the serum by a method which will be found described in Chapter IV., and it is upon their results that are alone based any accurate conclusions as to these three constituents of the serum.

The following facts may be taken as resting upon a firm foundation.

1. The serum contains a somewhat smaller proportion of inorganic salts than the liquor sanguinis, some being carried down with, or perhaps more intimately associated with, the fibrin when it separates.

2. The amount of inorganic matter left on the cautious ignition of serum amounts to from 0·7 to 0·9 per cent.

3. The principal inorganic constituent of the ash of plasma and serum, and not only of the ash, but of the liquids themselves, is common salt, sodium chloride, NaCl; the amount present in the liquid being about 0·5 per cent.

This salt constitutes, according to Lehmann, 61 per cent., and according to Schmidt 65·2 per cent. of the ash left by serum. That it is present before ignition is easily proved by the fact that crystals of salt separate on concentrating serum.

4. In addition to sodium chloride, the ash of plasma and serum contains, as its next most abundant ingredient, sodium carbonate.

It appears probable that the plasma and the serum before ignition contain not sodium carbonate Na_2CO_3, but sodium hydric

[1] Pribram, "Eine neue Methode zur Bestimmung des Kalkes und der Phosphorsaüre im Blutserum." Ludwig's *Arbeiten*, 1871.

[2] Gerlach, "Ueber die Bestimmung der Minerale des Blutserums durch directe Fallung." Ludwig's *Arbeiten*, 1872.

carbonate $NaHCO_3$. The grounds for this supposition are (*a*) that the plasma and serum contain considerable quantities of carbon dioxide held partly in simple solution and partly in a state of feeble combination as it is in sodium acid carbonate: (*b*) that when the proteid matters are separated from the serum by adding to it a large quantity of alcohol, and then solution of mercuric chloride is added, there is produced a brown crystalline precipitate of oxychloride of mercury (probably $HgCl_2$, $4HgO$), such as separates when mercuric chloride is added to solutions of sodium acid carbonate. If the sodium existed as normal sodium carbonate, a yellow precipitate of HgO would be thrown down instead. (Liebig.)

5. The ash of plasma and of serum contains about 4 per cent. of potassium chloride which, there is every reason to believe, exists as such in these fluids before they are subjected to chemical treatment.

The great preponderance of salts of sodium as contrasted with salts of potassium in the plasma and serum is one of the incontrovertible and most interesting facts relating to the saline constitution of these liquids.

Salkowsky found, in two cases, in the serum obtained from the blood of healthy men, that the ratio of potassium to the sum of potassium and sodium in the ash was respectively as 13·9 : 100 and 10·4 : 100, and A. Schmidt found the proportion to be in two cases 7·6 : 100 and 8·6 : 100.

6. In addition to the inorganic constituents referred to, the ash of plasma and serum is found to contain sulphuric and phosphoric acids and magnesium and calcium; and arranging the results of the analyses in accordance with the rules followed in such cases it would result that the serum contains ortho-phosphates of calcium, magnesium and sodium, as well as a small quantity of potassium sulphate.

We can, however, have no certainty from the results of such analyses as to the constitution of phosphates existing in the unaltered liquids. Admitting for instance that sodium compounds of orthophosphoric acid (H_3PO_4) exist in the plasma and serum, analysis in no way allows us to decide whether the compound present is the neutral trisodium phosphate Na_3PO_4 or the alkaline hydrogen di-sodium phosphate HNa_2PO_4 or H_2NaPO_4, of which the second, for other reasons, has been supposed to exist in the blood and probably actually does so. Again, the serum contains appreciable quantities of lecithin or some other derivative of glycerin-phosphoric acid. When that body is ignited it leaves metaphosphoric acid, which reacting upon alkaline and earthy carbonates would produce salts which in the analyses would be reckoned as phosphates. The question therefore arises whether the phosphoric acid which is found as a constituent of the ash really exists as such in the liquor sanguinis or whether it is there present as one of the products of the oxidation of such an organic body as lecithin.

The observations of Pribram and Gerlach allow us to decide the question. These observers have proved that calcium, magnesium

and phosphoric acid may by suitable methods be directly precipitated from, and correctly estimated in, the serum. They find that whilst the amount of calcium and magnesium estimated by direct precipitation agrees exactly with that determined by analysis of the ash of the same quantity of serum, the phosphoric acid does not so agree; in other words the amount of phosphoric acid found in the ash of serum is much larger than that directly precipitated from it. Furthermore Pribram, and after him Gerlach, found that after precipitating directly all the phosphoric acid in the serum, and precipitating all proteids by absolute alcohol, on igniting the filtrate its ash contained phosphoric acid; the amount thus found added to that directly precipitated agreed very closely with the total quantity of phosphoric acid determined in the ash of serum. Thus in one experiment,

100 c.c. of serum yielded on direct ignition	0·056	p.c. P_2O_5
„ „ by direct precipitation	0·0108	„
„ „ in the alcohol extract	0·0325	„
„ „ by ignition of the extracted residue	0·0060	„
	0·0493	p.c.

From this experiment, which may be taken as a type of many others, it results that serum contains a *much* smaller quantity of phosphoric acid than is present in its ash and that the greater part of the phosphoric acid in the latter is derived from an organic compound soluble in alcohol. The latter result had to a considerable extent been anticipated by Sertoli[1].

The phosphoric acid obtained on direct precipitation of the serum doubtless exists as an inorganic compound, but we cannot assert its precise condition; probably it does not exist as calcium phosphate. At any rate the whole of the phosphoric acid existing in the serum is not sufficient to combine with the calcium of that fluid to form the compound $Ca_3 2(PO_4)$, as will be learnt by a study of the following results obtained by Pribram.

RESULTS OF SEVEN DETERMINATIONS OF THE PHOSPHORIC ACID (CALCULATED AS P_2O_5) AND LIME (CaO) IN 100 c.c. OF SERUM.

	By direct precipitation.		Determined in Ash.	
	CaO	P_2O_5	CaO	P_2O_5
A	0·0174	0·0124	0·0177	0·0387
B	0·0216	0·0121	0·0230	0·0448
C	0·0150	0·0106	0·0170	0·0320
D	0·0173		0·0171	
E	0·0200		0·0195	
F	0·0188		0·0200	
G	0·0155	0·0108	0·0170	0·0559

[1] Sertoli, "Ueber die Bindung der Kohlensäure im Blute und ihre Ausscheidung in der Lunge." *Medicinisch-Chemische Untersuchungen* von Hoppe-Seyler. Heft III. (1868) p. 350 et seq.

If we calculate what quantity of P_2O_5 would have been found had the compound $Ca_3 2(PO_4)$ existed in the serum we obtain the following results.

A	would have contained	0·0146	P_2O_5
B	„	„ 0·0182	„
C	„	„ 0·0127	„
G	„	„ 0·0131	„

The following tabular views represent the constitution of the saline constituents of the liquor sanguinis and serum as derived from the most reliable researches. It is to be remembered that in reference to the amount of phosphoric acid the older analyses of Lehmann and Schmidt are unreliable, and that for this the results of Pribram and Gerlach are to be taken.

I. COMPOSITION OF ASHES OF SERUM (LEHMANN).

100 parts contain	
Sodium chloride	61·087
Potassium chloride	4·085
Sodium carbonate	28·880
Sodium phosphate	3·195
Potassium sulphate	2·784
	100·031

II. COMPOSITION OF THE SALTS OF THE PLASMA (SCHMIDT).

1000 parts of plasma yield	
Sodium chloride	5·546
Sodium phosphate calculated as Na_3PO_4	0·271
Sodium in other states of combination calculated as Na_2O	1·532
Potassium chloride	0·359
Potassium sulphate	0·281
Calcium phosphate	0·298
Magnesium „	0·218
	8·505

III. COMPOSITION OF THE SOLUBLE SALTS YIELDED BY SERUM OF OX'S BLOOD (SERTOLI).

1000 grammes of serum yielded	
Cl	3·270
Na calculated as in combination with Cl	2·120
Na calculated as Na_2O but existing as chloride	1·291
K calculated as K_2O	0·224
P_2O_5 representing the phosphoric acid actually existing in the liquid	0·025
SO_3 representing the sulphuric acid existing in the liquid	0·305

Sertoli arranges the results of the above analysis as follows, so as to represent the probable constitution of the soluble salts of the serum :—

1000 parts of serum yield	
Sodium chloride	5·39 grms.
Sodium sulphate	0·24 ,,
Disodium hydric phosphate (Na_2HPO_4)	0·05 ,,
Na, calculated as Na_2O (existing as carbonate or bicarbonate)	1·16 ,,
Potassium sulphate	0·414 ,,

IV. RESULT OF THE ANALYSES OF PRIBRAM OF THE CALCIUM AND PHOSPHORIC ACID EXISTING IN SERUM (DETERMINED DIRECTLY).

1000 grammes of serum yield	
Phosphoric acid corresponding to	0·179 grms. P_2O_5
Calcium corresponding to	0·173 of CaO

V. RESULTS OF THE ANALYSES OF GERLACH OF THE MAGNESIUM EXISTING IN SERUM.

1000 grms. of serum yielded	
(1) Magnesium corresponding to	0·025 grms. of MgO
(2) ,, ,, to	0·027 ,, ,,

THE GASES OF THE PLASMA AND SERUM.

It will be convenient to postpone a lengthened consideration of this subject to the section which treats of the gases of the blood as a whole, and to the chapter on Respiration. In this place it will suffice to make the following remarks :

When the blood is heated in a Torricellian vacuum it gives up more than half its volume of a mixture of gases, composed of oxygen, carbonic acid, and nitrogen, which in the blood itself were contained

partly in a state of solution and partly in the form of feeble chemical compounds.

Whilst the oxygen which is contained in the mixed gases is derived wholly from the decomposition of the oxy-haemoglobin of the blood corpuscles, the greater part of the carbonic acid and the whole of the nitrogen are derived from the plasma, in which they exist mainly in a state of simple solution, though the carbonic acid is *in part* in a state of feeble combination, probably in the form of sodium hydric carbonate, $NaHCO_3$.

Sect. 4. The Coloured Corpuscles of the Blood.

Shape, size, &c. of coloured corpuscles.

The red colour of the blood of vertebrates is due to the suspension in a colourless or slightly coloured liquid of large numbers of solid bodies, of which the principal solid ingredient is a red colouring matter, haemoglobin.

In the blood of man and the mammalia generally[1], the coloured blood corpuscles are non-nucleated biconcave disks, whilst in the blood of birds, reptiles, and most fishes they are nucleated, elliptical, biconvex bodies.

The size of the coloured blood corpuscles of the various orders of mammals varies somewhat, though with some exceptions not within very wide limits. The red blood corpuscles of man are amongst the largest, being for instance larger than those of any domestic animal inhabiting Europe.

The diameter of the average red blood corpuscles of human blood is about the $\frac{1}{126}$th of a millimetre, or about $7{\cdot}9\mu$[2], and the thickness about $1{\cdot}8\mu$; expressed in English measurements the average coloured blood corpuscle measures about $\frac{1}{3200}$th of an inch in diameter and about $\frac{1}{12400}$th of an inch in thickness (Gulliver)[3].

By means of a method which could only yield rough approximations to the truth, Welcker[4] determined the approximate volume of a human coloured blood corpuscle to be $0{\cdot}000000072$ MM. Cube or seven ten-millionths of a cubic millimetre, and the approximate superficial area to be about 0·000128 or rather more than one ten-thousandth of a square millimetre.

[1] In the blood of the Camelidae the red corpuscles are oval.

[2] The Greek letter μ is now employed to represent the *micro-millimetre*, or 1000th part of a millimetre, which is taken as a convenient unit for microscopic measurement. The micro-millimetre corresponds to about $\frac{4}{100000}$ths of an English inch or more accurately to 0·00003937 of an inch.

[3] By far the most complete set of measurements of the corpuscles of the vertebrata was made by Mr Gulliver, and the results of his researches were collected together and published as a note (CXVIII) to his edition of Hewson's works. They are transcribed, with some additions, by Milne-Edwards, *Leçons sur la Physiologie*, Vol. I. p. 84 et seq.

[4] Welcker: for an account of his method followed see Stricker's *Human and Comparative Histology*, Vol. I., Article "Blood," by Rollett, p. 383.

The views of observers respecting the structure of coloured blood corpuscles.

The structure of the red blood cell has been a matter of the greatest interest to histologists and physiologists, chiefly, it must be confessed, on account of the important part the red blood corpuscle has played in the Theory of Cells. A brief review of the opinions which have been held will here be attempted.

One view of the structure of the red disk in man is to be mentioned rather because it is curious than because it is important. Della Torre[1] sought to explain the well-known optical characters of the centre of the disk by supposing that the red corpuscles were in reality little rings. Excluding this view, the remaining discussions of the structure of red blood corpuscles may be grouped under two main questions and a subsidiary one. *Has the red corpuscle of man a nucleus? Has the red corpuscle generally a cell-membrane?* And the corollary question to the latter, *Does the red colouring matter reside in the contents of the vesicle or in the membrane?*

A nucleus was early detected by Hewson in the red blood corpuscles of the frog, and for some time it was tacitly assumed that a similar structure was to be found in the corresponding corpuscles of mammalia. But it should be clearly kept in mind that, from the first, it was analogy rather than direct observation which supported this view. Accordingly we find the red corpuscles of man playing the part of a non-nucleated cell in the reform of the old Cell Theory which was consummated in 1861. In more recent days the only supporter which the original doctrine has found has been Böttcher[2]. For the present, therefore, we may regard the red corpuscles of mammals as non-nucleated.

The question of the membrane was longer and more hotly debated than that of the nucleus. As early as 1685 the red corpuscles were spoken of as vesicles[3]; and by many subsequent writers, among whom we may mention Hewson and Wells[4], the same doctrine is openly held. Wells is said to have been the first to discuss the question systematically, bringing in support of his view the facts of the action of water and saline solutions upon the red cell. To the names of Hewson and of Wells we must add that of C. H. Schultz, who was believed by Schwann[5] to have been the first to demonstrate the vesicular nature of the blood cells. Schwann himself (*loc. cit.*), as is well known, maintained the same view; and for many years afterwards it was the prevailing doctrine. Excluding the botanical prejudices of Schwann's Cell Theory, the only definite grounds for the belief in the vesicular nature of the red cell were the appearance of the cells when irrigated with water and solutions of salts. In the case of the former, the cells swell up and become globular, with a diameter less than the long, and greater than the short, diameter of the original flattened disk. In the case of saline solutions of greater density than the normal blood plasma the outer surface of the corpuscles assumes a

[1] Della Torre, *Nuove osservazioni microscopiche*. Naples, 1776. Milne-Edwards, *Leçons*, Vol. I. p. 63.

[2] Böttcher, Virchow's *Archiv*, Bd. XXXVI. and XXXIX. (See Article "Blood Corpuscles" in Stricker's *Handbook*.) *Quarterly Journal of Microscop. Science*, N. S., Vol. XVII. 1877, p. 377.

[3] Bidloo, *Anatomia humani corporis*, 1685. Milne-Edwards, *Leçons*, Vol. I. p. 66.

[4] Wells, "On the colour of the blood." *Phil. Trans.* 1797, p. 429. Milne-Edwards, *Leçons*, Vol. I. p. 66.

[5] Schwann, "Microscop. Researches." *Syd. Soc.* 1847, p. 67.

folded or creased appearance like an ill-fitting glove. It cannot be denied that these appearances are, at first sight, strikingly suggestive of a membranous envelope; but how little they prove the existence of such an envelope the reader will find in Brücke's criticism of the question, too long to be reproduced or even epitomized in this place[1]. With this undermining of the main support of the vesicular theory, its essential weakness became evident. In the first place it is almost inconceivable that a fluid-filled vesicle with walls which may collapse, should maintain the shape of a biconcave or biconvex disk. In the second place, notwithstanding the frequency of the search, no one has yet detected a structure at all resembling the empty husk or skin of a red blood corpuscle. Indeed, by alternately freezing and thawing blood, the coloured contents may be extracted from blood corpuscles, leaving a colourless structure often exactly resembling in shape and elastic property its red original. Of similar import is the observation that the red corpuscles of amphibia may be cut with a fine razor without the escape of any coloured contents[2]; as well as the observations that the corpuscles may be eviscerated of their nuclei, becoming non-nucleated, but still coloured, spheroids; and that two coloured cells may actually become fused into one[3]. When to these considerations we add that no one has ever observed a double contour around the red blood cells, even when they are swollen and spherical under the influence of water, and that the attention of Schwann himself was arrested by its absence[4], we may acknowledge how very doubtful the alleged membrane of red blood cells has always been.

Most observers who adopted the view of the vesicular nature of red blood corpuscles believed that the envelope included the coloured contents. But the membrane was acknowledged to be slightly tinted red by Schwann[5], who remarked that, were it not so, the biconcave centre of the red cell of man would appear colourless. On the other hand Prevost and Dumas[6], who succeeded in rupturing the corpuscles so as to permit the escape of the nucleus, advanced the opinion that the colouring matter was not in the contents, but in the skin.

While, however, the prevailing idea of the red cell was that of a vesicle, there were not wanting other ideas of it which approached the modern one. Blumenbach[7], in 1797, taught that the globules were small semi-solid or gelatinous, lenticular masses, as did also de Blainville[8]; and, later, Donné[9] adopted a similar view.

[1] E. Brücke, "Die Elementarorganismen." *Sitzungsber. d. k. Akad. Wien*, Vol. XLIV. Abth. II. p. 387.

[2] Krause, *Menschliche Anatomie*, 1876. 3rd edit. Vol: I. p. 328.

[3] Article "Blood," by Rollett in Stricker's *Handbook*. *Syd. Soc. Trans.* Vol. I. p. 391.

[4] Schwann, *loc. cit.* p. 69.

[5] *Loc. cit.*

[6] Prevost and Dumas, "Examen du sang et de son action dans les divers phénomènes de la vie." *Biblioth. univer. de Genève*, XVII. pl. III. fig. 3. Milne-Edwards, *Leçons*, Vol. I. p. 67.

[7] Blumenbach, *Institutions physiologiques*, traduit par Paget, 1797, p. 9. Milne-Edwards, *Leçons*, Vol. I. p. 67.

[8] de Blainville, *Cours de physiologie*, I. p. 214. Milne-Edwards, *Leçons*, Vol. I. p. 67.

[9] Donné, *Thèse sur les globules du sang*, 1831, p. 13. Milne-Edwards, *Leçons*, Vol. I. p. 67.

To-day, this simple notion is enlarged into the doctrine of the 'stroma.' The red blood cell consists of a cavernous mass or 'stroma,' denser at the periphery than at the centre, whose external limit or boundary appears as a sharp contour. It is colourless and highly elastic: it is albuminous in substance, and generally admitted to be non-contractile. In the central trabeculae of the mass the nucleus is embedded, in those red corpuscles which are nucleated. The interstices are quite filled by the coloured substance of the corpuscle, which, under certain conditions (*e.g.* cautious irrigation with water, or with boracic acid of 2 p.c.), retreats from the edge upon the centre in a more or less regular manner. The stroma has been called by Brücke the *oekoid*, and the contained coloured matter the *zoöid*. The special appearances upon which this view is founded have already been stated. The view is not inconsistent with any of the known reactions of blood corpuscles; and it is especially adapted to interpret the concentration of the zoöid in the interior of the oekoid[1].

Enumeration of the corpuscles.

Principle upon which all methods of enumeration are based.

It might at first appear hopeless to attempt to count the number of the blood corpuscles in the blood, especially when we mention at the outset that one cubic millimetre of blood is estimated to contain about 5 millions of corpuscles. But the possibility of carrying out the process so as to permit of a fair approximation becomes evident so soon as the principle is grasped, upon which all the methods are based, all being but modifications of the method suggested by Vierordt and first carried out in all detail by that observer and by Welcker, whose results have received full confirmation by the numerous researches carried on by the aid of the more easy methods of Malassez, Gowers, &c.

The principle, then, is to dilute a known volume of blood with a sufficient but definite and known quantity (say 100 times its volume) of some colourless transparent solution which will not destroy the blood corpuscles, and which will affect their shape as little as possible; thereafter to take a known and very minute volume of the diluted blood, and with the aid of suitable micrometric arrangements to count the corpuscles in it.

The methods will now be described in detail.

Vierordt and Welcker's method[2].

"A measured volume of blood is diffused as equably as possible in a thousand times its volume of an indifferent fluid (six grammes of NaCl in one litre of water, according to Welcker). A small quantity of the mixture is taken up in a capillary tube of known calibre, and the length of the thread of fluid is estimated under the microscope by means of a micrometer. When the contents of the tubule have thus been ascertained, they are quickly distributed

[1] For fuller information the student is referred to the article on "Blood" by Professor Rollett, in Stricker's *Handbook*.

[2] Welcker, "Grösse, Zahl, Volum, Oberfläche, und Farbe der Blutkörperchen bei Menschen und bei Thieren." Henle u. Pfeufer's *Zeitschrift für rat. Medicin*, Dritte Reihe, Vol. xx. Heft 1 and 2, page 257.

with a little solution of gum upon a slide, and the whole is allowed to dry. The preparation is covered with a micrometer divided into squares, and the corpuscles in the several squares can then be successively counted[1]."

Method of Malassez[2]. In this case, as in all other methods, the blood, which is generally obtained by pricking the finger, is mixed with a known volume of a suitable solution. This is effected by means of the little pipette A, which is specially constructed for the purpose; the longer portion of the tube through which fluid is sucked is of capillary diameter and bears the mark 1 near the bulb.

On the shorter tube of the pipette, to which an indiarubber tube is attached for facilitating aspiration, is marked the letter *C*. The bulb of the pipette contains a glass bead.

Blood is now drawn into the pipette to the mark 1, then a solution of sodium sulphate or Potain's solution is aspirated until it reaches the level of *C*; the blood and its diluent are then mixed by agitating the pipette, the bead facilitating the process. The mixture is next introduced into a flattened capillary tube which is connected to a glass slide (B) and which has been accurately calibrated so as to determine the cubic capacity of various lengths of the tube in fractions of a cubic millimetre. The results of the calibration are engraved on the glass slide. Thus in

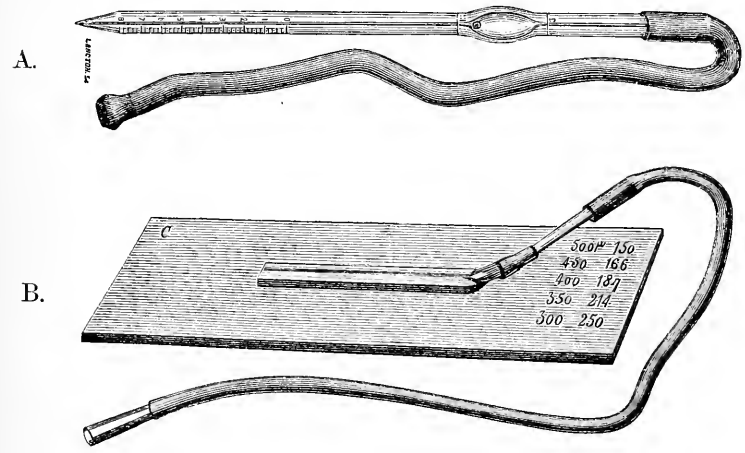


FIG. 12. MALASSEZ' APPARATUS FOR THE ENUMERATION OF BLOOD CORPUSCLES.

A. Pipette in which blood is measured and diluted (*Mélangeur*).
B. Flattened capillary tube fixed to slide, and calibrated.

[1] Stricker's *Human and Comparative Histology*, Art. "Blood," by Rollett, Vol. I. p. 383; see also for a description of Vierordt's original method, Gscheidlen's *Physiologische Methodik*, p. 378.

[2] Malassez, "De la numération des globules rouges du sang chez les mammifères, les oiseaux et les poissons." *Comptes Rendus des Séances de l'Acad. des Sciences.* 2 Décembre, 1872.

the diagram those to the left indicate lengths in micro-millimetres, those to the right capacities in fractions of the cubic millimetre.

Thus the numbers in the first row indicate that 500 μ of the tube contain $\frac{1}{150}$ of a cubic mm., again the numbers in the second row indicate that 450 μ contain $\frac{1}{166}$ of a cubic mm.

The number of corpuscles in a known length of the tube has now to be counted. In order to do so the slide B is placed upon the stage of a microscope whose eye-piece is provided with a micrometer ruled in squares. As a preliminary, however, observations must be made to find the value of the squares of the eye-piece micrometer in terms of a stage micrometer divided into millimetres. By drawing out or in the draw-tube of the microscope the side of the large square is made to correspond exactly with that of the number of micro-millimetres of the stage micrometer, placed on the slide.

For instance one side of the large square (which is divided into 100 smaller squares) is made to correspond to 500 or say 450 μ. The slide with the capillary tube is now substituted for the stage micrometer and the number of corpuscles contained in a certain number of the smaller squares is counted.

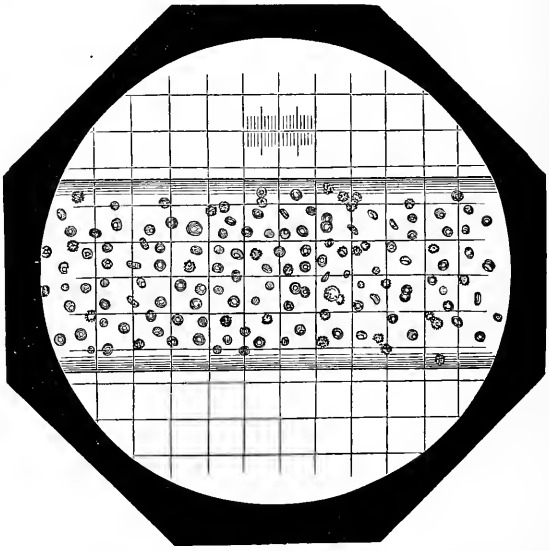


FIG. 13. THE CAPILLARY TUBE OF MALASSEZ' APPARATUS. Viewed in a microscope provided with an eye-piece micrometer ruled in squares. Magnifying power 100 diameters. (Copied from Ranvier.)

Knowing first the length of the tube covered by the squares, second the capacity of this length of the tube, we can by an easy calculation ascertain the number of corpuscles in a cubic millimetre of the diluted, and therefore also of the undiluted, blood. According to Malassez his method

occupies from first to last not longer than ten minutes and the mean possible errors amount to 2 or 3 per 100.

Method of Hayem and Nachet. This method is almost exactly similar to that to be next described at length as Dr Gower's. The diluted blood is introduced into a cell of exactly known depth, and the number of corpuscles is counted by means of an eye-piece micrometer similar to that used in the method of Malassez.

Method of Dr Gowers. (The Haemacytometer.) Dr Gowers has modified the instrument of MM. Hayem and Nachet and to it has given the name of the Haemacytometer[1].

The following description of his method is taken from an article by Dr Gowers in the *Lancet* for December 1, 1877 :—

"The Haemacytometer consists of (1) A small pipette, which, when filled to the mark on its stem, holds exactly 995 cubic millimetres. It is furnished with an indiarubber tube and mouthpiece to facilitate filling and emptying. (2) A capillary tube marked to contain exactly 5 cubic millimetres, with indiarubber tube for filling, &c. (3) A small glass jar in which the dilution is made. (4) A glass stirrer for mixing the blood

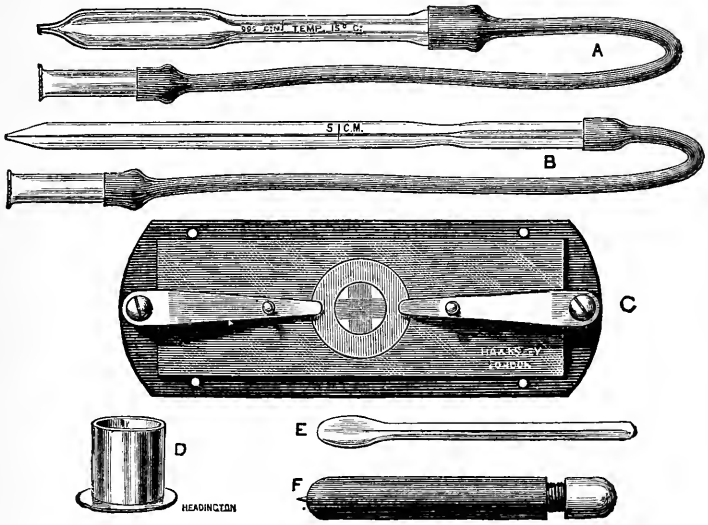


FIG. 14.

A. Pipette for measuring the diluting solution.
B. Capillary tube for measuring the blood.
C. Cell with divisions on the floor, mounted on a slide, to which springs are fixed to secure the cover-glass.
D. Vessel in which the solution is made.
E. Spud for mixing the blood and solution.
F. Guarded spear-pointed needle.

[1] The Haemacytometer is made and sold by Mr Hawksley, Surgical Instrument Maker, 300, Oxford Street, London, W.

and solution in the glass jar. (5) A brass stage plate, carrying a glass slip, on which is a cell, $\frac{1}{5}$ of a millimetre deep. The bottom of this is divided into $\frac{1}{10}$ millimetre squares. Upon the top of the cell rests the cover-glass, which is kept in its place by the pressure of two springs proceeding from the ends of the stage plate.

"Various diluting fluids[1] have been recommended in order to change as little as possible the aspect of the corpuscles. It is not well, however, to observe the characters of the corpuscles during the numeration. Whatever solution be employed, the corpuscles are more or less changed by it. One which answers very well is a solution of sulphate of soda in distilled water, of a specific gravity of 1025.

"The mode of proceeding is extremely simple. 995 cubic millimetres of the solution are placed in the mixing jar; 5 cubic millimetres of blood are drawn into the capillary tube from a puncture in the finger, and then blown into the solution. The two fluids are well mixed by rotating the stirrer between the thumb and finger, and a small drop of this dilution is placed in the centre of the cell, the covering glass gently put upon the cell, and secured by the two springs, and the plate placed upon the stage of the microscope. The lens is then focussed for the squares. In a few minutes the corpuscles have sunk to the bottom of the cell, and are seen at rest on the squares. The number in ten squares is then counted, and this multiplied by 10,000 gives the number in a cubic millimetre of blood.

"The average of healthy blood was decided by Vierordt and Welcker to be 5,000,000 per cubic millimetres, and later results agree with this sufficiently nearly to justify the adoption of this number as the standard, it being remembered that in a healthy adult man the number may be a little higher, in a woman a little lower."

By employing the methods previously described the following results have been obtained:

Welcker found a cubic millimetre of the healthy blood of man to contain 5,000,000 corpuscles. Malassez found the number to vary between 4,000,000 and 4,600,000, the average being about 4,500,000.

Malassez has determined the total number of corpuscles in the blood of a variety of animals, and he has determined for each the number of corpuscles corresponding to a unit weight (one gramme) of the body. This number he proposes to designate as the 'corpuscular capacity' ('capacité globulaire') of the blood. In the case of man the corpuscular capacity amounts to 341,000,000,

[1] The following diluting solutions have been employed:—

a. A solution of sulphate of soda and distilled water of specific gravity 1025.

b. Potain's Solution:—Solution of gum acacia sp. gr. 1020. One volume. Equal parts of sulphate of soda and chloride of sodium, in solution of specific gravity 1020. Three volumes. Mix.

c. Keyes' Solution:—

"Take urine slightly phosphatic, easily obtainable after a meal, about 1020 sp. gr., and make of it a saturated solution of borax. Clouds of earthy phosphates are thrown down. Filtration yields a clear alkaline fluid of sp. gr. about 1030. Add water enough to reduce the specific gravity to 1020, and the fluid is ready for use."

whilst the total number of corpuscles amounts to about 22,500 milliards[1]. Admitting the superficies of each blood corpuscle to be $\frac{128}{1000}$ths of a millimetre square, then the total superficies of the blood corpuscles of men would amount to about 2880 square metres, *i.e.* to the area of a square, each of whose sides is about 53·66 metres long.

Density and weight of the coloured corpuscles. According to C. Schmidt the coloured corpuscles of the blood have a specific gravity of 1089, and according to Welcker of 1105.

The student may feel some curiosity to know the method which was followed in making these determinations, and it may be said at once that the above results were obtained by calculation, and that their correctness depends upon the reliability of several data. Assuming that we know 1st the weight of the moist corpuscles in a known volume of blood, 2nd the specific gravity of the same blood defibrinated, 3rd the specific gravity of the serum, and if we further assume that the specific gravity of the serum does not differ sensibly from that of the plasma, we have all the data required. Thus to take an example from Lehmann's *Physiological Chemistry:* If we assume, for instance, that a specimen of blood contains 496 parts of moist cells per 1000, besides 4 parts of fibrin, that the specific gravity of the serum is 1028, and that of the defibrinated blood 1057·4, then we may very readily determine the density of the blood cells by the following considerations:

1000 − 4 = 996 parts of defibrinated blood occupy the space of 941·93 parts of water,
500 parts of serum ... „ 486·38 „ „
hence 496 parts of blood cells ... „ 455·55 parts of water.

455·55 : 496 :: 1000 : 1088·8.

The density of the blood cells in this specimen of blood must therefore be 1088·8[2]. It must be remarked that the weight of the moist blood corpuscles can only be determined in an approximately accurate manner.

Welcker has calculated the approximate weight of the coloured blood corpuscle to be 0·00008 or $\frac{8}{100,000}$ ths of a milligramme[3].

Summary of the composition of the coloured corpuscles.

Before describing the principal organic and mineral constituents of the red blood corpuscles it will be convenient to place before the reader the following analytical data.

[1] A milliard (Fr.) is one thousand millions.
[2] Lehmann, *Physiological Chemistry*, Vol. II. p. 163.
[3] Welcker, *loc. cit.* p. 274.

I. According to Carl Schmidt 1000 parts of moist blood corpuscles contain—

Water		688	parts.
Solid constituents	Organic .	303·88	,,
	Mineral .	8·12	,,

II. According to Hoppe-Seyler and Jüdell[1]
100 parts of dried corpuscles contain—

	Human Blood. I.	Human Blood. II.	Blood of Dog.	Blood of Goose.
Proteids .	12·24	5·10	12·55	36·41
Haemoglobin	86·79	94·30	86·50	62·65
Lecithin .	0·72	0·35	0·59	0·46
Cholesterin .	0·25	0·25	0·36	0·48

We shall now more in detail examine the various proximate constituents of the corpuscles.

The Stroma and the proteids associated with it.

Mode of obtaining the stroma for microscopic examination.

It has been asserted (p. 74) that histologists have abandoned the view that the coloured corpuscle is a vesicular body possessed of a cell-wall enclosing more or less liquid contents, and have come to consider the coloured corpuscle as being a viscous solid formed of a stroma or framework in which are imbedded the other proximate principles.

In order to demonstrate the existence of the stroma, defibrinated blood is allowed to flow drop by drop into a platinum or silver dish, which is cooled to -13° C., by immersion in a freezing mixture, care being taken that the blood contained in the capsule is frozen before more is added. The frozen blood is then thawed and heated to 20° C. The process of freezing and thawing may with advantage be repeated several times. The blood will be then found to have lost its opaque red colour and to present the appearance of a transparent lake-coloured fluid. On microscopic examination the non-nucleated coloured blood corpuscles are found deprived of all colour, sometimes retaining their original shape, but more frequently either more globular or more shrivelled than normal. The stromata retain, according to Rollett, the extensibility and the elasticity of the original blood corpuscles. Under the influence of the changes of temperature the haemoglobin has entirely dissolved in the serum, leaving the colourless stroma in which it had been deposited.

The stroma is insoluble in serum, dilute solution of salt and of sugar, and in distilled water at a temperature below 60° C., but readily soluble in serum containing alcohol, ether, or chloroform: in solutions of caustic alkalies: and in solutions of alkaline salts of the bile acids (Kühne).

[1] Jüdell, "Zur Blutanalyse." Hoppe-Seyler's *Med. Chem. Untersuchungen.* Heft III. p. 386.

Mode of separating the proteids of the stroma.

When defibrinated mammalian blood is mixed with ten times its volume of a solution of sodium chloride (made by mixing 1 volume of a saturated solution of NaCl with nine parts of H_2O) and set aside for a day, the blood corpuscles are, for the most part, deposited as a slimy precipitate. The fluid is decanted from the precipitate, which is again treated with the same weak solution of common salt and set aside for a day, when, after decanting the fluid, the corpuscles are obtained almost absolutely free from adhering serum. By employing the centrifugal machine in effecting this separation the whole process from first to last may occupy only a few hours. If the precipitate obtained in this way is now treated with water without being disturbed, the haemoglobin contained in the corpuscles is dissolved and there remains behind a gelatinous mass, which may be shaken with water and ether, and then separated by filtration. The body thus obtained is insoluble in water, soluble for the most part in a very weak solution of sodium chloride, and in water which contains 0·1 p.c. of HCl, and in weak solutions of alkalies. This body possesses all the characters of the globulins, and is said by Kühne to act fibrinoplastically[1]; he considers it to be paraglobulin. Instead of employing the above method, which we owe to Hoppe-Seyler[2], we may adopt a simpler method recommended by Kühne[3], and having separated the corpuscles as completely as possible from the serum (in this case too the centrifugal machine should if possible be used) these are treated with a large quantity of water. The solution is then subjected to a stream of CO_2 as long as white flakes continue to separate. The portion of this precipitate which is soluble in water holding oxygen in solution is composed of paraglobulin.

According to Kühne the red blood corpuscles were to be looked upon as the chief source of the paraglobulin of the blood, and this view was at one time shared by A. Schmidt. This author now, however, refers all the paraglobulin of the serum to the breaking down of the colourless corpuscles[4].

Peculiarities of the stroma of the nucleated coloured corpuscles.

If the blood of the newt or frog be placed in a microscopic gas chamber[5] and subjected to the action of a stream of CO_2, the nucleus, which was at first scarcely, if at all, visible, becomes beautifully distinct and somewhat granular; if a stream of oxygen or atmospheric air be then substituted for the CO_2 the nucleus

[1] We have seen that, according to Hammarsten, there is reason to doubt the existence of any specific fibrinoplastic substance, the separation of fibrin being brought about under certain circumstances by other bodies than paraglobulin. See p. 51 et seq.

[2] Hoppe-Seyler, *Handbuch d. physiologisch- und pathologisch-chemischen Analyse.* 3te Auflage, Berlin, 1870, p. 318.

[3] Kühne, *Lehrbuch der physiol. Chemie*, p. 193.

[4] "Ueber die Beziehungen des Faserstoffes zu den farblosen und den rothen Blutkörperchen und über die Enstehung der letzteren." Pflüger's *Archiv*, Vol. IX., p. 353—358. Maly's *Jahresbericht*, Vol. IV., p. 122.

[5] See "Blood Corpuscles," by Dr Klein, *Handbook for the Physiological Laboratory*, p. 17.

disappears. Occasionally this appearance and disappearance may be observed to occur many times in succession. It is pretty obvious that this phenomenon is due to the coagulation by the CO_2 of a proteid existing around the nucleus, and which is probably identical with paraglobulin, the re-solution under the influence of oxygen agreeing with the known characters of that body.

The Nuclei of the Red Corpuscles.

If we except the blood of adult mammals that of all other vertebrates contains red corpuscles possessed of a nucleus. This may, whilst the corpuscle is living and unaltered, be scarcely if at all perceptible, but readily comes into view when weak acids or carbon dioxide exert their action.

Composed according to Brunton of a mucin-like substance.

In order to investigate the chemical composition of the nuclei of coloured blood corpuscles, the blood of birds (and also of snakes) has been employed. In his researches, carried on under the direction of Kühne, Dr Lauder Brunton[1] followed the following process. Defibrinated blood from the bird was treated with ten or twelve times its volume of 3 per cent. NaCl solution, and the corpuscles separated by filtration and decantation. On shaking the residual mass of corpuscles with water and ether, the nuclei of the corpuscles are set free from the stroma, and float at the junction between the water and ether.

In order further to separate the nuclei from adhering stroma and colouring matter, the agitation with ether and water may be repeated several times and the residual matter washed with dilute hydrochloric acid, hot alcohol and ether[2]. From his observations Brunton came to the conclusion which Kühne had previously arrived at, viz. that the nuclei of the blood corpuscles are composed of a substance closely resembling, if not actually identical with *mucin*. He found that they were insoluble in HCl of 0·1 to 1 per cent., but soluble in solutions of the alkalies, the solutions thus obtained being precipitated by the addition of mineral acids, the precipitate being redissolved by an excess of acid. The solutions were precipitated by acetic acid, the precipitate not being soluble in excess, but were not precipitated by solution of mercuric chloride.

Nuclei of blood corpuscles said to contain Nuclein.

Plosz, repeating these experiments of Brunton, confirms the statement that the body composing the nuclei resembles mucin in its properties; on subjecting it to analysis, however, he found that it contained phosphorus, and he therefore considers it to be identical

[1] Brunton, "On the chemical composition of the nuclei of Blood-corpuscles." *Journal of Anatomy and Physiology*. Second series. Vol. III., p. 91.

[2] Plosz, "Ueber das chemische Verhalten der Kerne der Vogel- und Schlangenblutkörperchen," Hoppe-Seyler, *Med. Chem. Untersuchungen*, Heft IV. (1871) p. 460.

with the body separated by Miescher[1] from the nuclei of pus-corpuscles and by him termed NUCLEIN. This body, which will be treated of fully under 'pus,' is unacted upon by gastric juice, so that bodies composed of it (*e.g.* the nuclei of the red blood corpuscles) may be purified by subjecting them to artificial digestion.

The Nuclein of Miescher contains 9·59 p. c. of P, and to it the formula $C_{29}H_{49}N_9P_3O_{22}$ has been ascribed. This formula must be received with great caution.

Fatty matters containing Phosphorus (Lecithin, Protagon?).

Berzelius and Lehmann were aware that the corpuscles contained a fatty body or bodies containing phosphorus, and the second of these observers determined that the ash of the blood corpuscles contains phosphoric acid and has an acid reaction. A closer study of the phosphorized proximate principle of the coloured corpuscles was, however, made by Gobley[2], and afterwards by Hermann[3] and Hoppe-Seyler[4].

Having dissolved the blood corpuscles in water, Hermann agitated the solution repeatedly with ether. The ethereal solution was decanted and evaporated, when it was found to leave a crystalline deposit consisting of cholesterin and tufts of a body which Hermann considered identical with the substance shortly before separated by Liebreich from the brain and called by him PROTAGON.

In order to purify this substance Hermann added water to the mixed crystalline deposit left by the ether; the effect of the water is to cause the protagon to swell and to become less soluble in ether; by the latter reagent the substances soluble in ether are then separated. The residue is dissolved in alcohol heated to 50°; and from the alcoholic solution protagon is obtained in a crystalline form.

Of late years Hoppe-Seyler and, after him, nearly all physiological chemists have come to consider protagon as not being a definite proximate principle but as a mixture of a phosphorized body called lecithin $C_{44}H_{90}NPO_9$, with a body termed cerebrin $C_{21}H_{43}NO_3$, and it is the former substance which, according to Hoppe-Seyler, is contained in the red blood corpuscles. These surmises in reference to the non-existence of protagon have however been disproved by the author, who has shewn that protagon is a perfectly definite proximate principle. The observations of Hoppe-Seyler and Jüdell, however, would appear to be irreconcilable with the view that the coloured corpuscles contain protagon[5].

[1] Miescher, "Ueber die chemische Zusammensetzung der Eiterzellen." Hoppe-Seyler, *Med. Chem. Untersuchungen*, Heft IV. (1871) p. 441.

[2] Gobley, *Journ. de Pharm. et de Chemie*, Ser. III., T. XXI., p. 250.

[3] Hermann, *Archiv f. Anat. u. Physiol.*, 1866, p. 33.

[4] Hoppe-Seyler, "Ueber das Vorkommen von Cholesterin und Protagon und ihre Betheiligung bei der Bildung des Stroma der rothen Blutkörperchen." *Med. Chem. Untersuchungen*, Heft I. (1866) p. 140. Also Gustav Jüdell: "Zur Blutanalyse," Hoppe-Seyler's *Med. Chem. Untersuchungen*, Heft III. (1868) p. 386.

[5] Gamgee and Blankenhorn, "On Protagon." *Journal of Physiology*, 1879.

According to Jüdell, who worked under Hoppe-Seyler's direction, 100 parts of the dried blood corpuscles of man contained (1) 0·35 and (2) 0·72 of lecithin; 100 parts of the dried corpuscles of the dog contain 0·59, 100 parts of the dried corpuscles of the goose 0·46 of lecithin.

Cholesterin.

This body, which will be treated of fully under 'nervous tissue,' is an invariable constituent of the red blood corpuscles and can be separated from them by ether. For the method to be followed the reader is referred to the Appendix. According to Jüdell[1], 100 parts of the dried blood corpuscles of man contain 0·25 of cholesterin. In the dried corpuscles of the goose the cholesterin attained the amount of 0·48 per cent.

It was formerly supposed that the neutral fats were contained in the coloured corpuscles. Hoppe-Seyler[2] has however found that such is not the case.

OXY-HAEMOGLOBIN.

For a long time the opinions of chemists and physiologists were divided as to the nature of the colouring matter of the red blood corpuscles, and for the most part this was supposed to consist of a body termed HAEMATIN, which, as we now know, is but a product of decomposition of the true blood-colouring matter—HAEMOGLOBIN, or as we now term it when loosely combined, as it always is in the blood, with a certain quantity of oxygen, Oxy-Haemoglobin.

Crystals of a beautiful red colour had under certain circumstances been observed to separate from the blood of different animals by Leidig[3], Reichert[4], and Kölliker[5], and had been afterwards more carefully described by Funke, Kunde, and Lehmann.

The researches of several observers, but especially those of Hoppe-Seyler, soon proved that the blood crystals are in reality crystals of the true blood-colouring matter, which forms the chief part of the solid constituents of the red corpuscles, and methods were soon found for obtaining them in large quantities and in a very pure condition. Thanks to these and to the application of varied methods of physical and chemical research, we now have more definite knowledge as to the part played by the blood-colouring matter in the processes of the economy than we possess with regard to any other of the proximate principles of its tissues and organs.

[1] Jüdell, *loc. cit.*

[2] Hoppe-Seyler, *Handbuch d. physiologisch- u. pathologisch-chemischen Analyse.* Dritte Auflage (1870), p. 318, note.

[3] Leidig, *Zeitschrift für wiss. Zoologie.* Bd. I. (1849) p. 116.

[4] Reichert, Müller's *Archiv* (1849), p. 197.

[5] Kölliker, *Zeitschrift für wiss. Zoologie.* Bd. I. (1849) p. 216.

Methods of preparation of Oxy-haemoglobin.

It must be stated *in limine* that great difference exists in the ease with which this body can be obtained in an *obviously pure* condition from the blood of different animals. By *obviously pure* condition we mean to indicate in the form of well-defined crystals.

The principle upon which nearly all methods of separating oxy-haemoglobin is based is the following: to effect the solution of the haemoglobin of the red corpuscles either in the serum or in water added to the separated corpuscles, and then either by the addition of alcohol or by the agency of cold, or of both conjointly, to cause the oxy-haemoglobin (which is sparingly soluble in dilute alcohol and at low temperatures) to crystallize out.

From the blood of some animals, and especially of the rat, oxy-haemoglobin can be obtained for microscopic examination in two or three minutes by receiving a drop of blood on a glass slide, adding to it a drop of distilled water, and after mixing the two together covering with a microscopic covering-glass. Needle-shaped crystals form almost at once. In order to separate considerable quantities of oxy-haemoglobin or even to obtain large crystals for microscopic observation it is advisable to follow one or other of the following methods, of which the fifth and seventh are those which are most easily carried out and most uniformly successful[1].

I. Blood is allowed to coagulate and the clot is allowed to contract so as to separate the serum as completely as possible. (This end is naturally most readily attained by employing a centrifugal apparatus.) The clot is finely divided and then squeezed in a cloth; in this way the corpuscles are separated from the fibrin of the clot.

Water is added to the expressed grumous liquid (*cruor*) in quantity equal to one or one and a half times its volume. A stream of oxygen gas is now passed through the liquid for half an hour, and then a stream of CO_2 for ten minutes. After about five minutes a turbidity appears, crystals commence to form, a large quantity separating out in the course of two hours. By this method crystals are obtained only from the blood of the guinea-pig, the rat, and the mouse. In order to obtain them from the blood of the dog and other animals, before and during the passage of the gases, dilute alcohol is added in small quantities to the fluid, which then often yields a magma of crystals. Crystals thus obtained are, however, not pure, and in order to separate them from adhering impurities they must be washed with distilled water, or water holding a little alcohol in solution, until the filtrate is no longer precipitated by solutions of silver nitrate or of mercuric chloride[2].

Preyer has found that by merely passing air free from carbonic acid through the defibrinated blood of the dog crystallization ensues, even though the temperature of the blood be as high as 35°—38° C.

[1] The description of the first six methods of preparing oxy-haemoglobin is based upon that given by Preyer (in his admirable work entitled *Die Blutkrystalle*, Jena, 1871) as abridged in Maly's *Jahresbericht*, Vol. I. (1873) p. 57; the seventh the Author learned from Professor Kühne; he can highly recommend it.

[2] Lehmann, *Ber. d. königl. sächs. Ges. d. Wiss. zu Leipzig*, 1853. Also *Physiological Chemistry*. Translation by Day. Cavendish Society, 1854. Vol. III., p. 489 et seq.

II. A platinum capsule is placed in a freezing mixture and then freshly defibrinated blood is poured into it, so as to convert it into a red lump of ice. After being in this freezing mixture for half an hour, the blood is allowed to thaw gradually, and the contents of the basin are then poured into a glass vessel of such dimensions that the bottom is covered by the lake-coloured blood to a depth of 15 millimetres; the glass vessel is then set aside in a cool place. In a short time the blood of guinea-pigs and of squirrels furnishes by this method well-formed crystals. According to Rollett, cat's blood is next in the order of facility of crystallization. Then follow dog's blood, human blood, and the blood of rabbits. The blood of the pig and of the frog yield *by this method* no crystals, though their oxy-haemoglobin is crystallizable. In order to obtain crystals from the blood of these animals, the process of freezing and thawing must be frequently repeated[1].

This method is, according to Preyer, very convenient in winter, especially when comparative crystallographic and optical investigations of the oxy-haemoglobin of the blood of many different animals have to be carried on.

III. In this method, the stroma of the coloured corpuscles is dissolved by the addition to the blood corpuscles of a watery solution of crystallized bile (a mixture of sodium glycocholate and taurocholate).

A. The blood of the horse is collected in a cylinder and at once cooled. As soon as the plasma and subjacent stratum of colourless corpuscles have separated, these are separated from the red corpuscles, and the mass of residual red corpuscles is treated with a 0·5 per cent. watery solution of crystallized bile. Then the mixture is allowed to coagulate. The fibrin as it separates encloses the yet undissolved corpuscles, so that the surrounding deeply lake-coloured fluid is entirely free from them. To the fluid, which is kept continually stirred, there is then added 90 p.c. alcohol containing a trace of acetic acid, as long as the precipitate which is produced continues to redissolve. After some hours the fluid is converted into a magma of crystals which are collected on a filter and washed, first with diluted alcohol and then with iced water. Instead of this method we may use:

B. 100 c.c. of dog's blood is allowed to coagulate in a shallow basin; the clot is then separated from the sides of the vessel and set aside for 24 hours. (The centrifugal apparatus might be used with advantage.) The serum is then decanted and the clot washed with water; it is then finely divided and diffused by the help of a syringe through 50 c.c. of water, and after standing for 24 hours is filtered through linen and the residual fibrin washed with 10 c.c. of water. The mixture thus obtained of diluted serum and blood corpuscles is treated with 2 c.c. of a syrupy solution made by dissolving 1 part of crystallized bile in 3 parts of water; after 24 hours every blood corpuscle has disappeared. Nevertheless the fluid filters very slowly. On adding 20 c.c. of 90 p.c. alcohol for every 100 c.c. of the filtrate, the latter is converted into a magma of crystals which are washed first in dilute alcohol and then in iced water.

[1] Rollett, "Versuche und Beobachtungen am Blute." *Sitzungsber. d. math. naturw. Classe der kaiser. Akad. d. Wissenschaft.* Vol. XLVI. (1863). Abth. II., p. 77.

These methods are not to be recommended.

IV. The defibrinated blood of the dog is mixed with its own volume of distilled water and the diluted fluid is treated with one fourth of its volume of alcohol. The mixture is kept for 24 hours at a temperature of 0° C. or below. The crystals which separate are dissolved in as small a quantity as possible of water at 25° to 30° C., and the solution being cooled to 0° C. a fourth of its volume of alcohol is added.

It is better to place the fluid in a freezing mixture at a temperature of $-10°$ to $-20°$ for 24 hours. The whole fluid then becomes filled with a magma of crystals. The process of recrystallization may be several times repeated.

V. Blood is collected in a capsule and, having coagulated, is allowed to stand undisturbed for some hours or, better still, for a whole day. The serum is then decanted, the clot washed with water and cut into small pieces, and these also are repeatedly washed with distilled water. When the washings are no longer strongly precipitated by solution of mercuric chloride, the pieces of clot are treated with water heated to 30°—40° C., and the fluid is filtered, the filtrate being collected in a cylinder surrounded by ice. A known fraction of the red solution is then treated little by little with alcohol (poured out of a burette), the fluid being continually stirred, until a slight precipitate is formed. In this way is ascertained how much alcohol may be added without a precipitate resulting. Having thus found out how much alcohol would have to be added to the whole quantity of filtrate, a somewhat smaller quantity is actually added, and the fluid is placed in a freezing mixture. After some hours crystals separate abundantly. As much water has been employed in the process, the crystals can easily be filtered. These are washed, first with water holding alcohol in solution and afterwards with iced water. The crystals thus obtained may either be at once used or be purified by further crystallization. At a temperature below 0° C. they can be dried in the air without decomposition.

VI. Defibrinated blood is mixed with ten times its volume of a solution of sodium chloride (made by diluting 1 volume of saturated solution of NaCl with from 9—19 volumes of water), and allowed to stand for one or two days in a cool place so as to allow of the greater part of the blood corpuscles to settle. The supernatant liquid is decanted and the corpuscles are placed with a little water in a flask and then ether is added. After repeated agitation, the ether is decanted, and the fluid is filtered through a plaited filter as rapidly as possible. The filtrate is cooled to 0° C. and treated with $\frac{1}{4}$ its volume of spirits of wine; the mixture is then maintained for some days at $-5°$ or $-10°$ C. The crystals which separate may be purified by recrystallization[1].

VII. 500 c.c. defibrinated dog's blood are treated with 31 c.c. of ether and the mixture shaken for some minutes. It is then set aside in a cool place. After a period varying from 24 hours to 3 days the liquid has become converted into a thick magma of crystals. These may be separated by placing in tubes and using the centrifugal apparatus.

[1] Hoppe-Seyler, *Handbuch der physiologisch- und pathologisch-chemischen Analyse.* 3te Aufl., 1870, p. 215.

The cakes of crystals thus obtained are mixed with water holding one-fourth of its volume of alcohol in solution and again centrifugalized. By repeating this process the crystals are obtained free from serum-albumin. If requisite the crystals are dissolved in water and recrystallized by the method mentioned under IV.

VIII. In order to obtain very large crystals of oxy-haemoglobin for microscopic examination, Gscheidlen[1] seals defibrinated dog's blood which has stood in the air for 24 hours in narrow glass tubes (vaccine tubes answer well), and keeps the tubes for some days at a temperature of 37°C. On opening these tubes and emptying their contents into a watch-glass, and allowing some time for evaporation to take place, there are formed crystals of extraordinary size.

Elementary composition of oxy-haemoglobin.

The analyses of Carl Schmidt and Hoppe-Seyler have shewn that crystallized oxy-haemoglobin is a body of perfectly constant composition. Unlike any other of the proximate constituents of the animal body it contains the element iron.

The following table exhibits the mean results of the analyses of Hoppe-Seyler of oxy-haemoglobin from various animals and from the horse. The former were published in 1868[2], the latter in 1878[3]; the latter are so different from the former as to be not above suspicion, especially as they were not actually obtained by Professor Hoppe-Seyler but by an assistant.

PER-CENTAGE COMPOSITION OF CRYSTALLIZED OXY-HAEMOGLOBIN DRIED AT 100° C.

	Water of Crystallization.	C	H	N	O	S	Fe
Crystals from dog's blood	3—4	53·85	7·32	16·17	21·84	0·39	0·43
„ goose's blood	7	54·26	7·10	16·21	20·69	0·54	0·43
„ guinea-pig's blood	6	54·12	7·36	16·78	20·68	0·58	0·48
„ squirrel's blood	9	54·09	7·39	16·09	21·44	0·40	0·59
„ horse's blood		54·87	6·97	17·31	19·73	0·65	0·47

From the analyses of Hoppe-Seyler (excluding that of horse's blood, which is more recent) and of C. Schmidt, Preyer deduced the following as the mean per-centage composition of oxy-haemoglobin :

[1] Gscheidlen, "Darstellung von Hämoglobin Krystallen zu mikroscopischer Beobachtung." *Physiologische Methodik*, p. 361.

[2] Hoppe-Seyler, "Beiträge zur Kenntniss des Blutes des Menschen und der Wirbelthiere." *Med. Chem. Untersuchungen*, Heft III. (1868) p. 370.

[3] Hoppe-Seyler, "Weitere Mittheilungen über die Eigenschaften des Blutfarbstoffs." *Zeitschrift f. phys. Chemie*, Vol. II., p. 150.

	In 100 parts.
C	54·00
H	7·25
N	16·25
Fe	0·42
S	0·63
O	21·45
	100·00

and assuming that the molecule contains one atom of iron the following would be the empirical formula:

$$C_{600}H_{960}N_{154}FeS_3O_{179}.$$

Crystalline form. Oxy-haemoglobin obtained from the blood of man and the majority of the lower animals, crystallizes in prisms or rhombic plates of a beautiful blood-red colour, which belong to the rhombic system; the oxy-haemoglobin of the squirrel crystallizes in six-sided plates which belong to the hexagonal system. The

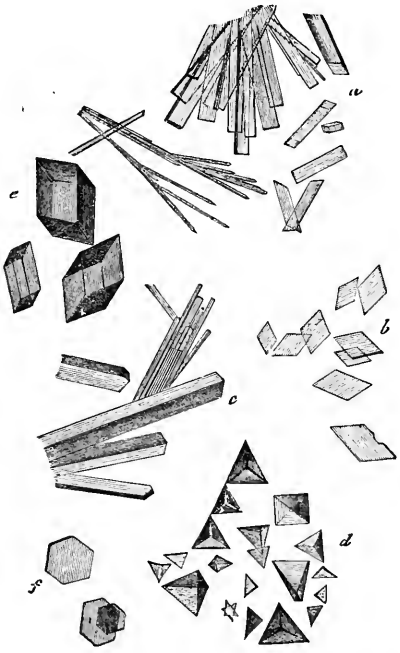


FIG. 15. CRYSTALS OF OXY-HAEMOGLOBIN.

a, *b*, *c* and *e* illustrate the forms in which haemoglobin separates from the blood of man and the majority of mammals. *d* are tetrahedral crystals from the blood of the guinea-pig. *f* are hexagonal crystals from squirrel's blood.

oxy-haemoglobin of the guinea-pig crystallizes in the form of tetrahedra or of tetrahedra with truncated edges and angles, which were at one time supposed to belong to the regular system; they have been proved by Lang[1] to belong to the rhombic system.

Crystals of oxy-haemoglobin, of whatever form, are doubly refracting and pleochromatic; when examined in polarized light the crystals, according to the position of their axes in reference to the observer, appear of a dark reddish-blue or of a bright scarlet colour.

Certain chemical reactions of oxy-haemoglobin.

Oxy-haemoglobin, as obtained by any of the processes above described, presents when moist the appearance of a pasty mass of a cinnabar-red colour; it may be dried *in vacuo* over sulphuric acid at temperatures below 0° C. without undergoing decomposition, and the dried crystals thus obtained are found to be perfectly soluble in water, yielding a solution which presents the optical properties afterwards to be described. The crystals of oxy-haemoglobin dried *in vacuo* still retain 3 or 4 per cent. of water of crystallization, which is driven off by heating to 110° or 120° C. If the crystals of oxy-haemoglobin have been thoroughly dried at a temperature below 0° C., the dried substance may be heated to 100° without undergoing decomposition; the slightest trace of moisture suffices, however, to effect decomposition at much lower temperatures—a decomposition evidenced by the assumption of a brown colour, by the substance becoming incompletely soluble in water, and by a modification of the optical properties (formation of methaemoglobin).

Solutions of oxy-haemoglobin in distilled water if sealed in vessels with no perceptible air-space may be kept for many months, or perhaps years, without undergoing any further change than the reduction or loss of oxygen to be afterwards referred to. The fact is one of importance practically, as enabling standard solutions of haemoglobin to be preserved almost indefinitely[2].

Oxy-haemoglobin obtained from different animals differs in its solubility. That obtained from the guinea-pig is comparatively little soluble, whilst that of bullock's and pig's blood is very soluble. Gautier gives the following order of solubility of the haemoglobin obtained from several animals—cat, dog, horse, man: the degree of solubility increasing according to the order named.

Haemoglobin is readily soluble without decomposition in very weak solutions of the caustic alkalies or of the corresponding carbonates; an excess of alkali, however, very readily induces decomposition.

All acids and salts having an acid reaction decompose haemoglobin with the formation of haematin.

[1] *Sitzungsber. d. Wiener Akad.* Vol. XLVI., p. 85.

[2] Hoppe-Seyler, "Weitere Mittheilungen über die Eigenschaften des Blutfarbstoffs. 2. Ueber die Fähigkeit des Hämoglobins der Fäulniss sowie der Einwirkung des Pankreasferments zu widerstehen." *Zeitschrift f. physiol. Chem.*, p. 125, et seq.

Potassium carbonate added to solutions of haemoglobin precipitates the body without decomposing it, if the temperature be low.

Solutions of haemoglobin are not precipitated by solutions of lead acetate even after the addition of ammonia, nor by silver nitrate, though these reagents soon lead to its decomposition.

Alcohol precipitates haemoglobin, the precipitate having at first a red colour, but soon changing to brown, indicating that decomposition has taken place.

When heated to 70° or 80°, dilute solutions of oxy-haemoglobin undergo, for some time, no decomposition; soon however the liquid becomes turbid and brown, in consequence of the decomposition of the oxy-haemoglobin and the separation of insoluble products.

These reactions will however be studied with greater advantage after a careful investigation of the optical properties of oxy-haemoglobin, as revealed by an examination of the spectrum of light which has traversed crystals of oxy-haemoglobin, solutions of the body, or which has merely been passed through dilute blood.

We have used the term oxy-haemoglobin to denote the colouring matter as it exists in the living blood or as it is obtained by the processes we have described: viz. under circumstances in which it exists in combination with a very small proportion of oxygen—oxygen which is linked to it by ties so easily broken that it can be transferred to other easily oxidizable bodies existing by its side, that it can be given up when its solutions are gently heated in a *Torricellian* vacuum, or are agitated at moderate temperatures with large quantities of inactive gases such as nitrogen or hydrogen—oxygen which may with appropriateness be spoken of as the *respiratory oxygen of haemoglobin.*

The absorption spectrum of oxy-haemoglobin.

It has long been known that if homogeneous white light be passed through certain coloured gases, liquids or solids, and then through a prism, the spectrum instead of being *continuous*, is seen to be intersected by dark lines or bands which are termed *absorption bands*, the spectrum which manifests such bands being designated an *absorption spectrum.* The situation of such absorption bands, being perfectly constant, often affords a valuable means of identification and a ready means of determining the occurrence and course of changes in composition effected in the body which exhibits them.

The blood was shewn by Hoppe-Seyler to exhibit when white light is passed through it a very characteristic absorption spectrum, which he was able to shew is identical with the spectrum of pure oxy-haemoglobin, supplying by this discovery the absolute proof that the blood crystals which had by many observers been suspected to be the pure colouring matter of the corpuscles, actually did consist of that substance.

In examining the absorption spectra of blood or any other solution it is convenient to dilute the liquid sufficiently and then to pour it into a glass vessel with parallel faces, which are a definite width apart. Such

vessels are made, after the plan of Hoppe-Seyler, for the purpose of the physiological chemist, and sold under the name of Haematinometers[1]; the glass plates are exactly one centimetre apart, so that when the apparatus is filled with liquid, the observer knows that he is examining a stratum 1 cm. broad. Instead of such a vessel the Haematoscope or Haemoscope of Hermann[2], shewn in the accompanying woodcut, may be employed. *F* is a plate of glass, and the piston *C* is a metallic tube closed at its inner end by a plate of glass. By sliding the piston *C* in and out of the tube *D* the capacity of the vessel *DFB* and the depth of a stratum of fluid contained between the two glass plates may be modified at will within wide limits. The depth of the stratum is read off by means of a millimetre scale engraved on the sliding tube *C*.

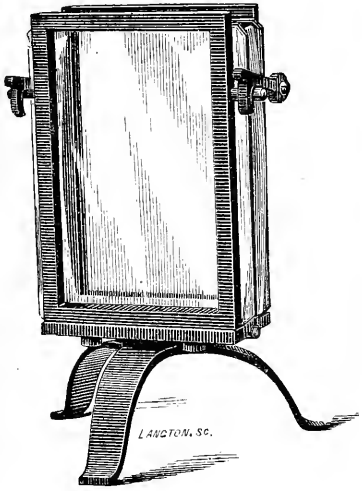


FIG. 16. THE HAEMATINOMETER.

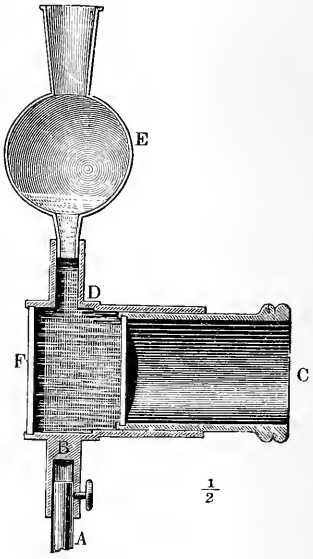


FIG. 17. HAEMATOSCOPE.

Whichever the exact form of vessel containing the blood to be examined, it is interposed between a source of light and a suitable spectroscope.

Various forms of spectroscope may be employed in these researches. Any ordinary spectroscope adapted to the requirements of the chemist will answer; it is advisable, however, that the instrument shall be provided with an arrangement for observing simultaneously two spectra, and with a scale.

[1] These are sold by Schmidt and Haensch, Berlin.

[2] Hermann, "Notizen für Vorlesungs- und andere Versuche." Pflüger's *Archiv*, Vol. IV. (1871) p. 209.

In the annexed drawing the arrangement of the whole apparatus is shewn.

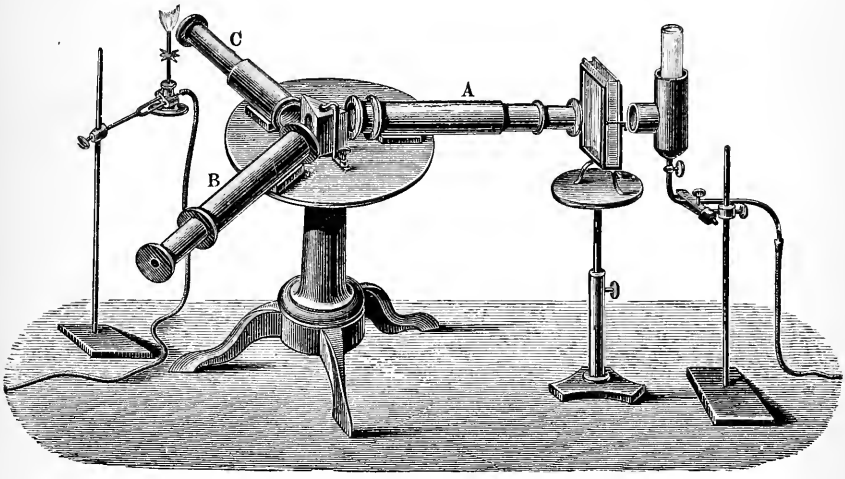


FIG. 18.

1. At *A* is a tube bearing at its distal end a slit which may be made narrow or wide at will and which is provided with a reflecting prism by means of which the spectrum of light from two sources may be simultaneously observed; at its proximal end it is furnished with an achromatic lens. 2. In the centre of the instrument is a flint glass prism which receives the parallel rays which have passed through the slit and collimating lens. 3. At *B* is a telescope into which penetrate the rays which have been dispersed by the prism. 4. At *C* is shewn a tube bearing at its distal end a scale photographed upon glass and which is illuminated by a lamp as shewn in the engraving.

In actually working with such an instrument the observer, having thrown a dark cloth over the prism, commences by adjusting the lights so as to illuminate the slit and the scale, and by adjusting the slit at the end of tube *A*, and focusing the scale in tube *C* and the telescope *B*, he endeavours to get a sharply defined spectrum, and immediately above or below it a well-illuminated image of the scale.

In working with the spectroscope it is of great importance to be able to fix more or less precisely the locality of any line or band which has been observed, and in order to do so various plans may be followed. One most commonly followed is to examine very carefully the spectrum of sunlight and to determine the position of the principal Frauenhofer lines in reference to the scale of the instrument. The observations are tabulated, or a map drawn shewing the position of these lines, which are to serve as land-

marks for future observations. A very useful method of recording the position of lines and bands in the spectrum, lately suggested by Dr MacMunn[1], is thus described in his own words:

"The slit of the spectroscope being illuminated by some light, it is sufficiently narrowed, and the eye-piece focussed, till the Frauenhofer lines are seen distinctly; a camera-lucida is then slipped over the eye-piece, and a point marked—on a piece of paper placed beneath the camera—just beyond the extreme red, and another beyond the extreme violet. A number of blank spectrum maps are then made of this length, and again brought beneath the camera; the position of the Frauenhofer lines is marked on the top one, and afterwards on all the others. In this way a number of solar maps are made, from which any required number can afterwards be copied.

"When an absorption spectrum has to be mapped, a test-tube containing the solution, illuminated by means of a strong light, is placed before the slit, the right-angled reflecting prism is made to cover half the slit, and a Bunsen burner, with a salt of sodium introduced into its flame, is placed so that its light shall fall upon the right-angled prism. On looking into the instrument two spectra are seen, one the absorption spectrum, the other the spectrum of sodium—a yellow line on a dark background. The camera lucida is then slipped over the eye-piece, two maps with the Frauenhofer lines marked on them brought beneath it, and the paper shifted till the bright-yellow sodium line covers the D line on the maps; with a lead pencil the position of the bands and the amount of shading is marked on the maps, care being taken to keep the paper from slipping. It must be remembered that the maps have to be turned upside down while being made, otherwise the A line would be on the right-hand side and the H on the left in the solar maps, and the absorption bands in the wrong place in the others."

Spectroscopes with scales indicating wave-lengths.

For some time past physicists have been in the habit of recording the position of bright or dark lines observed in the spectrum by stating the wave-length of the region in which they occur. Usually the observations have been made with instruments furnished with an arbitrary scale only. Having determined the position of certain lines on the solar spectrum (of which the wave-length is precisely known) in reference to the arbitrary scale, data are obtained for constructing, by an easy geometrical process, a curve which represents the relation of any point on the arbitrary scale to a scale of wave-lengths. The observations which have been made with the arbitrary scale are then reduced to wave-lengths. Though the reduction is somewhat troublesome the observer knows that, when made, his observations have acquired a definiteness which they otherwise would never have possessed. Usually wave-lengths are now expressed in 10-millionths of a millimetre, but other units of measurement may be employed.

Recently Herr Carl Zeiss, the eminent optician of Jena, has, at the suggestion of Professor Abbe, constructed spectroscopes provided with an illuminated scale, which is divided and numbered so as to permit of the

[1] MacMunn, *Studies in Medical Spectroscopy*. Reprinted from the *Dublin Journal of Med. Sc.*, June, 1877.

direct determination of the wave-length of any region in the visible spectrum.

The scale is similar to that shewn below, except that the position of the

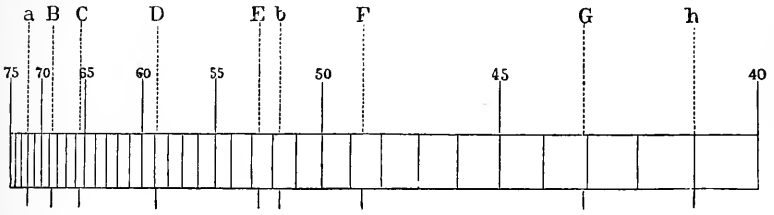


Fig. 19.

Scale of wave-lengths, similar to that in Zeiss's spectroscopes.

lines of Frauenhofer is not marked on the scale. The numbers attached to the divisions on the scale indicate wave-lengths expressed in 100,000ths of a millimetre; and each division indicates a difference in wave-length equal to one hundred thousandth of a millimetre (0·00001 mm.). By the eye, the position of any line situated between two divisions of the scale can be estimated to one-tenth of a division, so that its wave-length can be expressed in millionths of a millimetre. In using Zeiss's instruments, the observer commences by causing Frauenhofer's line D, or the sodium line, to coincide exactly with that part of the scale which expresses its wave-length, that is to say to correspond to division 58·9 of the scale (which expresses a wave-length of 589 millionths of a millimetre or 0·000589). Having done this the scale is accurately set for all other points.

Let us suppose that the observer wishes to determine the wave-length of Frauenhofer's line E. He will at once see that the line is placed between divisions 52 and 53 and he would determine its position between two divisions to within one-tenth of a division, but probably much nearer. The reading would probably be 52·7, which would give for the wave-length of E 527 millionths of a millimetre, a result which is only three ten-millionths below the value deduced from the observations of Angström[1]. As a result of many experiments with one of Zeiss's spectroscopes the Author has found that the mean error in his case is not greater than ± 0·000001 mm.

Printed blank maps accompany Zeiss's instruments, which correspond exactly to the scale of the spectroscope. There is therefore not the slightest

[1] The following are the wave-lengths corresponding to Frauenhofer's lines A, B, C, D, E, F, G, according to the most recent measurements, expressed in millionths of a millimetre:

A 760·4
B 687·4
C 656·7
D 589·4
E 527·3
F 486·5
G 431·0
H 396·8

difficulty in drawing up a map which shall represent the relative and *absolute* position of any lines or bands observed in a given spectrum.

The absorption bands which form the characteristic features in the spectra of blood and certain other animal liquids do not admit of having their limits determined with the same sharpness and precision as is possible in the case of the bright lines in the spectra of incandescent metals or in that of the lines of Frauenhofer in the solar spectrum. It would therefore be mere pedantry to express their position or extent on a wave-length scale to one ten-millionth of a millimetre. In this work all drawings of spectra will be accompanied by a scale of wave-lengths, and the position and extent of bands usually expressed in millionths of a millimetre.

Micro-spectroscopes. Where very small quantities of a solution are to be examined these may be introduced into small cells made by cementing sections of barometer tubing of various lengths and diameters to glass slides. Such a cell may be made which only requires two or three drops of fluid in order to fill it. Instead of employing an ordinary spectroscope we may in this case with advantage employ some form of micro-spectroscope.

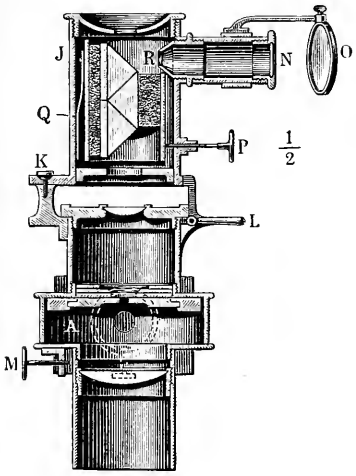


Fig. 20.

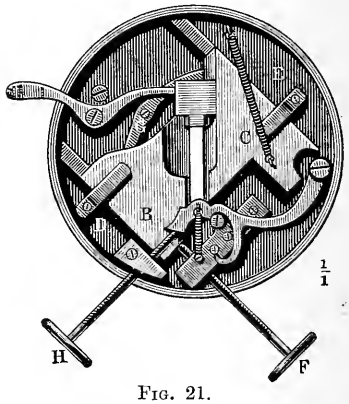


Fig. 21.

Zeiss's micro-spectroscope.

The instrument consists of a drum *A* (Fig. 20) interposed between the field-lens and eye-lens of an eye-piece. Within the drum there is a slit which by means of screws *H* and *F* (Fig. 21) can be lengthened or shortened and made wider or narrower; it also contains a prism whereby light coming from an aperture in a stage at the side of the drum is totally reflected in the direction of the optic-axis of the eye-piece. Over the eye-lens of the eye-piece is situated the combination of prisms with the measuring apparatus; this, which is the spectroscope proper, revolves around the eccentric *K* (Fig. 20); it can either be moved away from the eye-lens or brought over it, and retained there by the catch *L*. At *N* is placed the scale of wave-lengths (see Fig. 20), which is illuminated by the mirror *O*. The screw *P* and the spring *Q* are employed to alter the relation of the scale to the spectrum. The former is always set by the observer so that Frauenhofer's line D corresponds to division 58·9.

The first to apply a spectroscope to the microscope was Mr Sorby[1], and very numerous modifications of his original micro-spectroscopes have been made. In all cases the micro-spectroscope consists of a modified microscopic eye-piece which has superadded to it a direct-vision prism, an arrangement of slits for allowing definite quantities of light to reach the prism, usually arrangements for comparing two different spectra, and finally some micrometric arrangement. In consequence of the admirable nature of the micrometric arrangement we give the preference to the instrument made by Zeiss and of which a vertical and horizontal section are given in Figs. 20 and 21.

Being provided, then, with one or other of the spectroscopes previously described, or with a similar instrument, let the observer interpose between it and some source of light a solution of blood, say made by diluting defibrinated blood with ten times its volume of distilled water contained in a haematinometer (Fig. 16, p. 92) 1 centimetre wide. It will then be found that the whole of the more refrangible portion of the spectrum has been cut off but that the red end of the spectrum remains visible, or rather, those rays having a wave-length greater than about 600 millionths of a millimetre.

If now a stream of hydrogen or nitrogen be passed for a considerable time through the diluted blood it will be observed that the absorption is least between Frauenhofer's line a (W. L. 718) and Frauenhofer's line B (W.L. 686·7), but that the rest of the spectrum is less bright than before the gas was passed. The effect of the N or H has been to drive more or less of the *respiratory oxygen* from the haemoglobin, and in consequence there is more light absorbed; this difference in the spectrum corresponds to the change which the blood undergoes from a bright vermilion colour to a brown-red when it passes from the arterial to the venous condition, in other words from a condition in which its haemoglobin is nearly saturated with its respiratory oxygen, to one in which a portion of that oxygen has been given up.

If now the blood solution be rendered much more dilute so as to contain ·8 p.c. of haemoglobin, on examining a stratum 1 centimetre wide the spectrum becomes distinct up to Frauenhofer's line D (W. L. 589), *i.e.* the red, orange and yellow are seen, and in addition also a portion of the green between b and F. Immediately beyond D and between it and b, however (between W. L. 595 and 518), the absorption is intense. (See Fig. 22, 4.) On diluting still further, what appeared one wide black band between D and E is seen to resolve itself into two beautifully distinct absorption bands separated by a green interspace (Fig. 22, 3). Of these absorption bands, the one next to D is narrower than its fellow; it has more sharply defined edges and is undoubtedly blacker; its centre corresponds with wave-length 579, and it may conveniently be distinguished as the absorption band α in the spectrum of oxy-haemoglobin. The second of these absorption bands, *i.e.* the one next to E, which we shall designate β, is broader, has less sharply defined edges, and is not so dark as α. Its centre corresponds approximately to W. L. 553·8.

[1] Sorby, *Quarterly Journal of Science*, 1865, XI. p. 198.

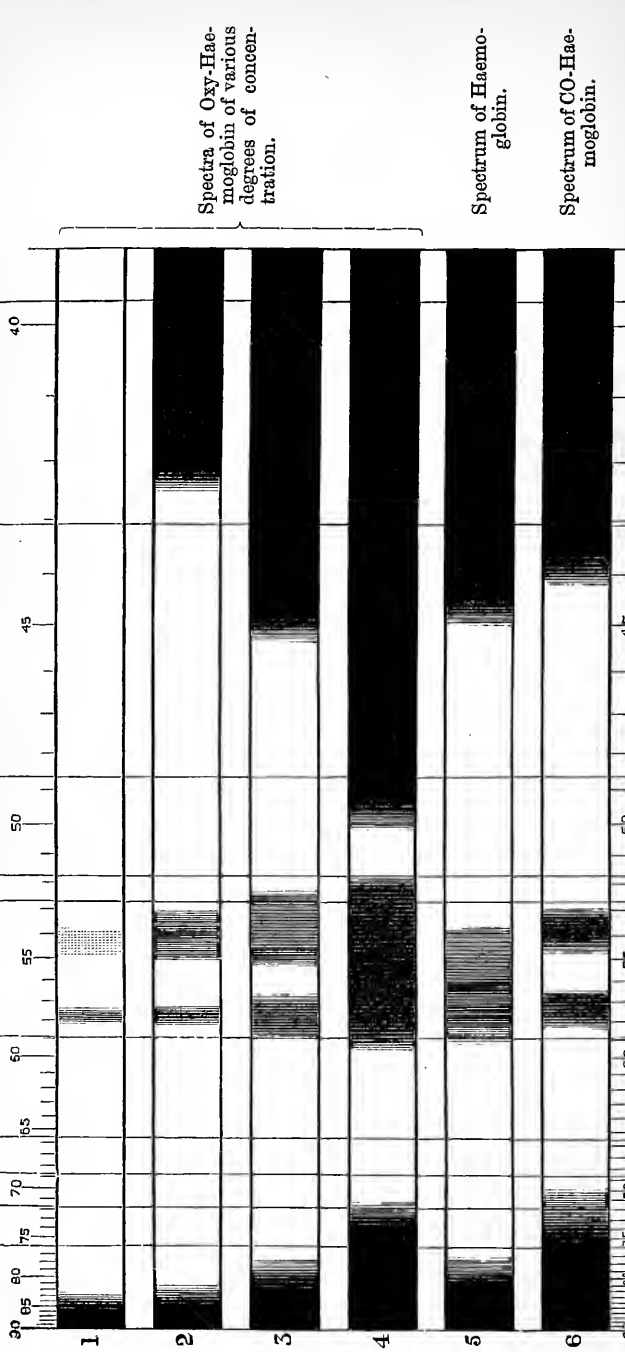


FIG. 22. THE SPECTRA OF OXY-HAEMOGLOBIN, HAEMOGLOBIN, AND CO-HAEMOGLOBIN.
(Copied from the original illustrations of Preyer, but with the addition of a scale of wave-lengths. Each measurement has been repeated by the Author. The numbers attached to the scale indicate wave-lengths expressed in 100,000ths of a millimetre. Each division of the scale represents a difference of wave-lengths of 1-100,000th of a millimetre.)

FIGURE 22.

Spectrum 1. (Preyer, Plate 1, sp. 2 *modified.*) Solution of oxy-haemoglobin containing less than 0·01 p. c. In this as in every other case, a stratum 1 centimetre thick was examined. One distinct, though faint, absorption band (α) between W. L. 583 and 575·1. According to Preyer[1] there is no trace of the band β seen; the Author finds, however, that whenever α is visible he perceives a faint shadow in the position of wave-lengths 538—550. There is no absorption at either violet or red end of the spectrum.

Spectrum 2. (Preyer, Pl. 1, sp. 4.) The solution contains 0·09 p. c. of oxy-haemoglobin, α extends from 583—571 and β from 550—532. The violet end is absorbed to about 428. The red end is scarcely affected.

Spectrum 3. (Preyer, Pl. 1, sp. 6.) The solution contains 0·37 p. c. of oxy-haemoglobin, α extends from 589—567, and β from 553—523. The red end of the spectrum is perceptibly shortened. The violet is entirely, and the blue partly, absorbed, to about 453.

Spectrum 4. (Preyer, Pl. 1, sp. 8.) The solution contains 0·8 p. c. of oxy-haemoglobin. The two absorption bands have amalgamated and one broad band is seen extending from 595 to 518. The green is just visible between 518 and 498; the slightest increase in the strength of the solution causes the green to disappear.

Spectrum 5. (Preyer, Pl. 1, sp. 9.) Solution of haemoglobin (*Syn. reduced haemoglobin*) of about 0·2 p. c. A single broad band with diffuse edges, between 595 and 538; the band is darkest at about 550. Both ends of the spectrum are more absorbed than by a solution of oxy-haemoglobin having the same degree of concentration.

Spectrum 6. (Preyer, Pl. 1, sp. 14.) Solution of carbonic oxide haemoglobin. Two absorption bands very similar to those of oxy-haemoglobin, but moved somewhat nearer to E. α extends from 587 to 564 and β from 547 to 529. The blue and violet are less strongly absorbed than by a solution of oxy-haemoglobin of the same strength.

On diluting very largely with water nearly the whole of the spectrum appears beautifully clear except where the two absorption bands are situated. If dilution be pursued far enough even these disappear; before they disappear they look like faint shadows obscuring the limited part of the spectrum which they occupy. The last to disappear is the band α.

The two absorption bands are seen most distinctly when a stratum 1 cm. thick of a solution containing 1 part of haemoglobin in 1000 is examined; they are still perceptible when the solution contains only 1 part of haemoglobin in 10000 of water.

Haemoglobin (Reduced haemoglobin).

The spectrum of oxy-haemoglobin had been described by Hoppe-Seyler when Professor Stokes made the remarkable discovery that when diluted blood is treated with certain reducing agents the colour of the liquid and its spectrum undergo remarkable changes; the former loses its bright red and acquires a brown colour, whilst the green interspace which

[1] *Die Blutkrystalle: Untersuchungen von W. Preyer; mit drei farbigen Tafeln*, Jena, 1871.

had existed between the absorption bands α and β of oxy-haemoglobin disappears, and instead of the two bands there appears a single one, less deeply shaded and with less finely defined edges, extending between D and E. This band we may distinguish as absorption band γ. (See Fig. 22, Spect. 5 for description.)

Hoppe-Seyler[1], who has described the spectrum of reduced haemoglobin with great care, remarks that when a strong solution of that body is diluted with water, avoiding at the same time the access of oxygen, before the distinct absorption band which we designate γ comes into distinctness there is seen some green light between b and F. As the solution is diluted, the dark absorption band, which extends at first from D to b, diminishes in width, and the blue rays of the spectrum become more distinctly visible.

On further diluting, the single absorption band is observed not to exhibit any trace of subdivision, but to diminish rapidly in intensity, so that in a solution of such concentration that both absorption bands of oxy-haemoglobin would be quite distinct, the single band γ of reduced haemoglobin has disappeared from view. Further, reduced haemoglobin existing in solution is distinguished from oxy-haemoglobin by its stronger absorption of the light between C and D, as well as by its weaker absorption of the blue light above F.

If now the solution which presents this spectrum be shaken with air or oxygen, the single band at once gives place to the two original bands, whilst the liquid loses its brown and reacquires more or less of its florid red colour. The process of reduction and subsequent oxygenation may be repeated many times in succession.

From his experiments Stokes concluded that "*the colouring matter of blood, like indigo, is capable of existing in two states of oxidation, distinguishable by a difference of colour and a fundamental difference in the action on the spectrum. It may be made to pass from the more to the less oxidized state by the action of suitable reducing agents, and recovers its oxygen by absorption from the air*[2]." This surmise has been proved to have been perfectly correct, and to blood-colouring matter after it has lost the oxygen which it can give up to reducing agents, the name of *reduced haemoglobin* is given. By many, as by Hoppe-Seyler, it is termed simply *haemoglobin*, to distinguish it from the body as it exists combined with its respiratory oxygen and which is then termed *oxy-haemoglobin*.

Methods of reducing oxy-haemoglobin to haemoglobin.

Before proceeding further, it is advisable to consider how the blood or a solution of oxy-haemoglobin may be reduced so as to exhibit the spectrum of haemoglobin.

The following are the methods which may be followed:

[1] Hoppe-Seyler, "Beiträge zur Kenntniss des Blutes des Menschen und der Wirbelthiere. Das reducirte Hämoglobin oder der venöse Blutfarbstoff." *Med. Chem. Untersuchungen*, Heft III. (1868) at p. 374 et seq.

[2] Stokes, "On the reduction and oxidation of the colouring matter of the blood," by Professor Stokes, F.R.S. *Proceedings of the Royal Society of London*, Vol. XIII. (1864) p. 357, paragraph 8. Also *Philosophical Magazine*, 1864, p. 391.

1. To a solution of a ferrous salt, as for instance of $Fe\,SO_4 + 7H_2O$, a small quantity of tartaric or citric acid is added, and then ammonia until the reaction is alkaline. In consequence of the presence of the vegetable acid, ammonia does not throw down a precipitate of ferrous hydrate, but a clear light-green solution is obtained which readily darkens by absorption of oxygen from the air. Such a solution when freshly prepared exerts a powerfully reducing action upon oxy-haemoglobin. When added in small quantities to a solution of this body or to blood, the colour and spectrum change almost instantly, to be restored again on agitation with air. Often we may observe that on shaking up the reduced solution with air the spectrum of oxy-haemoglobin is restored, though on leaving the solution a moment or two at rest the two bands again disappear, and the single band of reduced haemoglobin appears, proving that when existing side by side with a ferrous salt, reduced haemoglobin can more readily take possession of oxygen than that substance, to which however it afterwards cedes it.

2. Instead of ferrous sulphate we may employ a solution of a stannous salt prepared after the same fashion, by mixing a solution of stannous chloride, $Sn\,Cl_2$, with tartaric acid and then adding ammonia to neutralization. In this case, as in 1, by rendering the liquid alkaline we prevent it profoundly decomposing the blood-colouring matter, whilst its oxidizing power is increased. The tin presents the advantage over the iron solution of not becoming deeply coloured as it absorbs oxygen, and therefore not absorbing light passed through it.

3. The blood or solution of oxy-haemoglobin is boiled at a temperature of 40^0 C., in a vessel in which a Torricellian vacuum is established by means of a mercurial pump. Very shortly the colour of the liquid and the change in spectrum evidence the complete removal of oxygen.

4. The blood or solution of haemoglobin is subjected for a long-continued period to the action of a stream of washed hydrogen or nitrogen. The same apparatus may be employed for this experiment as is used in preparing Haemochromogen.

Whilst oxy-haemoglobin or its solutions very rapidly undergo change at temperatures above 0^0C. this is not the case with reduced haemoglobin. Hoppe-Seyler has discovered that when a solution of pure oxy-haemoglobin is sealed up in a glass tube (care being taken to include very little air) after undergoing reduction, as exhibited by its change of colour and spectrum, it suffers no further change and may be kept for years. When such a solution is brought in contact with oxygen oxy-haemoglobin is again formed and may even be crystallized. This discovery of Hoppe-Seyler's is of great practical importance to the physiological chemist, as it enables him to prepare standard solutions of oxy-haemoglobin, when temperature and other circumstances are favourable, and to keep them indefinitely for subsequent use.

Hoppe-Seyler has also shewn that reduced haemoglobin resists the action of pancreatic ferment[1].

[1] Hoppe-Seyler, "Ueber die Fähigkeit des Hämoglobins der Fäulniss sowie der Einwirkung des Pankreasferments zu widerstreben." *Zeitschrift f. phys. Chemie*, Vol. I. p. 125.

The facts which have been narrated above supply the chief materials for forming an opinion in reference to the nature of the compound of haemoglobin with oxygen. From them it would appear that this compound is of so remarkable a nature that it may be formed with exceptional facility by the mere contact with atmospheric oxygen, and that it is one which readily undergoes dissociation—the decomposition being one in which the molecule of haemoglobin is left intact and ready to combine again with fresh molecules of oxygen.

The amount of the respiratory or loosely combined oxygen of oxy-haemoglobin.

What is the quantity of oxygen which reduced haemoglobin can link to it as respiratory oxygen?

Preyer[1] as a result of three determinations found that 1 gramme of haemoglobin can link to itself 1·27 cub. cents. of oxygen measured at 0° C. and 1 metre pressure (or 1·671 c. c. measured, as is more usual in England and France, at 0° C. and 760 mm. pressure), and more recently Hüfner[2] has determined the amount again by a different method and has obtained a result almost identical with that of Preyer. According to Hüfner and as the mean of ten separate determinations, 1 gramme of haemoglobin fully saturated with oxygen is associated with 1·28 c. c. of oxygen gas (measured at 0° C. and 1 metre pressure.)

Dissociation-tension of the respiratory oxygen of oxy-haemoglobin.

Oxy-haemoglobin is one of those compounds which at particular temperatures and pressures undergo *dissociation*. At 40° C. the dissociation-tension is equal to about 30 mm. of mercury[3]. The Author attempted some time ago to ascertain the dissociation-tensions of oxy-haemoglobin for various temperatures, but the results which he obtained were not sufficiently accordant to allow of conclusions being drawn from them. The subject will be discussed again under 'Respiration.'

Action of oxy-haemoglobin upon the resin of Guaiacum.

Before leaving this division of our subject we have to refer to a reaction which is possessed by oxy-haemoglobin and by some of its derivatives, though not by reduced haemoglobin, and to which at one time considerable theoretical importance was attached, and which still is of great practical value inasmuch as it affords us the most delicate, though by itself not a conclusive, test for detecting exceedingly minute quantities of these bodies.

It was found by A. Schmidt that when diluted blood is dropped upon a filter paper which has been moistened with tincture of guaiacum and then dried spontaneously in the air, a blue ring forms at the edge of the drop; it is best in this experiment to use blood diluted with 20 times its volume of water, and it may be well to remember that the reaction is one which is not produced by all

[1] Preyer, *Die Blutkrystalle: Untersuchungen von W. P.*, Jena, 1871, p. 134.

[2] Hüfner, "Ueber die Quantität Sauerstoff welche 1 Gramm Hämoglobin zu binden vermag." *Zeitschrift f. physiologischen Chem.* Vol. 1. p. 317.

[3] Worm Müller, "Ueber die Spannung des Sauerstoffs der Blutscheiben." Ludwig's *Arbeiten*, 1870, p. 119.

specimens of tincture of guaiacum. This blueing of the resin of guaiacum is due to its oxidation and is also observed when ozone acts upon it, but not when common oxygen does so.

When the respiratory oxygen of haemoglobin has been expelled from blood by the action of carbonic oxide, as will be afterwards described at length, it no longer possesses (in the absence of oxygen) the power of blueing guaiacum. If atmospheric oxygen, however, comes in contact with the drop of CO blood and guaiacum, the blue ring appears.

Oxy-haemoglobin shares with many other organic bodies and also with many inorganic bodies, such as spongy platinum, the power of decomposing hydrogen peroxide, H_2O_2, as is proved by the effervescence produced in a solution of the latter by the addition of a few drops of blood or of a solution of haemoglobin; if to a mixture of blood and tincture of guaiacum some solution of H_2O_2 be added, the fluid assumes a blueish tint.

Does the oxygen of oxy-haemoglobin possess peculiar activity?

These facts were formerly explained by A. Schmidt on the hypothesis that haemoglobin possesses in an intense degree the power of ozonizing oxygen and of rendering it therefore infinitely more active than atmospheric oxygen. Against this view Pflüger[1] has raised the most serious, and it appears to us the most reasonable objections, which will have to be considered in detail in another section. According to Pflüger when blood is poured upon filter paper, as in the guaiacum experiment previously referred to, the haemoglobin almost instantly undergoes decomposition, and it is the products of decomposition which occasion the reaction. According to Pflüger haemoglobin in no way modifies the properties of the oxygen which it links to itself.

Proportion of haemoglobin in the blood of man.

In former times, when blood-letting was highly prevalent, a large number of analyses of blood were made by competent observers who had no means, such as we now possess, of determining directly the amount of haemoglobin, but who ascertained the amount of iron contained in the blood. Since we now know the exact proportion of iron which haemoglobin contains, we may calculate the amount of this substance found by the older observers. Preyer[2] has taken the trouble to do this in the case of a large number of the most reliable analyses, and from his tables we take the following extract:—

QUANTITY OF IRON AND HAEMOGLOBIN CORRESPONDING TO IT CONTAINED IN 100 GRMS. OF BLOOD.

A. Blood of woman (in health).

	Iron.	Hämoglobin.
Minimum . . .	0·048 grm.	11·57 grm.
Maximum . . .	0·057 „	13·69 „

[1] E. Pflüger, "Kritik über die Angaben vom Ozon im Thierkörper," Pflüger's *Archiv*, Vol. x. p. 252.

[2] Preyer, *Die Blutkrystalle*, p. 117, et seq.

B. Blood of man (in health).

	Iron.	Hämoglobin.
Minimum . . .	0·0508 grm.	12·09 grm.
Average of 11 cases .	0·056 ,,	13·45 ,,
Maximum . .	0·063 ,,	15·07 ,,

The variations which the amount of haemoglobin undergoes in disease will be considered in a future chapter.

Relation of haemoglobin to the number of the blood corpuscles.

By employing methods which will be subsequently described, it is possible to determine with comparative readiness not only the number of corpuscles contained in a certain volume of blood, but also the amount of haemoglobin, and the relation between the weight of haemoglobin and the number of the blood corpuscles. Thus Malassez found the number of red corpuscles in a cubic millimetre of the blood of healthy men to vary between 4,000,000 and 4,600,000, and the amount of haemoglobin between 0·125 and 0·134 of a milligramme[1]. Malassez has actually expressed the mean amount of haemoglobin in each blood corpuscle of man in billionths of a gramme (the billionth of a gramme he represents by the letters $\mu\mu$ gr.); his estimate is that each corpuscle contains on an average 30 $\mu\mu$ gr.

By μ cub. Malassez[2] designates the 1000th part of a cubic millimetre; he takes this as the unit of cubic capacity of the matter of red blood corpuscles, and expresses the amount of haemoglobin in billionths of a gramme ($\mu\mu$ gr.) contained in one μ cub. of corpuscles of various animals, as is shewn below—

	Volume of each corpuscle according to Welcker.	Haemoglobin contained in one μ cub. of corpuscles.
Man . . .	72 μ cub.	0·416 $\mu\mu$ gr.
Dove . . .	125 ,,	0·416 ,,
Lacerta agilis .	201 ,,	0·348 ,,
Rana fusca . .	629 ,,	0·343 ,,
Proteus . . .	9200 ,,	0·115 ,,

These numbers must, however, be received with the greatest caution, and as being very crude approximations to the truth, as will be obvious when we consider that the number of corpuscles found in the healthy blood of man by Malassez differs very notably from that found by other equally competent observers, whose methods were probably more accurate.

Action of certain gases which displace the Oxygen of Oxy-haemoglobin.

Carbonic oxide, CO.

It had been observed by Claude Bernard that the blood of animals poisoned with carbonic oxide uniformly becomes of an intensely florid arterial hue, and that this differs from the normal colour of arterial blood by its persistence.

[1] L. Malassez, "Sur les diverses méthodes de dosage de l'hémoglobine et sur un nouveau colorimètre." *Archives de Physiologie*, 2 ser. vol. IV.

[2] Malassez, "Sur la richesse en hémoglobine des globules rouges du sang." *Gaz. méd. de Paris*, p. 534.

He demonstrated, further, that when blood is shaken up with carbonic oxide, not only does it become florid, but an exchange of gases takes place, the loosely combined oxygen of the blood being expelled from it, and its place taken by an equal volume of carbonic oxide.

After the discovery by Hoppe-Seyler and Stokes of the remarkable spectroscopic properties of the blood-colouring matter, attention was paid to blood which had been treated with CO, and it was found that whilst the spectrum of such blood is almost identical with that of oxy-haemoglobin, it possesses the property of resisting the action of reducing agents.

Subsequently, Hoppe-Seyler found that after passing a stream of CO through a solution of oxy-haemoglobin, and then adding alcohol, on exposing the mixture to cold, crystals separated which were identical in form with those of oxy-haemoglobin, but the solution of which was unacted upon by the agents which reduce oxy-haemoglobin.

From all these observations it resulted that carbon mon-oxide possesses the power of displacing the respiratory oxygen which exists in a state of loose chemical combination with haemoglobin, and of forming a compound possessed of nearly the same physical properties but differing from it in being much more stable; further from the fact that, in the formation of this compound, one volume of oxygen is exactly replaced by one volume of carbon mon-oxide, it follows that a molecule of the latter takes the place of a molecule of the former.

Although the spectrum of CO-haemoglobin very much resembles that of oxy-haemoglobin, there are minute differences which are shewn by comparing the spectra of the two bodies existing in a solution of the same strength, and examined under precisely similar circumstances. It will be seen (Fig. 22, spect. 6 compared with spect. 2), that in the CO-haemoglobin both the bands α and β are moved *very slightly* nearer the violet end of the spectrum. Amongst other points of difference between the CO- and O- compound, we have to mention that the crystals and solutions of the former have a tinge of blue which is wanting in the latter, and that the crystals of CO-haemoglobin are slightly less soluble than those of O_2-Hb.

So far as the Author is aware, Jäderholm[1] and Sorby are the only observers who have stated the position of the bands of oxy-haemoglobin and of CO-haemoglobin in wave-lengths. According to Jäderholm the centre of oxy-haemoglobin α corresponds to W. L. 5730, of β to 5370. On the other hand the centre of CO-haemoglobin α corresponds to W. L. 5690 and of β to 5340. These determinations do not agree with those of Preyer, nor with independent observations of the Author. In the first place the centre of these bands is not constant for solutions of different strengths, for it will be found that the band β extends more towards the blue than the green as the concentration of the solution increases. According to Sorby the centre

[1] Jäderholm; see abstract by Hammarsten in Maly's *Jahresbericht*, vol. IV. p. 106.

of oxy-haemoglobin α corresponds to W. L. 5830, of β to 5450; of CO-Hb α corresponds to W. L. 5755 and β to 5420.

From our own observations we conclude in the case of the band α of oxy-haemoglobin that its centre certainly corresponds almost exactly with W. L. 5780 (expressed in 10 millionths of a mm. for comparison with Jäderholm). The band α of CO-haemoglobin corresponds, on the other hand, approximately to wave-length 5720. The centre of CO-haemoglobin β is from 5340 to 5380 according to concentration. It will be seen that these determinations differ very materially from those of Jäderholm and Sorby.

It has been shewn by the researches of the Author[1], of Donders[2], and of Zuntz[3], that although the compound of CO and haemoglobin is much more stable than that of O, it yet can be decomposed, and CO expelled. By passing a neutral gas, or air, through solutions of CO-haemoglobin for long periods the gas may be gradually driven out, and the haemoglobin again becomes reducible. The same happens if the blood be boiled in the mercurial pump.

The great stability of CO-haemoglobin enables us to detect it in the blood of animals poisoned by this gas or by gaseous mixtures which contain it.

The blood in these cases presents sometimes an unusually and persistently florid colour; whether it does so or not, it however is in great part irreducible, *i.e.* after acting upon it with reducing agents two bands yet remain in its spectrum.

It has recently been shewn by Hoppe-Seyler[4] that the CO-haemoglobin resists putrefaction for very long periods of time, so that two bands remain visible for months and even years, whilst when normal blood putrefies, the reduction of its O_2-Hb to Hb takes place at once. According to Hoppe-Seyler the fact that long-kept blood exhibits two bands is a proof by itself that its haemoglobin has been combined with CO.

In a later section of this chapter it will be mentioned that a useful test for CO-blood is the production of a cinnabar-red precipitate on the addition of caustic soda; this is believed by Jäderholm[5] to be due to the formation of a compound of CO with haematin.

Nitric oxide, NO.

So great is the affinity of this gas for oxygen that the moment it comes in contact with it, deep red fumes of nitrogen peroxide, NO_2, are formed, and when these meet water the decomposition indicated in the following equation results:

$$3NO_2 + H_2O = 2HNO_3 + NO.$$

As has been previously said, all free acids, and salts with acid reaction, *ipso facto* decompose the colouring matter of the blood, and

[1] Gamgee, *Journal of Anatomy and Physiology*, vol. I. (1867) p. 346.

[2] Donders, "Der Chemismus der Athmung, ein Dissociations-Process." Pflüger's *Archiv*, v. 20—26.

[3] Zuntz, "Ist Kohlenoxydhaemoglobin eine feste Verbindung?" Pflüger's *Archiv*, v. 584—588.

[4] Hoppe-Seyler, "Unveränderlichkeit des Kohlenoxyd-Hämoglobin bei Einwirkung von Fäulniss oder Pankreasferment; Werth dieses Verhaltens für den Nachweis der Kohlenoxydvergiftung." *Zeitschrift f. phys. Chem.*, Vol. II. p. 131.

[5] Jäderholm, *loc. cit.*

therefore in investigating whether NO can form a compound with haemoglobin, analogous to the oxygen and carbonic oxide compounds, certain precautions had to be taken; for firstly, by combining with the respiratory O of haemoglobin, NO_2 would be formed, and next, by the reaction of water upon this body, nitric acid would result, which would immediately decompose the haemoglobin.

Hermann added ammonia to blood and then passed a stream of NO through it; all the acid generated in the reaction, at the expense of the oxygen of haemoglobin, was neutralised by the ammonia, and thereafter a compound of NO with haemoglobin was formed.

Again when CO-haemoglobin was placed in a vessel from which the air had been expelled and then a stream of NO was passed through the liquid, this gas displaced the CO, and combined with the haemoglobin in its stead.

Hermann found that the body thus produced yielded crystals isomorphous with those of the oxygen and carbonic oxide compounds, and that its spectrum presented a spectrum closely resembling theirs, though like that of the CO-haemoglobin undergoing no change after the addition of reducing agents.

We are therefore acquainted with three compounds of haemoglobin with gases which are isomorphous, and in which presumably 1 molecule of haemoglobin is linked with 1 molecule of the gas. The least stable of these compounds is that with oxygen, for it can be decomposed by CO, which then takes its place, forming a compound of intermediate stability, which in its turn can be decomposed by NO. That in each case a molecule of the gas takes part in the reaction is argued from the facts that CO displaces an equal volume of O (O_2 occupying the same volume as CO) and that the three compounds are isomorphous, so that the constitution of the NO-compound will almost certainly be similar to that of the CO body[1].

Acetylene, C_2H_2. In the case of both CO and NO we have unsaturated bodies which presumably satisfy their free affinities by linking themselves to the complex molecule of haemoglobin, and it is quite conceivable that other similarly constituted bodies might exert a similar action. It has indeed been surmised[2] that Acetylene or Ethine, C_2H_2, actually does so form a very unstable compound with haemoglobin, easily reducible by ammonium sulphide or reducing tin solutions. An investigation made with a view of testing the results in Hermann's laboratory has not confirmed the existence of this acetylene compound.

Assumed compound of Hydrocyanic acid with haemoglobin. Upon very slender evidence it has been advanced[3] that hydrocyanic acid forms an easily broken up compound with haemoglobin.

If the acid be added to a solution of haemoglobin, on crystallizing out the latter it retains some of the hydrocyanic acid,

[1] See Hermann, "Ueber die Wirkungen des Stickstoffoxydgas auf das Blut." Reichert und Du-Bois-Reymond's *Archiv*, 1865, p. 469.

[2] Bistrow u. Liebreich, *Ber. d. deutsch. chem. Gesellschaft Berlin*, 1868, p. 220.

[3] Hoppe-Seyler, "Cyanwasserstoffhaemoglobinverbindungen." *Med. Chem. Untersuchungen.* Heft II. (1867) p. 207.

which can afterwards be obtained from it by distillation after acidulating with sulphuric acid. It is to be noted that the spectrum of the supposed hydrocyanic compound is identical with that of oxy-haemoglobin, and that the behaviour of the solution to reducing agents is absolutely the same as that of a solution of oxy-haemoglobin.

Those who advocate the existence of the compound however rely somewhat upon the fact that blood to which hydrocyanic acid has been added shews the bands of oxy-haemoglobin, or bands identical with them, for a much longer time than normal blood—a fact which they explain by supposing that the hydrocyanic compound is somewhat more stable than oxy-haemoglobin.

It appears to the Author that all proofs of the existence of such a compound are wanting. That some hydrocyanic acid should adhere to the haemoglobin as it crystallizes out is quite in accordance with a variety of experiences of a similar kind, and can by itself afford no evidence of an actual compound existing. The resistance of hydrocyanic blood to decomposition can on the other hand be easily explained by the unquestionable arrest or slowing of the process of putrefaction in the presence of hydrocyanic acid; it is undoubtedly the products of putrefaction which are the causes of the spontaneous reduction of the oxy-haemoglobin of blood confined in a vessel which has no access to air, so that an agent which will inhibit putrefaction and at the same time not decompose oxy-haemoglobin would be expected to act as hydrocyanic acid acts and cause the persistence of the oxy-haemoglobin bands.

Products of the decomposition of Haemoglobin.

When subjected to the action of various reagents, especially to that of acids and of salts having an acid reaction, the molecule of haemoglobin undergoes a profound decomposition, the ultimate products of which are, *amongst others*, a proteid substance or substances, and a body called HAEMATIN, which contains all the iron originally contained in the blood-colouring matter. The formation of haematin is, according to Hoppe-Seyler, necessarily dependent upon the presence of oxygen, in the absence of which the process of decomposition yields a proteid and a body to which he has given the name of HAEMOCHROMOGEN; the latter may by oxidation pass subsequently into haematin. Haematin is an interesting body which forms definite, well crystallized, compounds with hydrochloric, and apparently also with hydriodic acid.

Before describing the various bodies which are the products of a profoundly decomposing action exerted upon haemoglobin, it is essential to refer to a modification of haemoglobin which is brought about by the action of various agents, and concerning which very much difference of opinion still lingers, viz. methaemoglobin.

Methaemoglobin.

Spectrum of Methaemoglobin.

When a solution of haemoglobin is exposed to air for some time it loses its blood-red colour, assumes a brownish tinge, presents an acid reaction, is precipitated by solutions of basic lead acetate, and on examining its spectrum it is found that the two bands of oxy-haemoglobin have become faint, and that a new band has appeared in the red near C; this line occupies *nearly* though by no means exactly the position of a similar band in the spectrum of acid haematin. On now rendering the solution alkaline by the addition of ammonia, the band in the red disappears, and is replaced by a faint absorption band immediately near D.

The most remarkable phenomenon, however, relates to the action of reducing agents.

If to a solution which exhibits the last-mentioned spectrum there be added some sulphide of ammonium, it is observed that it manifests the spectrum of reduced haemoglobin. On shaking the solution containing the latter with air, oxy-haemoglobin is again formed.

Production of methaemoglobin under the influence of nitrites.

The peculiar and remarkable properties above mentioned were described by the Author in 1867[1] and more fully in 1868, as developed by the action of nitrites on solutions of haemoglobin and upon blood. It was shewn that besides presenting the remarkable optical properties and reactions previously referred to, as a result of the action of nitrites, the respiratory oxygen of haemoglobin had become irremovable by carbonic oxide or in a Torricellian vacuum, but that after undergoing the change the haemoglobin could be crystallized repeatedly, the body thus produced only differing from oxy-haemoglobin by its colour and its spectrum. On analysis it was found that the crystalline compound always retained some of the nitrite used, and the view was therefore expressed that in all probability the action exerted by nitrites consisted in the formation of a compound of those bodies with oxy-haemoglobin, which compound was decomposed by the reducing agent employed.

It was subsequently observed by Sorby[2], Lankester[3], and Jäderholm[4] that Gamgee's nitrite-haemoglobin spectra coincided with those of methaemoglobin prepared by the action of potassium permanganate, and the presumption has been established that his bodies really consisted of methaemoglobin generated by the action of nitrites. This change in the view as to the nature of the bodies produced under the influence of nitrites does not however affect the facts established by the researches above referred to. According to Sorby, however, methaemoglobin would be a per-oxy-haemoglobin, *i.e.* a more highly oxygenized haemoglobin,

[1] Gamgee, "Note on the action of nitric oxide, nitrous acid and nitrites on Haemoglobin." *Proceedings of the Royal Society of Edinburgh*, 1867, p. 168. "On the action of nitrites on blood." *Philosophical Transactions*, 1868, pp. 589—625.

[2] Sorby, *Quarterly Journ. of Micros. Sc.* 1870, p. 400.

[3] Lankester, "Abstract of a Report on the Spectroscopic Examination of certain animal substances." *Journal of Anat. and Phys.* Vol. IV. p. 123.

[4] Jäderholm, "Untersuchungen über den Blutfarbstoff und dessen Zersetzungs producte." Abstracted from the original Swedish by Hammarsten in Maly's *Jahresbericht*, Vol. VI. p. 85.

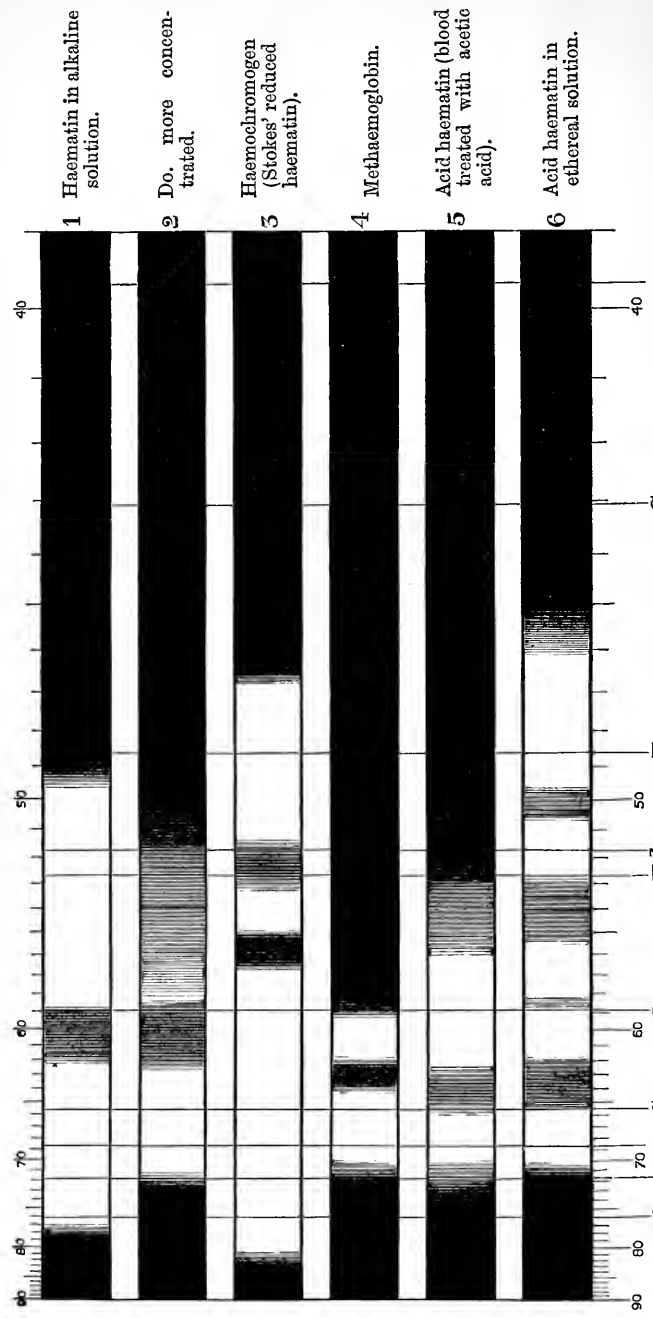


FIG. 23. TABLE OF THE SPECTRA OF SOME OF THE DERIVATIVES OF HAEMOGLOBIN.
(Drawn to a scale of wave-lengths after Preyer and from the measurements of the Author.)

in which the oxygen has become irremovable by a vacuum, but which is decomposed at once by reducing agents, which first liberate oxy-haemoglobin and subsequently form reduced haemoglobin. This view has lately received the support of Jäderholm.

Taking all the facts in consideration we must admit that under the influence of various agents the loosely combined oxygen of haemoglobin becomes irremovable by CO, and by a vacuum, whilst the new compound still preserves the crystalline form of oxy-haemoglobin, and the capability of being recrystallized. In this condition the body, which appears perfectly stable, can again be made to furnish haemoglobin. It is certainly convenient at present to retain for this body the term Methaemoglobin.

Hoppe-Seyler, who was the first[1] to describe briefly and to name methaemoglobin, long ago arrived at the conclusion that probably no definite body, such as is implied by the possession of a special name, exists, but that it represents an intermediate stage in the decomposition of haemoglobin into haematin and a proteid[2].

FIGURE 23.

Spectrum 1. (Preyer, Pl. 2, sp. 9.) Haematin in alkaline solution. A single absorption band between C and D, from wave-length 618 to wave-length 587. Strong absorption of the blue end.

Spectrum 2. (Preyer, Pl. 2, sp. 10.) The same as 1, but more concentrated. As the concentration of the solution increases the band extends more towards the red than the green. The red end of the spectrum is much absorbed.

Spectrum 3. (Preyer, Pl. 1, sp. 11.) Haemochromogen in alkaline solution (Stokes' *reduced haematin*). The spectrum is distinguished from all others by the extraordinary intensity and sharpness of the absorption band nearest to D. This extends from wave-length 567 to 547. The second absorption band, which is very much less intense and has less sharply defined borders, extends from about wave-length 532 to 518. The solution, even when concentrated, absorbs very little of the red. Violet and blue are strongly absorbed.

Spectrum 4. Methaemoglobin. In weak solutions of certain strengths four absorption bands may be made out. In a strong solution one is seen, the centre of which, according to the Author's measurements, corresponds to wave-length 632. According to Preyer this band would be a little nearer to C, the centre corresponding to wave-length 634.

Spectrum 5. Diluted blood treated with acetic acid. An absorption band in the red, the centre of which corresponds to wave-length 640. According to Preyer the centre of this band corresponds to 656·6.

Spectrum 6. Spectrum of acid haematin dissolved in ether. The position of the three bands between B and E agrees with the observations and drawings of Preyer. The centre of the band between b and F corresponds to wave-length 502. According to Preyer its centre corresponds to wave-length 505, *i.e.* it is somewhat nearer to b.

[1] Hoppe-Seyler, *Centralblatt f. d. med. Wissenschaften*, 1864.
[2] Hoppe-Seyler, *Med. Chem. Untersuchungen*, Heft III. (1868) p. 378.

Hoppe-Seyler's researches and views on methaemoglobin.

More recently Hoppe-Seyler has published fresh researches on the subject[1]. He opposes vehemently the view that methaemoglobin is to be looked upon as a peroxidized oxy-haemoglobin, resting his opposition very much on the facts (*a*) that when a solution of oxy-haemoglobin is introduced into the vacuum of the mercurial pump, so as to remove a part of its respiratory oxygen, and then is left at the temperature of the room, the fluid is found to contain a mixture of methaemoglobin and reduced haemoglobin, (*b*) that when a piece of palladium saturated with hydrogen is introduced into a flask filled with a saturated solution of oxy-haemoglobin, the whole of the colouring matter is very quickly converted into methaemoglobin, unless the quantity of the oxy-haemoglobin present was very large. In these two experiments conditions existed for removing a great part at least of the oxygen of the oxy-haemoglobin, and how therefore could a per-oxy-haemoglobin be formed?

Hoppe-Seyler has himself added lately the strongest proof of the possibility of reconverting methaemoglobin into oxy-haemoglobin by shewing that when a solution of methaemoglobin is allowed to decompose in sealed glass tubes, the band in the red of that body disappears and the spectrum of reduced haemoglobin appears. When some months have elapsed and the change has been completed, the tube is cooled to 0^0 until ice begins to form, then opened, and alcohol is added to the extent of $\frac{1}{4}$ of the volume of the solution; on afterwards lowering the temperature to -7^0 C. or -10^0 C. crystals of oxy-haemoglobin separate.

It is now admitted by Hoppe-Seyler that this possibility of reconversion into haemoglobin distinguishes methaemoglobin from haematin. According to this author methaemoglobin contains more oxygen than haemoglobin but less than oxy-haemoglobin, and this oxygen is in a more stable combination than in the latter body.

The Proteid matter derived from the decomposition of Haemoglobin.

When a solution of haemoglobin is boiled, the liquid becomes intensely turbid and a coagulum soon separates which possesses a dirty reddish-brown colour. Under the influence of heat the haemoglobin has been decomposed, and has yielded two substances insoluble in water, the first of which is a proteid, and the second is the body already referred to as *haematin*.

The same decomposition takes place when strong acids, or when large quantities of alcohol, act upon haemoglobin, though the rate at which it proceeds varies in these different cases.

Very little information is possessed concerning the proteid matter which results from this decomposition. According to Hoppe-Seyler it behaves as a normal proteid in reference to bases and acids, yielding alkali- and acid-albumins.

Preyer has described the proteid substance under the term of Globin, as a body which is free from all inorganic matter, which is insoluble in water, which swells in solutions of sodium chloride

[1] Hoppe-Seyler, "Die Zusammensetzung des Methämoglobin und seine Umwandlung zu Oxyhämoglobin." *Zeitschrift f. physiolog. Chemie*, Vol. II. (1878) p. 150.

and of sodium hydrate without dissolving. We agree with Kühne in holding that from the action of reagents one would conclude that a mixture of proteids, rather than a single proteid, results from the decomposition of haemoglobin.

Haematin.

When blood is treated with acetic acid it soon undergoes a change of colour, from red to brown, which indicates the decomposition of haemoglobin and the formation of haematin. If now the mixture of blood and acetic acid be shaken up with ether, the latter dissolves out a colouring matter, and on allowing the mixture to rest, the coloured ether may be decanted.

On examining the ethereal solution it is seen to present the spectrum represented in Fig. 23. 6, in which four separate absorption bands are to be observed. Firstly an absorption band in the red between C and D and corresponding to a wave-length of about 636, and secondly a very faint and narrow band, close to D, with an approximate wave-length of about 585, thirdly two much broader bands, one between D and E, and another nearly midway between b and F, the centres of which correspond approximately with wave-lengths 540 and 502 respectively. Of all these bands the one in the red is by far the most distinct.

If instead of experimenting in this way with ether holding acid haematin in solution we merely add acetic acid to a haemoglobin solution, we observe that as the liquid becomes brown in colour, the band in the red developes (Fig. 23. 5); the other absorption bands not being obvious. If we render the liquid alkaline by the addition of ammonia a single absorption band is seen, but much nearer to D, its centre corresponding to about 636 or 640. A marked shading of the blue end of the spectrum is noticed in addition. If now a reducing solution as of ferrous tartrate (Stokes' reagent) be added to the liquid, a spectrum is obtained which is marked by two bands which at first sight appear to the tyro to be identical with the bands of oxy-haemoglobin, but which are distinct from these; they will be found to be nearer the blue than are the bands of O_2-Hb. (See Fig. 23. 3.)

The first spectrum described is supposed to be that of haematin in acid solution, the second haematin in alkaline solution, and the third that of reduced haematin (Hoppe-Seyler's Haemochromogen). That the last is a less oxygenized product than the second is proved, not only by the fact that it is produced by the action of reducing agents, but likewise by the fact that on shaking the two-banded spectrum of reduced haematin with air or oxygen the two bands disappear and are replaced by the single bands of alkaline haematin.

As will be more fully stated when discussing haemochromogen, haematin is, according to Hoppe-Seyler, an oxidation product of haemoglobin; and it differs from *haemochromogen*, in that the latter

is a simple product of decomposition, which can be formed from reduced haemoglobin in the absence of oxygen.

Methods of preparation of Haematin.

I. Blood (defibrinated) is mixed with ether and then a large quantity of strong acetic acid is added; the two liquids are thoroughly shaken, and thereafter the dark-brown ethereal solution is decanted, filtered and set aside. The deposit which separates is washed with ether, alcohol, and water.

II. Blood is coagulated by the addition of an excess of cold alcohol; the precipitate is separated and boiled with alcohol holding sulphuric acid in solution. The hot filtered solution is set aside, and the matter which separates and adheres to the glass is washed with water and then with alcohol, and ether.

Although the above methods may yield haematin with which some qualitative experiments may be tried, we must employ the next process if it be desired to obtain the pure substance, viz. :—

III. Crystals of Hydrochlorate of Haematin or Haemin are dissolved in exceedingly dilute solution of pure potassium hydrate; the filtered solution is neutralized with hydrochloric acid, which throws down haematin in the form of a flocculent brown precipitate, which is washed with boiling water, until the washings are no longer rendered turbid by solution of silver nitrate. The haematin is then collected and dried, first at a gentle heat, and then at 120°—150° C. (Hoppe-Seyler[1].)

Properties of haematin.

Haematin, obtained by the method last mentioned, has a blue-black colour and a decided metallic lustre; it is free from crystalline structure, and when pulverized yields a dark-brown powder.

It can be heated to 180°C. without undergoing decomposition, but when heated more strongly it burns, evolving hydrocyanic acid, and leaving an ash which consists of pure oxide of iron, amounting to 12·6 per cent.

The following is the mean percentage composition of pure haematin, as determined by Hoppe-Seyler:—

Carbon	64·30
Hydrogen	5·50
Nitrogen	9·06
Iron	8·82
Oxygen	12·32
	100·00

These numbers agree well with the formula

$$C_{68}H_{70}N_8Fe_2O_{10}.$$

Haematin is insoluble in water, alcohol, and ether, easily soluble in solutions of the caustic alkalies, if these are not too concentrated, insoluble in diluted acids, and soluble with difficulty in hot alcohol holding sulphuric acid in solution.

Watery or alcoholic alkaline solutions of haematin when examined in thin layers by reflected light possess an olive-green colour; deeper

[1] Hoppe-Seyler, "Beiträge zur Kenntniss des Blutes des Menschen und der Wirbelthiere. Das Haematin." *Med. Chem. Untersuchungen*, Heft IV. 1871, p. 523.

layers possess a fine red colour and absorb strongly, as is proved by spectrum analysis, not only the violet rays, but also the yellow between Frauenhofer's lines C and D, especially near the latter. If alkaline solutions of haematin of sufficient dilution be examined, a distinct absorption band, the centre of which corresponds approximately to wave-length 603, is observed.

Haematin dissolves sparingly in alcohol holding sulphuric acid in solution, the solution assuming a dark-brown colouration.

Action of hot HCl on haematin.

When heated with fuming hydrochloric acid to 160° C., the iron which haematin contains is removed from it, and is found in the solution as a ferrous salt, whilst a body free from iron, termed *Haematoporphyrin*, is formed. Alkaline solutions of haematin, if pure, are not attacked by reducing agents. If, however, organic matters, such as proteids, be present, haemochromogen (*syn.* reduced haematin) is formed[1]. Haematin is scarcely, if at all, affected by putrefactive processes. (Hoppe-Seyler.)

Action of Potassium Cyanide on haematin.

When potassium cyanide is added to an ammoniacal solution of pure haematin, or to a solution of oxy-haemoglobin, a broad band somewhat resembling that of reduced haemoglobin, though by no means identical with it, is produced. This band extends from D to E. On adding reducing agents a spectrum with two well-marked absorption bands is obtained. These optical characters are supposed to depend upon the production of a compound of haematin and the cyanide employed, which has been denominated *cyan-haematin*. We are, however, merely acquainted with the spectroscopic characters of the supposed compound.

Hydrochlorate of Haematin—Haemin.

Mode of preparing crystals of Haemin for microscopic examination.

When a small drop of blood is boiled with a few drops of glacial acetic acid, the red colour almost instantly gives place to a brownish colouration. On evaporating down the fluid a residue is obtained, which on microscopic examination is found to be composed of reddish-brown

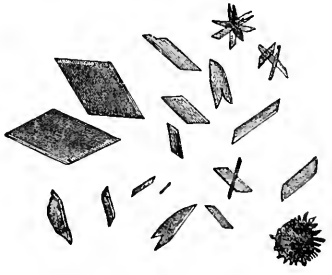


FIG. 24. CRYSTALS OF HAEMIN. (Frey.)

[1] Hoppe-Seyler, "Weitere Mittheilungen über die Eigenschaften des Blutfarbstoff's." *Zeitschrift f. phys. Chemie.* Vol. II. (1878) p. 154.

prismatic crystals[1]. Such crystals can be obtained from any blood stain, as on cloth or linen, by cutting out the stained tissue and heating it with glacial acetic acid, taking care to add a small crystal of sodium chloride. The evaporated residue contains the crystals.

Properties of haemin. These crystals are of a dark brown and sometimes of nearly a black colour, and present the form of rhombic plates sometimes arranged in radiating bundles.

Haemin is insoluble in water, alcohol, ether, chloroform, and in cold dilute acetic and hydrochloric acids. It is however soluble in caustic alkalies, in alcoholic solution of potassium carbonate, and in boiling acetic and hydrochloric acids. It dissolves in concentrated sulphuric acid, forming a violet-red liquid, which evolves hydrochloric acid when heated.

Hoppe-Seyler has prepared this body by a method to be afterwards referred to, and he considers it to be hydrochlorate of haematin and ascribes to it the formula $C_{68}H_{70}N_8Fe_2O_{10}2HCl$; he found the compound to contain 5·18 per cent. of chlorine.

It is held by Thudichum that haemin contains no chlorine, and he therefore looks upon it as crystallized haematin. Hoppe-Seyler however asserts that he has never obtained haemin crystals which were free from chlorine, and the statement agrees with the original observations of Teichmann who held the presence of chlorine to be indispensable to their formation.

Preparation of haemin in large quantities. Whilst it is very easy to prepare in a few minutes microscopic crystals of haemin, the difficulties attending the preparation of considerable quantities in a pure condition are considerable; the following method has been followed by Hoppe-Seyler:—

Defibrinated blood is mixed with a large excess of a solution of sodium chloride, containing $\frac{1}{20}$th its volume of saturated solution of NaCl, and set aside in a cool place so as to allow the corpuscles to subside; the clear supernatant fluid is decanted and the magma of corpuscles is mixed with some water, placed in a flask, and shaken up with ether; the ethereal solution is decanted, the solution of colouring matter is filtered and evaporated to dryness in shallow basins. The residue can be readily pulverized. The powder is passed through a sieve and then weighed. It is then mixed with glacial acetic acid in a mortar, the mass is washed into a basin by the aid of more glacial acetic acid, which is then added in such quantities that two litres of glacial acetic acid are employed altogether for every 100 grammes of the powder. The mixture, which has been mixed as well as possible, is then heated on the water bath, the temperature of which is allowed gradually to rise; the process of stirring is carried on from time to time and the mixture is allowed to remain for some hours at 100°C. Crystals soon commence to form, though long heating is required

[1] Teichmann, *Zeitschrift f. rat. Medizin f. Henle und Pfeuffer*, 1853, Vol. III. p. 375 and Vol. VIII. p. 141.

for the complete precipitation of the crystals and the solution of the proteids. The whole mixture is then poured into a large beaker and treated with many times its volume of water and set aside for many days. The magma of crystals which has then fallen to the bottom is washed many times in succession with water, and boiled with strong acetic acid, as long as the crystals appear to be mixed with masses of proteid matter; they are then washed with water, and collected on a filter and treated, first with alcohol, and then with ether. Haemin crystals may also be obtained by adding water and NaCl to a solution of haematin in alcohol which has been acidified with sulphuric acid and then heating. A method has been suggested by Gosdew for recrystallizing haemin, but it is not recommended by Hoppe-Seyler, as he found it to yield a mixture of haemin with haematin.

Haematoporphyrin.

Mode of preparation. When haematin is thoroughly mixed with concentrated sulphuric acid, the substance dissolves, and, after filtering through asbestos, a clear and beautifully purple-red solution is obtained. When this solution is treated with a large quantity of water, the greater part of the dissolved coloured body is precipitated in the form of a brown flocculent precipitate, the quantity of which increases if alkalies be added so as fully to neutralize the acid. In this operation the acid separates the whole of the iron from the haematin, and it is found in the solution in the state of a *ferrous* salt. In the process of decomposition of haematin by sulphuric acid there is no evolution of hydrogen gas.

Properties. The precipitate which is thrown down by water from the sulphuric acid solution is insoluble in concentrated solution of potassium sulphate, but soluble in water, and the watery solution possesses the same optical properties as the solution in sulphuric acid. It is also soluble in alkaline leys, yielding solutions possessed of a reddish-brown colour; in undergoing solution the substance appears to undergo some decomposition.

Both the original sulphuric acid solution and the dilute alkaline solutions of the body precipitated by water from it, possess characteristic and different spectra.

The first (solution in strong sulphuric acid) exhibits a pretty dark band immediately below D, and a very sharply defined band nearly intermediate between D and E.

The second (solution of precipitated body in alkaline leys) presents a four-banded spectrum: to wit—a weak band midway between C and D, an equally weak band between D and E but nearer to the former, a more strongly marked band nearer to E, and lastly a fourth band, darkest of all, which is not however very sharply defined, and extends through $\frac{4}{5}$ths of the space beween b and F.

To this iron-free body, obtained from haematin by the action of strong sulphuric acid, Hoppe-Seyler attaches provisionally the term of Haematoporphyrin, and ascribes the formula $C_{68}H_{74}N_8O_{12}$.

A second iron-free derivative from haematin has been obtained by Hoppe-Seyler, which differs from haematoporphyrin in being nearly insoluble in sulphuric acid and in caustic leys. To it he attaches the provisional name of Haematolin, and the formula $C_{68}H_{78}N_8O_7$[1].

Haemochromogen.

According to Hoppe-Seyler when reduced haemoglobin is decomposed in the absence of oxygen, instead of haematin, there is produced a body to which he gives the name of haemochromogen, whose solution presents a beautiful purple colour, but which is converted almost instantly into haematin when oxygen comes in contact with it. This body when in alkaline solution is, as proved by the most careful measurement of its absorption bands, identical with the so-called *reduced* haematin of Stokes.

The following are the two methods which at different times Hoppe-Seyler has employed for the preparation of haemochromogen:—

I. In the Woulff's bottle *A* (see annexed woodcut) hydrogen is evolved by the action of dilute hydrochloric acid upon zinc, and the gas is washed by passing through the wash-bottle *C*, which contains dilute solution of caustic soda. In order that the acid which is to act upon the zinc shall be free from oxygen, a piece of zinc is placed in the beaker *B* which contains the acid.

First of all having opened the clips *b* and *b'*, by aspirating at the *a* end of the wash-bottle, a sufficient quantity of acid is made to flow out of the beaker *B* to fill the tube *f* and then enter *A* where it evolves hydrogen, which gradually expels all the air from the apparatus. The clip *b* is then closed. After gas has been passing for about half an hour the bulb-apparatus *DEF* is attached to the wash-bottle in the manner represented in the diagram. This bulb-apparatus contains in the division *F* concentrated solution of oxy-haemoglobin, and in the division *D* alcoholic solution of sulphuric acid or potassium hydrate, or instead of these an aqueous solution of potassium hydrate. A stream of gas is now again allowed to pass through the apparatus by opening the clip *b* and raising the vessel *B* so as to allow a fresh quantity of dilute acid to enter *A* and act upon the zinc which it contains. (If *A* happens to be already full, the solution of $ZnCl_2$ which it contained might be removed by depressing the vessel *B* and allowing it to be on a lower level than *A*. The vessel *A* having been thus more or less completely emptied, and the clip *b* closed, a fresh stock of dilute acid may be placed in *BA*, and everything is ready for recommencing.) After a stream of H has passed through the whole apparatus, including the bulbs, for some considerable time (2 or 3 hours), the bulb-apparatus is sealed in a blowpipe flame at *d* and at *e*. By means of the spectroscope the observer determines

[1] The whole description of haematin and its derivatives is abridged from the memoirs of Hoppe-Seyler, of which the most important relating to this subject is the one entitled "Das Hämatin," under the general heading of "Beiträge zur Kenntniss des Blutes des Menschen und der Wirbelthiere." Hoppe-Seyler's *Med. Chem. Untersuchungen*, Heft IV. (1871) p. 523. See also "Ueber die Zersetzungen der Hämoglobine." *Ibid.* p. 377—385.

before sealing whether the froth which fills the division *F* exhibits, as it ought to do, the spectrum of reduced haemoglobin; if it does so, after

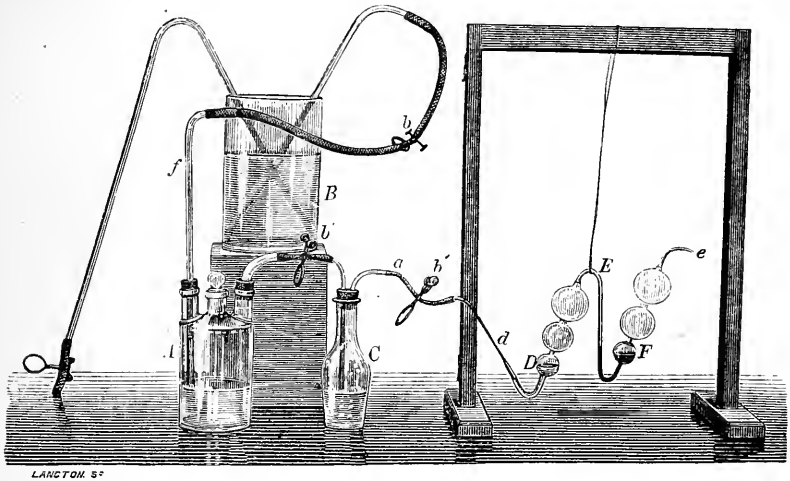


FIG. 25. APPARATUS FOR THE PREPARATION OF HAEMOCHROMOGEN.

sealing, the fluids contained in the bulbs *D* and *F* are mixed by reversing and shaking their contents together.

If in this way, in the complete absence of oxygen, acid alcohol has been mixed with a little haemoglobin, a precipitate forms, which soon loses its colour on being heated in the water bath, whilst the liquid becomes coloured purple. The liquid then exhibits four absorption bands, of which two are situated between C and D. A third absorption band of greater degree of sharpness and darkness extends between D and E, and a fourth is situated between b and F. The absorption band nearest C is, if the oxygen has been thoroughly expelled, exceedingly weak and may be due to a trace of haematin, as its position is identical with the band of acid haematin.

If instead of sulphuric acid, alcohol holding caustic alkali in solution has been employed, on mixing the fluids we obtain a rose-red or purple-red precipitate and a solution having the same tints. This exhibits two absorption bands which are identical with those of Stokes' alkaline haematin.

II. Lately Hoppe-Seyler has recommended the following method[1].

A solution of oxy-haemoglobin is placed in a glass tube, and then a smaller glass tube containing solution of dilute phosphoric or tartaric acid, or solution of potassium hydrate, is introduced into the larger tube, the open end of which is then drawn out and sealed; the large tube with its contained smaller tube is then heated gently for some time, care being taken that the contents of the two tubes do not mix. The oxy-haemoglobin

[1] Hoppe-Seyler, "Weitere Mittheilungen über die Eigenschaften des Blutfarbstoffs." *Zeitschrift f. phys. Chem.* Vol. I. p. 138.

first becomes reduced, and thereafter the oxygen contained in the air of the tube is removed by it. When many days have elapsed and the whole of the haemoglobin is reduced, the tubes are reversed and their contents mixed, when the optical properties of haemochromogen can be satisfactorily observed.

According to Jäderholm[1], Hoppe-Seyler's haemochromogen in alkaline solution is identical with the reduced haematin of Stokes, and haemochromogen in acid solution has a spectrum which is a combination of those of acid haematin and haematoporphyrin. The former statement is indeed admitted by Hoppe-Seyler, and is indisputable. Hoppe-Seyler urges, however, and as it appears to the Author, most correctly, that the term *reduced haematin* is a misleading one, haemochromogen being a mere product of decomposition of haemoglobin, whilst haematin is an oxidized product of decomposition.

Haematoidin.

This name has been assigned by Virchow[2] to a substance which occurs in the form of yellow microscopic crystals in old extravasations of blood, as for example in old apoplectic clots, and which was first observed by Everard Home[3].

FIG. 26. CRYSTALS OF HAEMATOIDIN (AFTER FUNKE). (Frey.)

These crystals appear to be identical in form with those of Bilirubin, the chief colouring matter of human bile, and when treated with fuming nitric acid give the same colour reaction (Gmelin's reaction).

Identity of haematoidin and bilirubin.

Opinions have been divided on the question of the identity or non-identity of haematoidin and bilirubin. On the ground of different deportment towards solvents Holm[4] asserted that haematoidin, prepared from the

[1] Jäderholm, "Untersuchungen über den Blutfarbstoff und dessen Zersetzungsproducte." Abstracted from the Swedish by Hammarsten in Maly's *Jahresbericht*, Vol. VI. p. 85.

[2] Virchow, *Archiv d. pathol. Anat. u. Physiol.* Vol. 1 (1847), p. 383—443.

[3] Sir Everard Home, *A short tract on the Formation of Tumours, &c.* London, 1830, page 22. In Figs. 1, 2 and 3 of Plate I., crystals of haematoidin are admirably figured as seen in an aneurismal coagulum. Home was, however, altogether ignorant of their nature and referred to them as 'crystallized salts.'

[4] Holm, "Haematoidin," *Journ. f. prak. Chemie.* Vol. c. p. 142.

corpora lutea of the cow, is not identical with bilirubin. Salkowski[1], on the other hand, found haematoidin prepared from the contents of a strumous cyst to be identical in all respects with bilirubin. Preyer[2], relying mainly though not entirely upon the spectra of the two bodies, denies the identity. According to this observer bilirubin possesses no definite absorption-band, whilst solutions of haematoidin when examined with the aid of magnesium light present a well-marked absorption-band between b and F, and a weaker one nearly midway between F and G.

The majority of physiological chemists are, however, now of the opinion that haematoidin and bilirubin are identical. This matter will be again referred to under 'bilirubin.'

The Mineral Constituents of the Red Corpuscles.

It was pointed out in discussing the salts of the serum and plasma that our information in reference to these was far from complete, in consequence of the inherent difficulties which attach to the methods of research. The same remark appears with still greater force to the mineral matters of the corpuscles. It is possible to obtain plasma and serum free from corpuscles (though certainly not free from all constituents of corpuscles, e.g. serum-globulin), but far from possible to obtain corpuscles free from the liquids in which they float. Comparative analyses, however, of the mineral matters of the serum and of the clot, and of the blood as a whole, do lead to certain results which are to be relied upon. They at once reveal, for instance, that the iron of the blood is, with the exception of the minutest traces, contained in the corpuscles, where we know it to exist as an essential constituent of haemoglobin; that the corpuscles are much richer in potassium salts than the serum, and that the amount of chlorine is very much greater in the latter than in the former. When, however, we enquire whether phosphates and sulphates exist in the blood-corpuscles, or whether these ingredients of the ash are not due to the oxidation of organic constituents, we can merely say that the experimental data for furnishing an answer to the question fail, though from the fact that the blood-corpuscles are rich in lecithin we cannot doubt that nearly the whole, if not the whole, of the phosphoric acid found in the ash, is derived from the oxidation of that body.

The analyses of C. Schmidt.

In order to impress upon the reader the difference between the mineral constituents of the blood-corpuscles and the plasma, the results of C. Schmidt's analyses of both are here appended :—

[1] Salkowski, "Zur Frage über die Identität des Hämatoidin und Bilirubin," Hoppe-Seyler's *Med. Chem. Untersuchungen*, III. p. 436.

[2] Preyer, *Die Blutkrystalle*, p. 187.

1000 parts of moist corpuscles yield:		1000 parts of plasma yield:	
Mineral matters (exclusive of Iron)	8·120	Mineral matters	8·550
Chlorine	1·686	CHLORINE	**3·640**
Sulphuric anhydride	0·066	Sulphuric anhydride	0·115
PHOSPHORUS PENTOXIDE	**1·134**	Phosphorus pentoxide	0·191
POTASSIUM	**3·328**	Potassium	0·323
Sodium	1·052	SODIUM	**3·341**
Calcium Phosphate	0·114	Calcium Phosphate	0·311
Magnesium	0·073	Magnesium Phosphate	0·222

One would be inclined to attribute too great an importance to the remarkable difference in the distribution of potassium and sodium in the blood corpuscles of man if one were in ignorance of the undoubted fact that this difference does not hold in the case of most animals.

Thus if we glance at the subjoined tabular view which contains the results of the analyses of Schmidt of the inorganic matters yielded by the blood cells and plasma of several animals, we come to the conclusion that the proportions of sodium and potassium in the corpuscles may vary within wide limits, and that in most animals the salts of sodium preponderate greatly over those of potassium.

TABLE SHEWING THE AMOUNT OF POTASSIUM, SODIUM AND CHLORINE PRESENT IN 100 PARTS OF THE INORGANIC MATTERS OF BLOOD CELLS AND PLASMA[1].

	Blood Cells.			Liquor Sanguinis.		
	K	Na	Cl	K	Na	Cl
Man (mean of 8 experiments)	40·89	9·71	21·00	5·19	37·74	40 68
Dog " " " "	6·07	36·17	24·88	3·25	39·68	37·31
Cat " " " "	7·85	35·02	27·59	5·17	37·64	41·70
Sheep " " " "	14·57	38·07	27·21	6·56	38·56	40·89
Goat " " " "	37·41	14·98	31·73	3·55	37·89	40·41

The much more recent researches of Bunge[2], whilst they differ in some respects materially from those of C. Schmidt, indicate that in some animals potassium and in others sodium preponderates. Thus Bunge found no sodium (!) in the blood corpuscles of the dog and of the cat, whilst he found nearly three times as much sodium as potassium in the blood of the ox. These differences perhaps will be explained, as some have surmised, by further researches proving that when considerable quantities of potassium salts are ingested, they replace sodium in the corpuscles, though probably before being able to do so the richness of the blood in potassium must attain a certain figure.

[1] Lehmann, *Physiological Chemistry*, Vol. II. p. 189. This table, which the Author has modified somewhat in form, is compiled from the observations of C. Schmidt.

[2] Bunge, "Zur quantitativen Analyse des Blutes." *Zeitschr. f. Biol.*, Vol. XII. p. 191—216.

The Gaseous Constituents of the coloured corpuscles.

In discussing the properties of oxy-haemoglobin we have studied with considerable minuteness the nature of that compound, and have shewn that it is produced by the union of oxygen from the air with the complex molecule of haemoglobin. We have shewn that under various circumstances oxygen can be expelled from its state of combination, as when blood is introduced into a Torricellian vacuum, when neutral gases such as H and N are passed through it, or when CO or NO act upon it.

Now, although the oxygen removed by these various means is derived from the oxy-haemoglobin of the corpuscles, in that body it exists in a state of actual combination—in a state very different from that in which a gas exists which is merely dissolved in a liquid or absorbed by a solid body, so that strictly we have as little right to speak of the O of the corpuscles as one of their gaseous constituents as we have so to designate the H or N which are essential constituents of haemoglobin.

We may however state that which we shall in succeeding sections comment upon at far greater length, viz. that of the mixed gases which are given up by blood when it is heated in a Torricellian vacuum and which consist of a mixture of O, CO_2 and N, practically the whole of the first is derived from the dissociation of oxy-haemoglobin, of which each gramme can give up as much as 1·28 c.c. of O (at 0° C. and 1 metre pressure). Of the carbonic acid thus obtained the greater part is derived from the plasma in which it is partly dissolved and partly loosely combined, a small quantity only being derived from the blood corpuscles. Probably the whole of the nitrogen, obtained from the blood, is held in solution in the liquor sanguinis.

In short, if we wish to be strict in our expressions, we should say that probably the only gaseous constituent *properly so called, i.e.* gas not existing in a state of chemical combination in the corpuscles, is carbonic acid.

Sect. 5. The Colourless Corpuscles of the Blood.

In addition to the red corpuscles, which have been already described, the blood of vertebrate animals contains a number of globules and particles of various sizes and characters, all included under the designations of *white corpuscles* and *intermediate corpuscles*.

The members of the first class are readily defined. They are nucleated masses of protoplasm destitute of any cell-membrane, and containing fine or coarse granules. They were first discriminated from the red corpuscles by Hewson: and they were for a long time spoken of as lymphatic corpuscles. In man they have a diameter of about 10 μ ($\frac{1}{2500}$ in.), while in batrachians they are much larger. Their most important property is, without question, that of amoeboid movement, which was first observed by Wharton Jones[1] in the blood of the skate. The recognition of the power of amoeboid movement of white blood corpuscles was one of the most

[1] *Phil. Trans.* 1846.

important steps in establishing the analogy between the sarcode of the lowest animals and the substance of the cells composing animals of higher grades.

Another interesting property of white blood corpuscles is that of enveloping and absorbing small particles of colouring matter, such as carmine, with which they are in contact.

The members of the second class, the *intermediate corpuscles*, are less clearly defined than the amoeboid corpuscles; and for a full description of all their varieties, the reader is referred to the larger text-books and memoirs on the Histology of the Blood. But among them must be mentioned some which seem to have a great importance in the phenomenon of coagulation. These are described by Semmer under the name of red *granular* corpuscles (*rothe Körnerkugel*[1]), and by Hayem[2] under the name of *haematoblasts*. According to Semmer, who examined the blood of the horse and other mammals, the granular red intermediate corpuscles are nucleated granular bodies, the granules largely obscuring the nucleus. They have about the same specific gravity as the white corpuscles; hence they subside in the uncoagulated plasma more slowly than the common red corpuscles. They possess the power of amoeboid movement. They become colourless and readily disintegrate during the act of coagulation; and the detritus appears to be soluble in the plasma. The disintegrating corpuscles in many cases form centres for the radiation of threads of fibrin through the coagulating liquor sanguinis (refer to p. 35, and fig. 10).

The number of the white corpuscles, though less than that of the red, varies with the many conditions of age, sex, period after food and region from which the specimen of blood was taken. On an average there is one white corpuscle to 330 or 350 red ones.

The proportion is—[3]

In boys	1 to 226
„ girls	1 to 389
„ men	1 to 346
„ old men	1 to 381
„ menstruating women	1 to 247
„ pregnant women	1 to 281
„ the morning fasting state	1 to 716
Half an hour after breakfast	1 to 347
Three hours after breakfast	1 to 1514
In splenic vein	1 to 60
„ splenic artery	1 to 2260
„ hepatic vein	1 to 170
„ portal vein	1 to 740.

Our knowledge of the physical and chemical characters of the colourless corpuscles is for obvious reasons very much more defective than that of the coloured corpuscles.

[1] Alex. Schmidt, "Ueber die Beziehung der Faserstoffgerinnung zu den körperlichen Elementen des Blutes." Pt. 2. Pflüger's *Archiv f. d. ges. Physiol.* Vol. xi. (1875) p. 560.

[2] Georges Hayem, *Recherches sur l'anatomie normale et pathologique du sang*, p. 108. Paris, 1878.

[3] The above figures are taken from Stricker's *Handbook*, Art. "Blood," by Alex. Rollett.

The colourless corpuscles are obviously much lighter than the coloured, as is evidenced (1) by their always being found in greater abundance near the upper surface of a blood clot; (2) by their forming a separate white layer on the surface of the red corpuscles, when horse's blood is cooled with the object of separating the corpuscles from the liquor sanguinis.

The colourless corpuscles exhibit obvious adhesiveness even when contained in the blood-vessels of the living body, an adhesiveness which causes them to cling one to the other when they meet, and to foreign bodies or blood clots which may happen to project into the blood stream.

The great mass of the protoplasm of the colourless corpuscle is undoubtedly proteid in its nature, the proteid matter having associated with it smaller quantities of other principles, and imprisoning the nucleus or nuclei which we may provisionally assume to be composed of that somewhat non-descript, phosphorus-containing, non-digestible, mucin-like body, Nuclein (see p. 82).

The protoplasm of the colourless corpuscles appears to undergo, at least partial, coagulation at 45° C. It swells and becomes transparent when treated with acetic acid, which renders the nuclei much more sharply defined and distinct.

The protoplasm swells and ultimately dissolves in 10 p.c. solution of Na Cl, leaving the nuclei undissolved. The salt solution thus obtained is precipitated by the addition of a large quantity of water, is coagulated by heat and by mineral acids.

The colourless corpuscles sometimes contain within them minute fat-granules.

Many of the white corpuscles of the blood present, when treated with a solution of iodine in iodide of potassium and water, a reddish mahogany colour, which is due to their containing Glycogen. The solution recommended to be used is one made by dissolving 1 gramme of iodine and 2 grms. of potassium iodide in 100 c.c. of water. "The main substance of the corpuscles is uniformly stained of a deep yellow, but many contain groups of mahogany-stained granules, and from others are seen to exude after a time pellucid drops of varying size, which become tinted of a mahogany or port wine colour, and no doubt contain glycogen[1]."

The average proportion of colourless to coloured corpuscles is liable to considerable variations consistently with health. It undergoes physiological fluctuations which are related to the process of digestion, viz. the colourless corpuscles increase after the ingestion of food, and diminish during fasting, a fact explained in great measure by the fact that in the former case a larger influx of colourless cells takes place through the thoracic duct. The origin and destination of the colourless corpuscles, though perhaps beyond the scope of this work, will be shortly treated of under 'lymphatic glands.'

[1] Schäfer, *A Course of Practical Histology*. Smith, Elder and Co., 1877.

Sect. 6. The Gases of the Blood as a whole.

Under the head of 'The Gases of the Liquor Sanguinis' and 'The Gaseous Constituents of the Coloured Corpuscles,' it has been shewn that from each of these constituent parts of the blood, there can be separated, by certain methods of treatment, gases, which are a mixture of carbonic acid, oxygen and nitrogen. We shall give a description of the methods employed in separating the gases of the blood in Chapter IV., and postpone a lengthened theoretical treatment of the gases of the blood to the chapter on Respiration. In this place it will suffice if we make the following brief statements.

(1) The blood, when admitted into an empty space and exposed to the temperature of the body, readily gives up more than half its volume of mixed gases, consisting of oxygen, carbon dioxide, and nitrogen.

(2) The first (oxygen) is present in much larger quantities than could be held in simple solution by the water of the blood, and, as will be afterwards proved, is mainly held in feeble combination by the haemoglobin of the coloured blood corpuscles; only a trace of it is, under ordinary circumstances, held in solution in the liquor sanguinis.

(3) The second (carbon dioxide), whilst not existing in larger quantity in blood than it could do if simply dissolved by the water of that fluid, is partly in a state of chemical combination but chiefly in a state of simple solution. It is contained in great part in the liquor sanguinis and serum, but in part also in the corpuscles.

(4) The nitrogen is held in a state of simple solution in the liquor sanguinis.

(5) Arterial blood of the dog of mean composition yields for every 100 volumes, 58·3 volumes of mixed gases (measured at 0° C. and 760 mm.), composed of 22·2 volumes of O, 34·3 volumes of CO_2, and 1·8 volumes of N, the maximum amount of oxygen observed having been 25·4 volumes (Pflüger[1]).

(6) As venous blood differs in composition according to the vascular area whence it is obtained, it is impossible to state the mean composition of its gases; the following facts are however correct:—the nitrogen is present in the same proportion as in arterial blood, the O is less in amount (from 8 to 12 volumes per 100 of blood) and the CO_2 greater (from 40—50 volumes per 100 of blood).

Summary of the Quantitative Composition of the Blood.

Having treated at length the properties of the individual constituents of the blood, we shall here append tables exhibiting the results of the elaborate researches of C. Schmidt and Lehmann on the blood of man, although some of the data have already been referred to in the preceding pages.

[1] Pflüger, "Die normalen Gasmengen des arteriellen Blutes nach verbesserten Methoden." *Centralblatt f. d. med. Wissenschaft*, 1868.

I.

RESULTS OF THE ANALYSIS OF THE BLOOD OF A HEALTHY MAN. (C. SCHMIDT[1].)

1000 parts of Venous Blood of a man aged 25, of specific gravity 1059·9.

Blood cells			513·02	
Water contained in cells			349·69	
Organic solids of cells (mainly haemoglobin)			159·59	Solid constituents of cells, 163·33
Mineral matters corresponding to	Chlorine	0·898	3·74	
	Sulphur trioxide	0·031		
	Phosphorus pentoxide	0·695		
	Potassium	1·586		
	Sodium	0·241		
	Calcium phosphate	0·048		
	Magnesium phosphate	0·031		
	Oxygen	0·206		
Specific gravity of blood cells			1088·6	

Plasma			486·98	
Water contained in the plasma			439·02	
Fibrin obtained from ,,			3·93	Solid constituents, 47·96
Albumin			39·89	
Mineral constituents corresponding to	Chlorine	1·722	4·14	
	Sulphur trioxide	0·063		
	Phosphorus pentoxide	0·071		
	Potassium	0·153		
	Sodium	1·661		
	Calcium phosphate	0·145		
	Magnesium phosphate	0·106		
	Oxygen	0·221		
Specific gravity of Plasma			1031·2	

[1] Carl Schmidt, *Charakteristik der epidemischen Cholera gegenüber verwandten Transudationsanomalien. Eine physiologisch-chemische Untersuchung.* Leipzig und Mitau, 1850, p. 29—31.

II.

TABULAR VIEW EXHIBITING THE RELATIVE COMPOSITION OF THE BLOOD CORPUSCLES AND THE LIQUOR SANGUINIS, AS DEDUCED FROM THE OBSERVATIONS OF C. SCHMIDT AND LEHMANN[1].

1000 parts of Blood Corpuscles contain :—		1000 parts of Liquor Sanguinis contain :—	
Water	688·00	Water	902·90
Solid constituents	312·00	Solid constituents	97·10
Specific gravity	1088·5	Specific gravity	10·28
Haemoglobin and proteids of the stroma	298·97	Fibrin	4·05
Fat	2·31	Proteids, chiefly *serum-albumin*	78·84
Extractive matters	2·60	Fat	1·72
Mineral substances	8·12	Extractive matters	3·94
		Mineral substances	8·55
Chlorine	1·686	Chlorine	3·644
Sulphur trioxide	0·066	Sulphur trioxide	0·115
Phosphorus pentoxide	1·134	Phosphorus pentoxide	0·191
Potassium	3·328	Potassium	0·323
Sodium	1·052	Sodium	3·341
Oxygen	0·667	Oxygen	0·403
Calcium phosphate	0·114	Calcium phosphate	0·311
Magnesium phosphate	0·073	Magnesium phosphate	0·222

[1] Lehmann, *Physiological Chemistry*, vol. II. p. 160.

Sect. 7. Characters presented by the Blood of Invertebrate Animals.

It has already been stated that with very few exceptions[1], the blood of all vertebrate animals is characterized by the possession of a red colour which is due to the presence within it of coloured corpuscles, which in all classes but one (that of the Mammalia) are nucleated. In addition to the coloured corpuscles, we have seen that the blood always contains a much smaller number of colourless cells, consisting of nucleated masses of protoplasm, endowed with contractility, and presenting many of the essential features of independent elementary organisms, and it has been incidentally remarked that there appears to be a much greater uniformity in the shape and size of the colourless than of the coloured corpuscles of the blood of different classes of Vertebrates.

When we pass from the vertebrate to the invertebrate sub-kingdoms we find that in all those organisms in which a differentiated blood-vascular system exists, the contained liquid presents floating in it nucleated masses of protoplasm closely resembling the colourless cells of vertebrate blood, but is generally, though not invariably, free from all representatives of the coloured corpuscles. In the immense majority of invertebrate animals this intra-vascular liquid is colourless, or presents a yellowish tint, though in a small minority it is coloured red, or green, or blue. Generally, however, the colour is diffused through the liquor sanguinis if it is not actually dissolved in it.

In the colourless liquid contained in the vascular system of most Invertebrates, we have probably a liquid which discharges only one half of the functions of the vertebrate blood—which serves merely as a common medium, supplying liquid and solid matters to the various tissues and organs, and washing away from them products of waste and decay, which it discharges through the agency of, or at, the various excretory organs. The other half of the functions of the vertebrate blood, the respiratory, are probably scarcely represented by the colourless blood of Invertebrata.

Such blood possesses, probably, no arrangement whereby the oxygen of the medium external to the body can be stored up by it, at certain points, to be carried away to tissues and organs far removed from that medium and then given up.

The respiratory exchanges in creatures provided with such blood probably take place by processes of diffusion directly between the tissues of the organism and the medium which it inhabits, and

[1] It is a matter of dispute whether the blood corpuscles of *Amphioxus* contain haemoglobin. According to Ray Lankester they do not. In *Leptocephalus* we have at any rate a fish whose blood is certainly free from haemoglobin. (Lankester: "A Contribution to the Knowledge of Haemoglobin." *Proceedings of Royal Society*, Vol. xxi. (1872) p. 71 *et seq.*

without the intermediation of any special arrangement such as is represented by the haemoglobin of the vertebrate coloured corpuscles.

In the Invertebrata whose blood is coloured, we have, however, undoubtedly, a clear indication of the blood discharging respiratory functions, for such blood, when red, contains oxy-haemoglobin, and when of other colours, sometimes undoubtedly does contain matters which are capable of acting as oxygen carriers.

The following are the most important facts which have been discovered in reference to the chemical composition of the blood of invertebrate animals:

Distribution of Haemoglobin through the vascular liquids of various groups of Invertebrata.

Our knowledge of this subject is mainly derived from the researches of Professor Ray Lankester[1]. The following are the chief conclusions to which he has arrived.

Haemoglobin is contained—

1. in special corpuscles:

a. In the perivisceral fluid of some species of the Vermian genera, *Glycera*, *Capitella* and *Phoronis*.

b. In the blood of the Lamellibranchiate Mollusk, *Solen legumen*.

2. Diffused in a vascular or ambient liquid:

a. In the peculiar vascular system of the Chaetopodous Annelids very generally, but with apparently arbitrary exceptions.

b. In the vascular system (which represents a reduced perivisceral cavity) of certain Leeches, but not of all (*Nephelis*, *Hirudo*).

c. In the vascular system of certain Turbellarians as an exception (*Polia sanguirubra*).

d. In a special vascular system (distinct from the general blood-system) of a marine parasitic Crustacean (undescribed) observed by Professor Edouard van Beneden.

e. In the general blood-system of the larva of the Dipterous Insect *Cheironomus*.

f. In the general blood-system of the Pulmonated Mollusk *Planorbis*.

g. In the general blood-systems of the Crustaceans *Daphnia* and *Cheirocephalus*.

In reference to Planorbis, Mr H. C. Sorby has made observations which lead him to doubt very strongly whether the red colouring matter be really haemoglobin[2]. Mr Sorby's doubts are based (1) upon the fact that the measurements of the bands in the spectrum of the blood of Planorbis differed slightly from those of oxy-haemoglobin; (2) that the red colouring matter in the blood of Planorbis seemed to resist the action of decomposing reagents (such as acids) longer than haemoglobin. According to Sorby the following are the centres of the bands of normal haemoglobin and of

[1] Lankester, *Op. cit.*, p. 76.

[2] H. C. Sorby, "On the Evolution of Haemoglobin." *Quarterly Journal of Microscopical Science*. Vol. XVI. N. S. (1876) p. 76 *et seq.*

the colouring matter of the blood of Planorbis, expressed in wave-lengths in millionths of a millimetre.

	Centres of Bands.	
Normal oxy-haemoglobin	581	545
Planorbis	578	542½.

According to the measurements of Preyer and the Author, the position of the bands in Planorbis as stated above really coincides almost exactly with that of the bands of oxy-haemoglobin.

It must not be concluded that all the red colouring matters found in invertebrate animals are identical with haemoglobin. Thus the perivisceral cavity of *Sipunculus nudus*, which is abundant in the Gulf of Naples, has a pale madder-like colour due to a large number of coloured corpuscles, varying in size between $\frac{1}{3500}$th and $\frac{1}{2000}$th of an inch, in which a pink colouring matter is deposited. This colouring matter, which is found in other tissues of that creature, is quite distinct from haemoglobin[1]. Whether certain crystals which are obtainable from the blood of insects consist of haemoglobin or not has been disputed, and yet deserves further investigation[2].

On the Green Blood of Certain Annelids. Chlorocruorin.

In 1838 Milne Edwards[3] had discovered that in certain Annelids of the genus *Sabella*, the blood possessed a green colour, and a similar observation was made by M. de Quatrefages in the case of the annelid *Chloronema Edwardsi*. Professor Ray Lankester[4] some years ago shewed that the green colour is due to a body to which he gave the name of CHLOROCRUORIN.

Lankester's researches were carried out on *Sabella ventilabrum* and *Siphonostoma*.

He found that the blood yielded an absorption spectrum with two distinct bands, viz. one between C and D, and a second much less distinct band in the green, almost midway between D and E. On reducing a solution of the blood by means of one of the reagents used with a similar object in the case of haemoglobin, Lankester found that the two bands were replaced by a single band having nearly the same position as the darker of the two, though fainter than it. On agitating with air the two bands returned.

The Author has reduced Professor Lankester's observations to a scale of wave-lengths, and finds that the first band of oxy-chlorocruorin, as drawn by Lankester, extends from wave-length 588·5 to 617, its centre being, therefore, 602·7. The second band extends from 560 to 570. The band of (reduced) chlorocruorin extends from wave-length 588·5 to 611·3, and its centre therefore corresponds to wave-length 600.

[1] Lankester, *Op. cit.*, p. 80.

[2] Landois, *Zeitschr. f. wiss. Zoologie*, Vol. XIV. pp. 55—70, Plates VII.—IX., (quoted by Preyer, *Op. cit.* p. 10). The Author has not seen the original paper.

[3] Milne Edwards: "Recherches pour servir à l'histoire de la circulation chez les Annelides." *Ann. des Sciences Natur.*, 1838. 2me série. Vol. x. p. 190.

[4] Lankester: *Journal of Anatomy and Physiology*, 1868, p. 114: *ibid.* 1870, p. 119.

To the green substance Lankester applied the term Chlorocruorin, and concluded that this body, like haemoglobin, was capable of existing in two states of oxidation; when oxygenized he proposed to designate it oxy-chlorocruorin. Furthermore Lankester found that the action of certain reagents upon chlorocruorin appeared to indicate that when decomposed it yields products which have identical spectra to those of certain haematin derivatives.

On the Blue Blood of certain of the Mollusca and Molluscoida.

1. The blood of the mollusca has received considerable attention. Usually the blood of animals belonging to this class presents a white colour, but sometimes it is distinctly of a blueish tint. C. Schmidt analysed the blood of the Pond-mussel (*Anodonta cygnea*) and found it to be colourless and slightly alkaline. It deposited a pale fibrinous coagulum; it contained 0·854 p. c. of solid constituents, and of these there were 0·033 of a fibrin-like body, 0·565 of albumin, 0·189 of lime, 0·033 of sodium phosphate, sodium chloride, calcium sulphate, and 0·034 of calcium phosphate[1].

2. The blood of the large shell-snail (*Helix pomatia*) was found by Harless and von Bibra[2] to contain 8·393 p. c. of organic and 6·12 p. c. of mineral matters, there being 0·055 of oxide of copper in the latter.

This blood acquired a blue colour on exposure to air which disappeared under the influence of CO_2. Alcohol precipitated a colourless coagulum and ammonia removed the blue colour, which reappeared on neutralizing the solution with hydrochloric acid.

Harless and von Bibra stated that the blood of *Helix pomatia* contained copper, but no iron, but v. Gorup-Besanez states that on having these observations repeated under his direction, in addition to copper, iron was also found in the ash[3].

3. Harless and von Bibra also investigated the blood of certain Cephalopods (*Loligo* and *Eledone*) and Ascidians, which they likewise found to contain copper but no iron. They assert that this blood possesses altogether opposite colour properties to that of *Helix pomatia*, *i.e.* that it is blue when free from oxygen but becomes colourless when shaken with air, again being bleached when oxygen is passed through it. v. Gorup-Besanez considers that this statement requires further proof before it can be accepted[4].

4. The whitish-blue blood of *Limulus Cyclops* was examined by A. Genth[5]. A few seconds after this blood is shed a yellowish-white coagulum separates from the liquid, which retains its blue colour. The latter is destroyed by boiling and by putrefaction. Genth analysed the ash of the blood of this creature and found it to contain in one case 0·081 p. c. of oxide of iron, and 0·085 of cupric oxide; in another case only a trace of iron, but 0·297 p. c. of metallic copper.

[1] C. Schmidt: see Lehmann's *Physiological Chemistry*, Vol. III., p. 256.

[2] Harless und von Bibra, Müller's *Archiv*, 1847, pp. 148—157. "Ueber das blaue Blut einiger wirbellosen Thiere und dessen Kupfergehalt."

[3] Gorup-Besanez, *Lehrbuch der physiologischen Chemie*, p. 369.

[4] Gorup-Besanez, *Op. cit.* p. 370.

[5] "Ueber die Aschenbestandtheile des Blutes von Limulus Cyclops." *Ann. d. Chem. u. Pharm.*, LXXXI. (1852), p. 68.

The Blue Blood of the Octopus. Haemocyanin.

However interesting the above facts may have been as rendering it most probable that the blue colouring matter of the blood of certain of the Mollusca is concerned in the function of respiration, and suggestive of the possibility that other metals may take the place of iron as constituents of the blood-colouring matter, they are infinitely less important than the observations of Frederique made upon the blood of the Octopus.

Rabuteau and Papillon[1] had described the blood of the Octopus, and had correctly pointed out that it becomes blue on exposure to air, doubtless in consequence of the action of oxygen. Their researches have been continued by Léon Frederique[2] with the following most interesting results:—

The blood of the Octopus has a specific gravity of 1047, and it contains between 13 and 14 per cent. of solid matters. The blood contained in the vessels going to the branchiae is colourless, whilst the blood leaving them is of a deep blue colour. If a large artery be exposed in a living octopus, whilst it is immersed in water, and breathing freely, it will be seen to have a deep blue colour, due to a substance dissolved in the plasma; if the animal be now withdrawn from the water, as the respiration becomes impaired, the colour of the artery is seen to become lighter and lighter, its contents becoming ultimately colourless.

The blue blood drawn from an artery, if placed in a closed vessel, undergoes, after some hours, a process of bleaching, the change of colour being analogous to the change of the tint of arterial blood when it is similarly treated. When the blue blood is boiled in the receiver of a mercurial pump the blue colour disappears. The same result follows when it is subjected to a stream of H_2S or CO_2.

The blue colour is due to a body to which Frederique has given the name of HAEMOCYANIN.

This body, like haemoglobin, is allied to the proteids, but still more complex, seeing that it yields a proteid substance as one of its decomposition products, but in addition a colouring matter. In the case of haemocyanin this colouring matter is blue, and contains copper. Following the analogy of haemoglobin the blue compound might be termed oxy-haemocyanin, and the colourless derivative simply haemocyanin.

Solutions of oxy-haemocyanin when examined with the spectroscope do not present any definite absorption-bands. Solutions of the body, when heated, exhibit slight opalescence at 65° C., and this

[1] Rabuteau et Papillon, "Observations sur quelques liquides, &c." *Comptes Rendus*, v. 77, (14 Juillet, 1873) p. 137.

[2] Léon Frederique, "Sur l'organisation et la physiologie du Poulpe." *Extrait des Bulletins de l'Académie Royale de Belgique*. 2me série, T. XLVI. N° 11; 1878.

increases to 73° C.; coagulation actually occurs at 74° C. They are likewise coagulated by alcohol, ether, mineral acids, and glacial acetic acid; and give the general reactions of the proteids.

Haemocyanin is a colloid, non-crystallizable body; in addition to it there appears to be no proteid or proteid derivative in the blood. When decomposed with mineral acids it yields a prismatic crystalline body.

It was said that the blue colouring matter of the blood of the Octopus was contained in the liquor sanguinis. The blood does contain a small number of corpuscles, but these are colourless.

The following table, extracted from Frederique's memoir, exhibits the results of the quantitative analyses hitherto made of the blood of Cephalopoda.

	Harless	Paul Bert	Schlossberger		Léon Frederique
	Eledone	*Sepia*	*Sepia*	*Octopus*	*Octopus*
Solid matters in 100 parts	7·23	10·9	18—20	12·6	13·689
Salts	2·63		3·205	2·225	3·014
„ soluble . . .	1·975		2·7918	1·940	2·33
„ insoluble . .	0·655		0·414	0·284	0·684
Organic matters . .	4·6		„	10·375	10·675
Proteids . . .	„	3·4	„		8·9

On certain coloured corpuscles found in the Perivisceral Fluid of certain Sea-urchins and Holothurians.

The perivisceral fluid of Sea-urchins and Holothurians has a more or less distinct reddish tinge, which is due to the admixture of a considerable proportion of coloured corpuscles[1]. These are large nucleated amoeboid cells, of which the fluid endosarc is filled with small highly refracting spherules of a rich mahogany-brown colour. They abound in the water-vascular system and in the intestinal blood-vessels of the Urchin, and are also to be found scattered throughout all the tissues, more particularly the integument. The following observations have been made by Mr Patrick Geddes and have been kindly communicated by him to the Author.

If an Urchin be divested of its spines and left exposed to the air, its warm hue soon becomes dingy, and, in the course of a few hours

[1] For descriptions and figures of these corpuscles see—
Erdl Wiegmann's *Archiv*, 1842.
Williams, On the Blood-proper and Chylaqueous Fluid of Invertebrate Animals. *Philosophical Transactions*, 1852. Part II. p. 595.
Semper, *Reisen im Archipel der Philippinen*, Bd. I. Taf. XXXIII.
Hoffman, *Niederl. Archiv*, 1871.
Geddes, "Observations sur le fluide périviscérale des Oursins." *Archives de Zoologie expérimentale*, 1878.

changes into a peculiar dark green. When a quantity of perivisceral fluid containing corpuscles in the dingy brown state is placed in the vacuum of the mercurial gas pump, it rapidly recovers its normal colour. Thus the colouring matter of these corpuscles is readily oxidised and deoxidised, and there is considerable probability that it may have a respiratory function. However, on account of the small number of brown corpuscles in the fluids of the Urchin, it is impossible to make satisfactory analyses of the evolved gases by means of the blood pump, nor has any attempt to isolate the pigment yet succeeded.

That this brown substance is nearly related to the purple colouring matter of the shell of many urchins, as well as to the yellowish-brown (biliary ?) pigment of the intestinal epithelium, is made evident by adding a mineral acid to their alcoholic solutions. All three immediately assume a green tint, very similar to that of the integument of the dead Urchin. Moreover, when a morsel of any of the highly pigmented tissues of *Spatangus purpureus*, for instance, the ovary, is slightly torn with needles, purple spots appear at the injured points, and, under the microscope, the brown corpuscles may be watched, one by one changing into purple.

Lemon-yellow amoeboid corpuscles are also found, though sparingly, in the fluids of certain of the regular Sea-urchins (*Dorocidaris*, *Arbocia*), and are exceedingly abundant in the perivisceral fluid of the *Spatangoidea*.

The greatest variety of colour is to be seen in the contents of the intestinal vessels of *Spatangus*, in a single preparation of which may be seen brown, purple, green, lemon-yellow, and indigo-blue amoeboid corpuscles, together with vast numbers of peculiar greyish vesicles of very variable size, from that of a *micrococcus* up to more than that of a coloured corpuscle.

CHAPTER III.

CHANGES WHICH THE BLOOD UNDERGOES IN DISEASE.

Introduction.

The blood may be looked upon as the internal medium whither tends the stream of matter which flows from the external world into the organism, and whence simpler combinations of matter, which are the result of the chemical processes of the organism, leave it to form again a part of the external medium. The blood represents a common reservoir which is continually being drawn upon by each tissue and organ for the materials which it needs, and to which, in its turn, each tissue and organ contributes its quota of useful manufactured products or of useless waste.

If we except the coloured corpuscles, whose function it is to act as the internal oxygen-carriers of the body, and the colourless corpuscles, which we have good reason to think are the precursors of the coloured, the blood represents a solution of organic and inorganic matters, which is continually being added to and taken from, in different ways and degrees, by the different tissues and organs, and at varying rates by each tissue or organ according to the degree of its functional activity.

The ancients looked upon the blood as essentially representing vitality: as that part of the matter of the body in which specially resided *the life*, and hence arose the natural wish to connect all the morbid processes of the body, processes tending towards death, with a perversion of the life-giving or actually vital liquid—a wish which found expression in the various phases of the *humoral pathology* which under one form or another reigned more or less imperiously over medicine until the fifth decade of the present century had passed.

If, however, we look upon the blood very much as a fluid contained in a reservoir which is contributed to by many sources, and whence at many points, by a variety of chemical and physical processes, matter is being continuously removed, we shall, naturally, be forced to admit that any changes which the blood undergoes are, in all

probability, nearly always dependent upon some modification of the organs which intervene between the external world and itself, of the organs through which certain of its materials have to pass in order to reach it, or of the organs through which other of its materials have to pass before they can be eliminated.

The progress of biological research has tended more and more to confirm this view of the relation of the blood to the organs of the body, and to transfer the vital processes to those elements of the various organs which we term *cells*, modified though these may be from the ideal conception of the cell in its primordial condition, as represented, for instance, by the mammalian ovum, or the cells of embryonal connective tissue.

Apparently, it is in connection with those extra-vascular *centres of nutrition, the cells*, that take place those chemical processes (nearly all of which are associated with oxidation) which result either in the assimilation of fresh matter for the body's use, or of elimination of waste matter which would accumulate to the body's detriment, or which *primarily* have for their object the evolution of the kinetic energy which the body needs, in order that it shall perform its internal and external work; so that the life of an organ, as evidenced by its ability to perform those acts which characterize it as *alive*, may be philosophically considered as the sum of the life of its constituent living centres, *the cells*, and the life of the organism as, in one sense, the sum of the life of all the constituent living centres of its various organs.

If this view be correct, disease will, in all probability, depend primarily upon modifications in the processes of *cells*, rather than of the fluid whence cells obtain their nourishment, and we shall be quite prepared to find (1) that a morbid process may seriously interfere with organs whose functions are essential to life, without influencing the composition of the blood in a manner perceptible by our methods of chemical and microscopic analysis, however delicate these may be, and (2) that when a marked change is revealed by these methods of enquiry it must be a difficult matter to trace the component causes of which the change is the resultant effect. The first proposition is proved by the paucity of results which have been obtained in spite of the assiduous labours of many scientific physicians, the second may be well illustrated by taking as an example that change in the blood which is better characterized than all others, viz. *anaemia*, or that condition in which the relative and absolute number of the coloured corpuscles of the blood is diminished.

It is a condition which may result from accidental losses of blood, or from some process (for example, abundant suppuration) which tends unnaturally to drain the blood of some of its constituents, or from a deficiency of proper food, or from causes so complex that we willingly hide our ignorance under the expression of *disorders of nutrition*. Where the actual fault primarily lies can, in many cases, be not even guessed at, and the physician knows little more than that the disorder

of nutrition is one which is often successfully overcome by the administration of iron, by fresh air, and an abundant diet.

Researches on the chemical changes which take place in the blood in disease were not possible until the chief proximate constituents of the blood had been studied, and methods devised for their reparation. Amongst the researches which proved of the greatest value in this respect were those of Berzelius[1], of Thénard and then of MM. Prévost and Dumas[2], which, by determining the mean composition of healthy human blood, first established a standard of comparison which might be referred to by those studying the changes induced in the blood by disease. Amongst the most complete of the systematic investigations which were made in the latter subject were those of Andral and Gavarret[3], of Becquerel and Rodier[4], of Simon[5], whilst the changes in particular diseases engaged the attention of certain distinguished writers, as of Christison[6], of Garrod[7], of C. Schmidt[8].

During the last thirty years comparatively little attention has been paid to the condition of the blood in various diseases, a fact which may be explained partly as due to the discontinuance of the practice of venesection, which has deprived the physician of the material required for these investigations, partly as a result of the change of views which has been explained at the commencement of this section.

Of late, however, attention has again been enthusiastically directed to the modifications which certain constituents of the blood undergo in disease, notably to the variation in the relative number of coloured and colourless corpuscles, and of the haemoglobin contained in the former, and we may therefore expect rapid accessions to the exact knowledge which we possess.

We shall in the first place consider categorically the changes which the various normal constituents of the blood undergo in disease, and then draw special attention to the results of investigations of the changes in *particular diseases.*

[1] Berzelius: see "General views of the Composition of Animal Fluids." *Transactions of Med.-Chir. Soc. of London*, Vol. III. p. 198.

[2] Prévost et Dumas, "Examen du sang et de son action dans les divers phénomènes de la vie." *Ann. de Chimie*, 1821, T. XVIII., p. 280. A second memoir, with the same title, was published in the *Annales de Chimie et de Physique*, 1823, Vol. XXIII. p. 50 and p. 90.

[3] Andral et Gavarret, "Recherches sur les modifications de proportion de quelques principes du sang (fibrine, globules, matériaux solides du sérum et eau) dans les maladies." *Annales de Chimie et de Physique*, Tome LXXV., p. 225—"Recherches sur la composition du sang de quelques animaux domestiques dans l'état de santé et de maladie,' (in conjunction with M. Delafond). *Annales de Chimie et de Physique*, 3me série, Vol. V. p. 304. Andral, *Essai d'Hématologie Pathologique*, Paris, 1843.

[4] Becquerel et Rodier, *Recherches sur les altérations du sang.* Paris, 1844.—*Traité de Chimie Pathologique appliquée à la Médecine Pratique.* Paris, 1854.

[5] Simon, *Animal Chemistry*, translated by G. E. Day, M.D. Sydenham Society, 1845.

[6] Christison, "On granular degeneration of the kidneys, and its connexion with dropsy, inflammations and other diseases. 8vo. Edinburgh, Adam and Charles Black, 1839.

[7] Garrod: see page 143.

[8] C. Schmidt, *Charakteristik der epidemischen Cholera gegenüber Transudationsanomalien.* Leipzig u. Mitau, 1850.

SECT. 1. *Variations in the proportion of the principal Constituents of the Blood in Diseases in general.*

Water.

I. Before considering the changes which the blood undergoes in different diseases, it is well to insist upon the fact that loss of blood very rapidly influences the composition of that which remains in the vascular system. It has been shewn by the concordant results of many trustworthy observers[1] that when an animal is bled, the portion of blood first obtained contains the largest quantity of solid matter, and that this gradually diminishes, so that the blood obtained at the commencement of a venesection has a slightly, but still perceptibly, different composition from that obtained at its termination, unless, of course, the total quantity of blood withdrawn be excessively small.

This diminution in the solid matter of the blood which is noticeable even in the course of venesection is naturally much more perceptible in cases of excessive and repeated accidental haemorrhages. The diminution of solid matter is partly due to actual loss of solids, but in great part to the blood becoming more rapidly diluted by lymph than in the normal condition.

The normal quantity of water in the blood of man may be estimated as varying between 780 and 800 parts per 1000 of blood. An increase in the water of the blood is much more frequent than the converse; this increase may be only slight or it may be considerable.

A slight augmentation of the water of the blood, *i.e.* to between 800 and 820 parts per 1000, occurs as a result of a temporary abstinence from food, in the early stages of nearly all acute diseases, and in the majority of chronic diseases.

A more marked augmentation, the water amounting to between 820 and 880 parts per 1000 of blood, occurs in starvation: after considerable haemorrhages; in cases of abundant suppuration, or in which some other considerable drain is taking place, as in chronic diarrhoea; in the course of malarial diseases; in lead poisoning; in chronic mercurial poisoning; in cancerous and tubercular affections: and we might add in *anaemia*, if it were not more correct to characterize the latter as the condition which really exists in all the morbid states just enumerated.

A decrease in the quantity of water of the blood has been observed in articular rheumatism, in erysipelas, in puerperal fever, and especially in cholera.

Coloured Corpuscles and Haemoglobin.

II. The coloured corpuscles are increased in the first stages of cholera; the increase is however not an absolute one, but merely dependent on a diminution of the water of the blood. A diminution of the coloured corpuscles occurs in the various forms of anaemia, including chlorosis; in Bright's disease; as a result of prolonged diarrhoea and dysentery; of continued and abundant suppurative discharges; in scurvy; in leucocythaemia; in the advanced stages of continued and of intermittent fevers; in chronic metallic poisoning; in cases of advanced heart disease; in chronic diseases generally.

[1] Prévost and Dumas, Becquerel and Rodier, Simon, and others.

In health, the amount of haemoglobin in the blood appears to be proportional to the number of corpuscles. This relation does not hold, however, in disease, as will be particularly mentioned in discussing the phenomena of anaemia.

The largest number of determinations of the amount of haemoglobin in the blood of various diseases has been carried out by Quincke[1], who made use of Preyer's method for the determination of haemoglobin. In the annexed table may be seen the results which he obtained. The letters V-S. in the second column indicate that the blood was obtained by venesection, and the letter H that it was obtained by Heurteloup's artificial leech.

Sex and Age.	Method by which blood obtained.	Specific gravity.	Haemoglobin in 100 grammes.	Disease.	Observations.
F. 35	VS.	1058	14·4	Angina pectoris.	Otherwise healthy, well nourished woman.
F. 60—70	VS.	1060·6	14·1	Cerebral Apoplexy.	Previously healthy and well nourished, V-S. two hours after the attack.
M. 44	H.	1060·8	14·6	Scorbutus.	Purpura haemorrhagica on lower extremities which quickly disappeared by rest in bed; state of nutrition good.
M. 20	H.	1049·6	10·1	Cirrhosis of the liver; haemophilia.	Pretty intense jaundice. Frequent epistaxis, profuse bleeding from any accidental wound.
F. 15	H.	1035·2	5·3	Chlorosis.	Well developed body; no complication. Date Nov. 14, 1869.
	H.	1049·1	9·92	,,	10 weeks later. Has been taking iron. Date Feb. 3, 1870.
M. 45	H.	1044·3	5·80	Splenic leucocythaemia.	
F. 28	VS.	1050·5	10·30	Parenchymatous Nephritis.	Patient died of acute oedema of the lungs.
M. 40	VS.	1047·3	10·70	Nephritis Uraemia.	Considerable general oedema. The patient died a few hours after.
M. 27	VS.	1048·7	11·40	Nephritis Uraemia.	Considerable oedema. Constitutional syphilis. V-S. during a uraemic convulsion. Sp. gr. of the serum, 1044.

[1] Quincke: "Ueber den Hämoglobingehalt des Blutes in Krankheiten." Virchow's *Archiv*, Vol. LIV. (1872), p. 537.

Sex and Age.	Method by which blood obtained.	Specific gravity.	Haemoglobin in 100 grammes.	Disease.	Observations.
M. 43	H.	1047·0	10·60	Bright's disease contracted stage.	Considerable oedema. Very abundant urine of light colour and low specific gravity.
M. 24	H.	1041·1	8·5	Bright's disease contracted stage.	Very considerable oedema. Chronic uraemia. Post-mortem examination revealed highly contracted kidneys.
M.	H.	1054·9	14·4	Diabetes Mellitus.	Appetite still very good. Total quantity of urine in 24 hours, 10 litres. Sp. gr. 1030.
M. 30	H.	1059·5	15·9	Diabetes Mellitus[1].	Enormously fat person. Good appetite. Urine in 24 hours from 3 to 4 litres. Sp. gr. 1020.
M. 22	H.	1056·6	12·9	Typhoid fever, 1st week.	A somewhat cachectic individual.
M. 25	H.	1059·6	12·7	Typhoid fever, 1st week.	Moderately strong man.
M. 25	H.	1062·1	14·6	Typhoid fever, 1st week.	Moderately strong man, an attack of medium severity.
	H.	1054·4	12·6	Typhoid fever, 4th week.	
M.	H.	1056·4	14·4	Relapsing fever, 5th day.	Strong man.
F. 50	VS.	1057·9	15·0	Cerebro-spinal meningitis of great acuteness.	A strong person. Apparently has been ill three days. Deepest coma. Death on the 5th day.
M. 56	H.	1050·5	11·3	Pyaemia, 2nd or 3rd week.	Following a phlegmonous abscess of the neck, there occurred phlebitis of the jugular vein and pyaemia.
F. 20	VS.	1056·7	14·9	Phosphorus poisoning.	Patient had four days before swallowed an infusion of lucifer matches. Intense icterus, enlargement and tenderness of the liver. Death 12 hours after venesection.

[1] This was probably a case which should have been termed glycosuria, rather than diabetes mellitus. Sugar not unfrequently occurs in the urine of very obese persons who present none of the other symptoms of diabetes. This statement the author makes upon the authority of a verbal communication from Dr Lauder Brunton.

Fibrin. III. The quantity of fibrin which separates from the blood during coagulation, and which normally amounts in the case of man to about 2·5 parts per 1000, may increase in disease to as much as 10 parts per 1000. This increase of fibrin is to a certain extent characteristic of acute inflammatory affections; it is clearly not to be ascribed to the pyrexia which is often a prominent feature of these diseases, seeing that in the fevers the proportion of fibrin is diminished instead of being increased.

According to Becquerel and Rodier the cases in which the amount of fibrin is increased may be divided into two categories. In the first category the augmentation is only slight, the amount of fibrin fluctuating between 3 and 5 per 1000 of blood. In the second it is considerable and is comprised between 5 and 10 parts per 1000 of blood.

A. Slight augmentation of fibrin occurs (1) in chlorosis; (2) in pregnancy, especially towards the close of utero-gestation; (3) in slight inflammatory affections, if accompanied by pyrexia; such as slight attack of erysipelas of the face &c.; (4) in certain cases of scorbutus.

B. Considerable augmentation of fibrin (amount varying between 5 and 10 per 1000 of blood) is characteristic of the more serious inflammatory affections. It is most marked for instance in pneumonia, pleurisy, and acute rheumatism. Whenever the parenchyma of organs is implicated in the inflammatory process the fibrin of the blood appears to increase. Whence comes the increase? Seeing that we are yet in ignorance as to the origin of the fibrinogen of the blood plasma, a solution of the above question is impossible. In the proliferation of cellular elements which accompanies the process of inflammation we have however a cause which will add to the number of colourless cells of the blood, and to the amount of serum-globulin which will be available in the process of coagulation. Whether we admit or deny Schmidt's theory there is no question as to the influence which serum-globulin exerts in increasing the amount of fibrin, and this is one way (though only one) in which we may conceive that inflammatory diseases cause the proportion of fibrin to increase.

A diminution in the proportion of fibrin (so that it sinks to between 1 and 2 parts per 1000 of blood) has been observed in certain acute and certain chronic diseases. Amongst the former are to be reckoned typhoid fever, small pox, scarlet fever and measles; amongst the latter, organic affections of the heart in their last stage, certain malarial cachexiae, chronic scurvy, and chronic mercurial poisoning.

Serum-Albumin. IV. The normal amount of serum-albumin in the serum of the blood of man amounts on an average to 80 parts per 1000, the limits varying between the numbers 70 and 90.

An augmentation of serum-albumin has been observed to occur in cholera and after the use of hydragogue cathartics[1]. To a less extent in acute rheumatism and in the early stages of some intermittents (?).

A diminution of serum-albumin occurs most markedly in Bright's disease, anaemia, scurvy, dysentery, and generally in chronic diseases which impair the general nutrition: for instance, in the advanced stages of some cardiac affections.

[1] C. Schmidt, *Charakteristic der Cholera*.

Fats. V. The norma amount of fatty matters in healthy blood varies, according to Becquerel and Rodier, between 1 and 3·3 parts in 1000. It is said that the fats of the blood are increased in pneumonia, in alcoholism, in diabetes, in Bright's disease, in the hepatitis of hot climates, in cases of chylous urine, in some cases of acute rheumatism, and in many acute and chronic cases of poisoning[1]. The information on many of these points is in the highest degree unsatisfactory.

Cholesterin and Lecithin. VI. The amount of cholesterin in normal blood varies probably between 0·5 and 2·0 parts per 1000. According to Becquerel and Rodier this constituent increases in quantity in all acute febrile affections, in all acute inflammations, and especially in cases of jaundice in which there is almost complete retention of bile.

We possess no information whatever as to the amount of lecithin present in the blood in disease; indeed our knowledge of the proportions present in health only rest on a very few analyses by Judell and Hoppe-Seyler.

Sugar. VII. Sugar is increased in the blood of diabetes, as will be mentioned under that disease.

Urea, Uric acid and other extractives. VIII. Amongst the so-called extractive matters present in the blood, urea, uric acid, and hypoxanthine require to be mentioned as being affected in disease.

The amount of urea in the blood is largely increased in the various forms of Bright's disease[2], as was first shewn by Christison, in cholera[3], and in yellow fever. It has been said that this is the case also in diabetes and febrile affections[4].

Uric acid[5], as will be more particularly referred to under Gout, is markedly increased in the blood in acute and chronic cases of that disease.

Hypoxanthine has been found in considerable quantities in the blood of leucocythaemia[3]; according to Salomon this body is a constituent of healthy blood.

Salts. IX. *The salts of the blood*, especially the alkaline salts, undergo certain changes in disease, though our knowldge is yet very imperfect on this matter. In cholera, the serum of blood, though it contains less salts than normal, contains a larger quantity of salts of potassium; in dysentery, the salts of the serum are said to be increased, and the same holds in the case of Bright's disease.

The Gases of the Blood. X. As yet few facts have been collected which throw any light upon the proportion of the gases in the blood in disease. From a knowledge of the changes which other constituents undergo in certain diseases, or from a knowledge of the

[1] Gautier, *Chimie appliquée à la physiologie, à la pathologie et à l'hygiène.* Vol. II. p. 314.

[2] Christison, *On granular degeneration of the kidneys*, &c. Edinburgh, 1839.

[3] Scherer, *Verh. d. physik.-med. Ges. zu Würzburg*, Vol. II. pp. 321—325, and Vol. VII. pp. 123—126.

[4] Picard, *Thèse de Strasbourg*, 1856.

[5] Garrod, *A Treatise on Gout and Rheumatic Gout.* Third ed., 1876, p. 84 *et seq.* The first researches of this author on this subject were published in the *Medico-Chirurgical Transactions*, Vol. XXXVII.

physical conditions of the patients, we can often surmise the way in which the gaseous exchanges of the blood must be affected. Thus from the amount of haemoglobin found in cases of anaemia and chlorosis, we can, with considerable accuracy, calculate the maximum amount of oxygen which such blood can contain, and we arrive at the conclusion that the amount is much below the normal.

Thus a healthy man's blood contains on an average say 13·5 grammes of haemoglobin in one hundred parts. Such blood in virtue of its haemoglobin would, if saturated with oxygen, be capable of absorbing 22·55 c.c. of oxygen measured at 0° C. and 760 mm. pressure.

On the other hand the blood in cases of chlorosis may contain as little as 5·3 grammes of haemoglobin per 100 of blood. Such blood could in virtue of its haemoglobin only take up 8·85 c.c. of oxygen if fully saturated. We see therefore that the respiratory capacity of such blood is reduced to less than one half that of healthy blood.

Again in cases where mechanical causes exist which interfere with the due amount of the gaseous exchanges in the lung, the cyanosis and the dyspnoea, sometimes culminating in asphyxia, point to a condition in which the oxygen of the blood is greatly diminished and the carbonic acid greatly increased. Actual determinations are, however, almost entirely wanting[1].

Attempts have been made by certain observers to determine the changes which the gases of the blood undergo in disease. Unfortunately the methods employed have been such as to deprive the results of all value. Thus Quinquaud determined the amount of oxygen in the blood of various diseases by means of a standard solution of sodium hydrosulphite[2]. The results obtained by this method are unfortunately in no way comparable with those obtained by the mercurial pump. Again, Brouardel[3] has published analyses of the gases of the blood in variola and scarlatina which would appear to shew that in these diseases the proportion of oxygen which the blood can absorb is very much diminished. As, however, the amount of nitrogen found is much greater than could possibly have been held in solution by the quantity of blood analysed, the legitimate conclusion to be drawn is that the analyses possess no value. Régnard[4] has attempted to determine the so-called '*respiratory capacity*' of blood in disease, *i. e.* the maximum amount of oxygen which a given quantity of blood will absorb. Blood is shaken with air and then subjected to analysis in the mercurial pump. According to Régnard the respiratory capacity of blood is not affected even by putrefaction; *i. e.* blood which is decomposed can absorb as much oxygen as it did before the process of putrefaction set in. Régnard's observations were all performed with blood taken from the dead body, the clot being broken up artificially. They led to the conclusion that in many diseases the respiratory capacity is immensely diminished; were the results reliable they would indicate that under the

[1] In a case of cyanosis due to a cardiac lesion Lépine found that 100 c.c. of venous blood contained 64 c.c. of CO_2. *Gazette Médic. de Paris*, 1873, p. 128.

[2] Quinquaud, "Sur un procédé de dosage de l'hémoglobine dans le sang." *Comptes Rendus*, Vol. LXXVI. p. 1489. "Sur les variations de l'hémoglobine dans les maladies." *Comptes Rendus*, Vol. LXXVII. p. 447.

[3] Brouardel: "Des gaz du sang dans différentes maladies." *Société médicale des hôpitaux*, 1870, quoted by Régnard.

[4] P. Régnard: *Recherches expérimentales sur les variations pathologiques des combustions respiratoires*. Thèse pour le Doctorat en Médecine. Paris, 1878 109 *et seq.*

influence of morbid processes the power which haemoglobin possesses of linking oxygen to itself is more or less affected. The conditions under which these observations were made appear, however, to the author, to deprive them of any value whatever.

Légerot[1] produced septicaemia in dogs by the injection of putrefied blood and compared the respiratory capacity before and after the induction of the morbid state. His results would appear to shew that an enormous diminution (sometimes to more than one half) of the respiratory capacity occurs.

Sect. 2. The Changes which the Blood undergoes in particular Diseases.

In the preceding section we have grouped together under each principal constituent or group of constituents of the blood, the variations which have been observed in diseases generally.

We must now consider in detail the changes of the several chief constituents of the blood in certain special diseases, which have been particularly studied from this point of view.

A. The Blood in Disorders of Nutrition.

Anaemia.

It has long been known that in various forms of anaemia the coloured corpuscles of the blood undergo a diminution, which to a certain extent appears to be proportionate to the intensity of the disease. The observations of the earlier French writers on this subject were definite enough, and although made by methods which did not furnish an absolutely correct estimate of the weight of the dry corpuscles, and gave no indication of the weight of the moist corpuscles, yielded results which might be compared one with the other. Thus Becquerel and Rodier[2] classified cases in which the coloured corpuscles of the blood are deficient, into the three following classes, each distinguished by a separate letter. We give, in the first instance, their account, but slightly abridged.

Becquérel and Rodier's classification of cases of Anaemia.

Class A. Slight diminution (weight of dry corpuscles between 100 and 120 per 1000 of blood). Individuals belonging to this class are pallid, there is some feebleness; sometimes, but by no means always, a blowing murmur is heard with the first sound at the base, and a murmur in the carotids.

[1] Légerot, *Études d'hématologie pathologique basées sur l'extraction des gaz du sang.* Paris, 1874, quoted by Régnard, *Op. cit.* p. 121.

[2] Becquerel et Rodier, *Traité de Chimie pathologique.* Paris, 1854, p. 50 *et seq.*

This degree of diminution of the coloured corpuscles occurs under the following circumstances;—in feeble individuals of the so-called lymphatic diathesis: under the influence of insufficient diet: in persons inhabiting marshy districts: as the result of a copious blood-letting: as a result of the persistent use of purgatives: in chronic Bright's disease: after some days of an acute disease, such as a fever: in the course of many chronic diseases, &c.

Class B. Medium diminution (weight of dry blood-corpuscles between 80 and 100 per 1000 of blood).

This state of the blood is accompanied by a much more marked debility of those subject to it. The skin is pale and slightly yellowish. Bodily exertion is irksome. There exists palpitation, and some dyspnoea may come on if the patient takes exercise. There is a soft bellows murmur in the aorta and carotids, which is rarely propagated along other arteries.

The causes enumerated under Class A may, if continuing in operation, lead to the case being classed under B. As special causes are to be mentioned;—considerable losses of blood: continued diarrhoea (or dysentery): malarial cachexia: the cancerous cachexia: lead poisoning: chronic Bright's disease: the last stage of chronic diseases: lastly, and chiefly, chlorosis.

Class C. Great diminution (weight of dry blood-corpuscles varying between 40 and 80 per 1000 of blood).

Cases belonging to this class are much rarer than the preceding. The skin is pale, and may present a greenish hue, the strength is diminished; sometimes the least movement occasions sensations of painful weariness, muscular pains, dyspnoea and palpitation. Cephalalgia, vertigo, *tinnitus aurium* and other nervous troubles appear, in varying degrees of intensity. Syncope is readily induced; the pulse is quick and dicrotic; there is a murmur with the first sound of the heart at the base. A very loud bellows murmur is heard in the carotids, and usually there exists, especially in chlorosis, a more or less loud venous murmur (bruit de diable).

The following causes specially lead to the condition observed in this class;—great or frequently repeated hemorrhages: chlorosis: malarial anaemia: the cancerous cachexia, especially where cancer affects the stomach.

If to the causes producing a diminution of the blood-corpuscles enumerated by Becquerel and Rodier, we add abundant and long-continued suppuration, scurvy, leucocythaemia and the affection designated by the term 'pernicious anaemia' we shall have before us a pretty complete catalogue of the various morbid states connected with a diminution of the blood-corpuscles.

As will be appreciated by the reader of the section in which the determination of the weight of the coloured corpuscles is described, the weight of the dry corpuscles as found by such a method as that employed by Becquerel and Rodier does not admit of absolute accuracy. For the purposes of the physician it would be better if we could express the

variation which the weight of the moist corpuscles undergoes in respect to the weight of the liquor sanguinis, in various diseases. The methods which we possess for effecting this determination with accuracy are, however, so complex and so difficult, that no large collection of data directly obtained by this method exists. We can, however, as was shewn by the researches of C. Schmidt, obtain a very close approximation to the true weight of the moist blood-corpuscles present in the blood, if we multiply the results obtained by Prévost and Dumas' method (which was employed by Becquerel and Rodier in their researches) by 4.

Since, however, methods have been devised (1) for the enumeration of the blood-corpuscles contained in a known volume of blood and (2) for the determination of the amount of haemoglobin, the physician has been placed in possession of methods which have thrown great light upon some of the diseases in which the blood-corpuscles are diminished—in which typically the condition of anaemia exists.

By means of any of the methods described at pages 74—78 a close *approximation* to the number of corpuscles contained in the blood may be made in a few minutes, by employing a single drop of blood. Similarly by methods as ready and as accurate, the amount of haemoglobin in the blood may be determined.

As we have seen, haemoglobin constitutes by far the most abundant constituent of the red blood-corpuscles, and it might be supposed that the second of the above determinations might be sufficient for the purposes of the physician; the richness or poverty of the blood in coloured corpuscles being judged of by its richness or poverty in haemoglobin. Such is however not the case, as will be now briefly shewn.

Changes which the corpuscles undergo in Anaemia.

It resulted from the labours of Welcker, the discoverer of all the fundamental facts concerning the relative number, weight, cubic capacity, superficies and colouration of the blood-corpuscles, that in the physiological condition the colour of the blood is proportionate to the number of its coloured corpuscles—in other words, that in the healthy state the amount of haemoglobin contained in the red blood-corpuscles is constant. That Welcker was correct in his statement, in so far as the healthy state is concerned, has been proved by the recent researches of Worm-Müller[1], and is, on the whole, confirmed by Malassez[2]. In his remarkable researches on the changes which the blood undergoes in cholera and some other diseases, C. Schmidt[3] had however pointed out that the composition of the blood-corpuscles is liable to vary in disease, and attention was still more forcibly drawn to this interesting fact by Johann Duncan in 1867[4]. This observer

[1] Worm-Müller, "Ueber das Verhältniss zwischen der Zahl der Blutkörperchen und der Färbekraft des Blutes." *Om Forholdet imellem Blodlegemernes Antal og Blodets Farvekraft.* Christiania, 1876. Abstracted in Maly's *Jahresbericht*, Vol. VII. (1878), p. 102.

[2] Malassez, "Sur les diverses méthodes de dosage de l'hémoglobine et sur un nouveau colorimètre." *Archives de Physiologie*, Ser. II., Vol. IV. (1877), pp. 1—43.

[3] C. Schmidt, *Charakteristik der epidemischen Cholera*, &c.

[4] Duncan, "Beiträge zur Pathologie und Therapie der Chlorose." *Sitzungsber. d. kais. Akad. d. Wissenschaften zu Wien. Naturwissenschaft. Cl.* 1867. 2 Abth., pp. 516—522.

counted the corpuscles contained in a given volume of blood in three cases of chlorosis, and compared the colouring power of a given volume of the same blood with the colouring power of the same volume of healthy blood. From his observations he concluded that whilst the coloured corpuscles were nearly as numerous in his chlorotic patients as in healthy women, the amount of colouring matter was remarkably diminished, being about three times less in amount. The more recent researches of MM. Hayem[1] and Malassez[2], but especially of the former, have brought out the interesting fact that in the various forms of anaemia the anatomical characters of the red blood-corpuscles are affected, and that the normal relations between the haemoglobin and the other constituents of the corpuscles are disturbed. The following is an epitome of the statements of Hayem.

The changes which occur in the anatomical characters of the coloured corpuscles in anaemia are appreciated if we compare successively the diseased with the healthy corpuscles; paying attention to *size, number, form* and *colouration.*

1. Size. In normal human blood we may, according to Hayem, conveniently classify the corpuscles into three orders, according to size, viz. large, medium, and small; the large blood-corpuscles having a mean diameter of $8{\cdot}5\mu$, the medium $7{\cdot}5\mu$, and the small $6{\cdot}5\mu$. Usually the proportions in which these various corpuscles are present is the following: the medium-sized amount to 75, the large to about 12 and the small also to about 12 per 100, so that the mean size of the average blood-corpuscle is (according to Hayem) $7{\cdot}5\mu$.

If we except acute cases where the disease is suddenly produced by hemorrhage, in all forms of anaemia the size of the corpuscles is modified. Firstly, the blood contains a certain proportion of unusually small coloured corpuscles, which have a diameter varying between $2{\cdot}2\mu$ and 6μ. Almost as frequently, the blood contains a certain number of unusually large corpuscles, which we may term giant-corpuscles, measuring 10μ or 12μ or even 14μ. Secondly, the relation between the corpuscles of different sizes is disturbed, so that the blood contains a much larger number of small corpuscles in relation to other sizes than healthy blood.

In all cases of chronic anaemia of considerable intensity, the mean diameter of the corpuscles is always below the normal. It may fall to 7μ, *to* $6{\cdot}8\mu$, *to* $6{\cdot}5\mu$, *and even to* 6μ.

But this diminution in the mean diameter corresponds to a diminution in the mean volumes of the corpuscles.

Thus the normal blood-corpuscle, having a mean diameter of $7{\cdot}5\mu$, has approximately the volume of 66μ c.c. (cubic micro-milli-

[1] Hayem, *Recherches sur l'Anatomie normale et pathologique du sang.* Avec figures et tableaux. Paris, 1878. Here will be found reprinted the various papers on these subjects, elsewhere published by this author.

[2] Malassez, "Sur les diverses méthodes de dosage de l'hémoglobine et sur un nouveau colorimètre." *Archives de Physiologie*, Ser. II., Vol. IV. (1877), pp. 1—43.

metres). The corpuscle whose diameter is only 7μ has the volume of 57μ c.c.; that of $6{\cdot}5\mu$ has a volume of 49μ c.c.

When in anaemia the mean diameter of the blood-corpuscles falls to 7μ, 100 corpuscles correspond in volume to only 80 healthy corpuscles; when the mean diameter falls to 6μ, 100 corpuscles correspond only to 65 healthy corpuscles.

Even assuming that the proportion of haemoglobin remained constant in anaemia, it is obvious from the above considerations, that important consequences must result from the diminution in the size of the corpuscles, but as will be shewn subsequently, the proportion of haemoglobin does not remain normal.

2. Number. Usually the number of the coloured corpuscles is diminished in anaemia, but by no means constantly so. In the most intense cases of anaemia the diminution is however always very marked. In a case of malarial anaemia Hayem found 1,182,750 corpuscles in 1 cubic millimetre, and in a case of purpura hemorrhagica only 1,000,000, *i.e.* a diminution of the blood-corpuscles to between $\frac{1}{5}$th and $\frac{1}{6}$th the normal amount.

3. Form. The corpuscles of anaemic blood assume modifications of shape which would seem to depend upon a diminished consistence, and which affect chiefly the medium-sized or small corpuscles, which often assume an elongated oval form.

4. Colour. The most important character which distinguishes the blood of anaemia from healthy blood is a comparatively pale colour of its red corpuscles, which depends directly upon a diminution in the amount of haemoglobin. If by 1 we represent the maximum amount of haemoglobin, as measured by its colorific intensity, present in the unit volume of healthy blood, we shall find that the amount may be as low as 0·85 without health being sensibly impaired; indeed the unit volume of blood of mean composition contains a quantity of haemoglobin which fluctuates between 0·85 and 0·90, as compared with 1 (the maximum amount of haemoglobin contained in healthy blood). In anaemia, the amount of haemoglobin diminishes so much that the amount present in the unit volume may be represented by fractions varying between the limits 0·66 and 0·125, so that in the most intense anaemia the quantity of haemoglobin may sink to one-eighth its normal amount. In cases of anaemia of moderate intensity the amount varies between one-half and one-fourth.

We have seen that most interesting conclusions may be drawn from a consideration of the size alone of the corpuscles in anaemia; still more interesting are, however, the results of simultaneous enumeration of the corpuscles and determination of their colorific intensity.

Thus if we find the number of corpuscles in a cubic millimetre of blood to be 4 millions and the colorific intensity to correspond only to that of 2 million healthy corpuscles, we at once come to the legitimate conclusion that, in respect to the amount of haemoglobin which it contains, each corpuscle, in the case under investigation, is equivalent to one-half a healthy corpuscle.

By pursuing such methods of investigation Hayem has obtained results which have led him to recognize four classes of cases of anaemia, as determined by changes in the blood.

Hayem's classification of cases of Anaemia.

1. *Slight anaemia,* characterized either by slight changes in the corpuscles or by none. Corpuscles *equivalent* to between 4 and 3 millions of *healthy* corpuscles (the actual number may be actually as large as in health). The individual value of the corpuscles varying between 1 and 0·70 (1 expressing the normal as determined by richness in colouring matter).

2. *Anaemia of medium intensity,* characterized by marked changes in the corpuscles, with a diminution in the size of the corpuscles, the total of which may be *equivalent to* between 3 and 2 millions of corpuscles in the cubic millimetre, though the actual number may vary between 5,500,000 and 3,000,000; the individual value of the corpuscles may vary between 0·30 and 0·80.

3. *Intense anaemia,* characterized by marked changes in the corpuscles and especially by corpuscles of very unequal dimensions, some being of very large size. The total number of corpuscles may be equivalent to between 2 millions and 800,000 healthy corpuscles, though the actual numbers may fluctuate between 2,800,000 and 1 million; the individual value of the corpuscles may vary between 0·40 and 1.

4. *Extreme anaemia,* characterized by altered corpuscles, of unequal dimensions, whose mean size approaches the normal, and may even exceed it. The total number of corpuscles may be equivalent to between 800,000 and 450,000 healthy corpuscles, and the actual number of corpuscles may be very small, even smaller than corresponds to the number of corpuscles, so that in these cases the amount of colouring matter in each corpuscle may actually be higher than the normal.

During the course of treatment of anaemia by preparations of iron the progress of the case can be studied by comparative determinations of the total number of corpuscles and their colorific intensity, and it is sometimes found that as recovery advances the corpuscles actually decrease in number whilst their richness in colouring matter augments.

The Blood in cases of Chlorosis.

An attempt has been made to establish a distinction between the changes in the blood in chlorosis and other forms of anaemia. It appears to the Author however that such a distinction is of the most artificial and useless kind.

Chlorosis may be defined as an intense form of anaemia occurring in women (usually in adolescents), evidenced by a yellowish-green tint of the pallid skin, often associated with marked nervous symptoms, and usually, if not always, associated with disorder of the menstrual function. It is, that is to say, a disorder of nutrition accompanied by intense anaemia, which in some yet unintelligible manner is specially connected with the menstrual function.

In man, unless suffering from grave organic disease, or subject to obvious drain of blood constituents, the conditions rarely exist which are requisite to induce so characteristic an anaemia; in him, indeed, anaemia from hidden functional disorders of nutrition is rare, and, from the less susceptible nervous organization of man, when occurring in him, anaemia wears a somewhat different aspect from that which it presents in the chlorotic girl. Nevertheless chlorosis may be taken as the very type of anaemia depending upon a purely functional disorder of nutrition, and in discussing the changes which the constituents of the blood undergo in anaemia, we may take as examples cases of chlorosis, as they have been most frequently studied and obviously present the least complicated instances of anaemia.

It has been generally observed that in chlorosis, the bulk of the clot as compared with the volume of serum is markedly diminished and that usually the clot presents a buffy-coat. This appearance has by some been interpreted as indicating that the blood of chlorosis contains an excess of fibrin, which is not however the case. Amongst the causes which may induce the appearance of a buffy-coat is undoubtedly the increase in the relative quantity of liquor sanguinis as compared with the corpuscles. Thus Buchanan shewed long ago that when recently shed blood is diluted with serum, the clot which separates always exhibits a buffy-coat.

If we except the diminution in the number of corpuscles and of haemoglobin and the consequent increase of water, the blood in chlorosis exhibits no other deviations perceptible by chemical analysis.

The following exhibits the maxima, minima and mean results of twelve analyses of blood made by Andral and Gavarret in nine cases of chlorosis[1]:—

Composition of Blood in 1000 parts.

	Water.	Solid residue.	Fibrin.	Corpuscles.	Solid residue of Serum.
Maximum	868·7	181·3	3·6	95·7	100·9
Minimum	818·5	131·5	2·1	38·7	75·4
Mean	853·2	146·8	2·9	56·7	88·0

The following table exhibits the mean composition of the blood of six chlorotic girls as determined by Becquerel and Rodier.

Density of defibrinated blood	1045·8
Density of serum	1028·1
Water	828·1
Solid constituents	171·8
Fibrin	3·4
Fat	1·5
Albumin	72·1
Blood-corpuscles	86·0
Extractive matters and salts	8·8

[1] Simon's *Animal Chemistry*, Vol. I. p. 314.

The iron amounted to 0·319 parts in 1000. This would correspond to 74·1 parts of haemoglobin in 1000 of blood.

Changes effected in the Blood by the medicinal use of Iron.

Experience has long shewn that in the treatment of all forms of anaemia and especially of chlorosis, the administration of preparations of iron possesses astounding efficacy and that they are often essential to recovery. The clinical fact is borne out in a striking manner by the results of chemical analysis, no less than by the results obtained by the methods of enumeration.

The following are the results of the analyses by Andral and Gavarret of the blood in two cases of chlorosis, before and after the administration of iron. The number of blood-corpuscles increased coincidently with the improvement in the complexion and general condition of the patients.

1st Case.	Previous to use of iron	After use of iron
Water in 1000 parts	866·7	818·5
Fibrin	3·0	2·5
Blood-corpuscles	46·4	95·7
Residue of serum	83·9	83·3

2nd Case.	Previous to use of iron	After use of iron
Water in 1000 parts	852·8	831·5
Fibrin	3·5	3·3
Blood-corpuscles	49·7	64·3
Residue of serum	94·0	100 9

Leucocythaemia (*Leukaemia*).

In this disease the colourless corpuscles of the blood are enormously increased in number, so that they may amount to one-sixth or, it is said, even one-third the number of the coloured corpuscles. The condition is most usually associated with great hypertrophy of the spleen (*splenic leukaemia*), but more rarely with a general hypertrophy of the lymphatic glands of the body (*lymphatic leukaemia*). Usually the colourless corpuscles do not differ in shape or size from those in normal blood, though in certain cases (*lymphatic leukaemia*), they appear to be smaller in size.

In certain cases of leucocythaemia, nucleated coloured corpuscles are found which are similar to the nucleated coloured corpuscles which occur in the blood of the embryo. It has been shewn by Neumann[1] that when these cells occur the marrow of the bones is affected (*myelogenic leukaemia*); there occur, indeed, cases of leucocythaemia in which an affection of the marrow is the primary and essential lesion, others, and these are probably the more numerous, in which it occurs as a secondary phenomenon[2].

[1] Neumann, "Ein Fall von Leukämie mit Erkrankung des Knochenmarkes." *Archiv d. Heilkunde*, Vol. xi. (1870), p. 1—15.

[2] Eichhorst, *Die progressive perniziöse Anämie*. Leipzig, 1878, p. 6.

According to Scherer[1] the blood in leucocythaemia, besides being poor in haemoglobin, contains considerable quantities of hypoxanthine, of lactic acid, and of an albuminoid substance whose solutions possess the power of gelatinizing, and which is therefore surmised to resemble, if not to be identical with, gelatine. These results have been confirmed by the more recent researches of v. Gorup-Besanez and Salomon. The whitish-red blood clot obtained from the vessels after death, was by the former observer boiled with water, and the aqueous extract, when concentrated and then allowed to cool, gelatinized. As the body when separated did not in the least deviate the plane of polarization, v. Gorup-Besanez[2] considered its identity with gelatine to be disproved.

Salomon found 0·116 grms. of hypoxanthin in 1550 c.c. of blood obtained after death; the same quantity of blood yielded 1·5 grms. of zinc lactate.

Neither hypoxanthine nor lactic acid can, however, according to Salomon, be looked upon as characteristic of the blood of leucocythaemia, as they probably occur in healthy blood and certainly in that of other diseases[3].

Charcot's Crystals. Charcot[4] discovered in the blood, the spleen and the liver of leucocythaemic patients, certain microscopic colourless elongated crystals, insoluble in water, but soluble in acids and alkalies, which he and Vulpian[5] were inclined to consider as consisting of a proteid body. Salkowski considered them to be composed of a mucin-like body. According to Zenker[6] these crystals are often observed to form within the colourless corpuscles.

These crystals are never observed in the blood during life, or immediately after it is drawn from the vessels; they usually separate in large numbers as the leucaemic blood decomposes.

They usually appear as very much elongated octohedra, or simply as spindle-shaped bodies, which are transparent, colourless, insoluble in ether, chloroform, and alcohol; soluble with difficulty in cold or hot water, but easily soluble in dilute acids and alkalies. They appear to consist of the phosphate of a base which has been discovered by Schreiner[7] in semen and in the spirit in which anatomical preparations have been kept. To the hydrochlorate of this base this author provisionally ascribes the formula C_2H_5N. HCl.

[1] Scherer, *Verh. d. physik.-med. Gesell. zu Würzburg*, Vol. II. pp. 321—325.

[2] v. Gorup-Besanez, "Untersuchung des Blutes bei lienaler Leukaemie." *Sitzungsberichte der physikal-medicin. Societät in Erlangen*, Mai, 1873. Abstracted at considerable length in Maly's *Jahresbericht*, Vol. IV. p. 126.

[3] Salomon, "Zur Lehre von der Leukämie." *Archiv f. Anat. u. Phys.*, 1876, p. 762.

[4] Charcot et Robin, *Comptes Rendus de la Soc. de Biologie*, 1853.

[5] Charcot et Vulpian, *Gazette Hebdomadaire*, 1860, p. 755.

[6] Zenker, "Ueber die Charcot'schen Crystalle im Blute und Geweben leukämischer und in den Sputis." *Archiv f. klin. Medicin*, XVIII. 125—135.

[7] Schreiner, "Eine neue organische Basis in thierischen Organismen." Liebig's *Annalen*, Vol. CXCIV., p. 68.

Results of analyses of blood of Leucocythaemia.

The following Table exhibits the results of six systematic analyses of the blood of leucocythaemia by various observers[1].

Authorities.	Density of Blood.	Density of Serum.	Fibrin.	Solid Matters of Serum.	Dry blood Corpuscles.	Total solid matters of Blood.	Water.
Robertson	1041·5	1026·5	6·0	72·0	67·5	145·5	854·5
,,	1036	1023	2·3	67·0	49·7	119·0	881·0
,,	1049·5	1029	5·0	95·0	80·0	180·0	820·0
,,	1043·5	1027	3·2	80·7	82·3	166·2	833·8
Robertson	1049·5	1029	5·0	95	80	180	820
Isambert			1·4	69	69·2	142	858

Progressive Pernicious Anaemia.

By the above term Biermer[2] has designated a remarkable form of anaemia which had already been recognized and graphically described by Drs Addison and Samuel Wilks[3].

Occurring more frequently in women than in men, in adult life than in adolescence or old age, this disease seems frequently to originate in pregnancy, or to have exhausting disease as a predisposing cause. Cases, however, undoubtedly occur in which no predisposing cause can be traced.

Commencing insidiously as one of the more ordinary forms of anaemia, this disease is distinguished by the rapidity with which all the phenomena of the most intense anaemia are developed—such as intense pallor, dyspnoea, inability to undergo the slightest exertion, tendency to syncope, dropsy. It differs from the ordinary forms of anaemia by the occurrence of more or less pyrexia, by a great proneness to retinal hemorrhages, but especially by the much greater tendency towards a rapid fatal termination.

The disease is not associated with any essential lesion of any

[1] This table is taken from Robin's *Traité des humeurs*, 2nd edition, p. 272, to which we were referred by Gautier, who also uses it. (Gautier, *Chimie Appliquée*, &c., Vol. II, p. 321).

[2] Biermer, "Vorläufige Mittheilung über fettige Degeneration des Herzens und der Gefässe in Folge von Anämie;" *Tageblatt d. 42. Versamml. deutsch. Naturforscher u. Aerzte in Dresden*, 1868. *Deutsches Archiv f. klin. Med.* vol. XIII., p. 209.

[3] Addison, *On the constitutional and local effects of disease of the suprarenal capsules*. London, 1855. Collected Works, New Sydenham Soc. 1868, p. 211.

Samuel Wilks, "Cases of idiopathic fatty degeneration. With remarks on arcus senilis." *Guy's Hospital Reports*, 1857, p. 203.

In reference to the claims of these two authors to the merit of having first recognized the disease under discussion, the reader is referred to an interesting paper by Dr Pye-Smith, entitled "Zwei Fälle von Anaemia idiopathica perniciosa" (Virchow's *Archiv*, Vol. LXV. (1875), p. 507).

important organ, though in many cases fatty degenerations of heart, liver, and kidneys—changes which must be considered as mere results of imperfect nutrition—have been observed.

The statements of authors who have described this remarkable disease vary as to the changes which the blood undergoes.

In several cases where an enumeration of the coloured corpuscles has been effected, a very remarkable diminution has been found. Thus in a case described by Lépine the coloured corpuscles sank on the day preceding the death of the patient to 378,750 in 1 cubic millimetre. In a case described by Ferrand the number of coloured corpuscles was found to be 500,000 in 1 cubic millimetre, and the amount of haemoglobin estimated by the colorific intensity had sunk to one-tenth that of normal blood.

Dr Hermann Eichhorst[1] has studied the anatomical changes which the blood undergoes in certain cases of progressive pernicious anaemia, and the following is a brief summary of his observations:

1. The blood has a serous, amber-coloured appearance, with scarcely a trace of red (?), and coagulates with difficulty.

2. The colourless protoplasmic granules, which are always to be found more or less distributed throughout healthy blood, are completely absent.

3. The colourless cells of the blood are extraordinarily few in number.

4. The coloured corpuscles of the blood are diminished in number. Those which retain the form of normal corpuscles are observed to be increased in size, having a diameter of 8—9μ, some being as large as 9·5μ and very few having a smaller diameter than 8μ.

5. *In addition to the ordinary corpuscles there occurs a second class of corpuscles.* These are much smaller than normal coloured corpuscles, having a diameter varying between 3μ and 3·5μ or 4μ; they are not biconcave but globular, and are of a deeper red colour than normal blood-corpuscles. In one case these smaller corpuscles were in the ratio of 1 : 5 of the normal corpuscles.

That cases of progressive pernicious anaemia occur in which the changes which Eichhorst has described are not seen appears certain from the observations which have already been published. He himself admits that there may be, and doubtless are, several classes of cases, which have been, and which may be, grouped under the term of Progressive Pernicious Anaemia, but he argues that during the progress of the case a diagnosis is alone possible where the changes which he has described are found to exist.

The observations of Dr Byrom Bramwell[2] on several cases of pernicious anaemia, whilst they in the main confirm Eichhorst's statements in reference to the presence in the blood of small non-

[1] Eichhorst, *Die progressive perniziöse Anämie. Eine klinische und kritische Untersuchung*, p. 378. Leipzig, Verlag von Veit u. Comp. 1878.

[2] Byrom Bramwell, "Idiopathic or Progressive Pernicious Anaemia, with cases." *Edinburgh Medical Journal*, Vol. XXIII. (1877), p. 408.

biconcave coloured corpuscles, seem to shew that, not unfrequently, nucleated coloured corpuscles are present. In these cases it is to be assumed that an affection of the marrow of the bones existed similar to that which occurs in cases of myelogenic leukaemia. Professor Grainger Stewart[1] failed to observe Eichhorst's corpuscles in two fatal cases of pernicious anaemia. There can be no doubt moreover that cases of pernicious anaemia do occur which terminate in recovery. On the other hand, these corpuscles have been observed in large numbers in the blood of a case of leucocythaemia[2].

Scurvy.

Very little reliable information is possessed in reference to the alterations of the blood in this disease. When humoral doctrines prevailed, scurvy was looked upon as preeminently a disease due to marked changes within the blood, and attempts were made to explain the hemorrhagic tendency by ascribing it to a definite change in the composition of the blood, notably to a great diminution of fibrin (Andral[3].)

At the present time we are naturally more inclined to consider the hemorrhages which occur in this disease as due to a morbid change of the walls of the blood-vessels, and we are therefore not surprised to find that in many cases where the blood of patients affected with scurvy has been analysed the results have been purely negative.

Becquerel and Rodier had the opportunity of analysing the blood of five patients affected with scurvy. They found it to be poor in solid constituents and to be decidedly poor in corpuscles. In these five cases the proportion of haemoglobin (which we have calculated from the iron which they determined) was respectively 121·33; 64·41; 90·9; 99·3; 67·4 grammes per 1000 of blood. The amount of fibrin was found in no case to be below the normal, whilst in three of the five it was above the normal.

Mr Busk analysed the blood in three cases of scurvy which occurred in the Dreadnought Hospital Ship, and he found a great diminution in the solid constituents of the blood as a whole, a marked increase in the proportion of fibrin, albumin, and salts, with a very great diminution in the proportion of blood-corpuscles[4].

From the evidence which has been accumulated, it would therefore appear, that in scurvy a condition of anaemia is a constant antecedent, using that term to designate a diminution in the total corpuscular richness of the blood. The increase in the quantity of fibrin which sometimes, though by no means always is observed, probably depends

[1] Grainger Stewart, "Note upon Professor Eichhorst's new pathognomonic symptom of progressive pernicious Anaemia." *British Medical Journal*, July 8th, 1876, p. 40.

[2] Heuck, *Zwei Fälle von Leukämie mit eigenthümlichem Blut- resp. Knochenmarksbefund.* Virchow's *Archiv*, Dec. 1879, p. 475.

[3] Andral, *Essai d'Hématologie pathologique*, Paris, 1843, p. 128.

[4] The Author has failed in his attempts to discover the medium through which Mr Busk's results on this subject were published. They are, therefore, quoted at second hand.

upon the intercurrent inflammatory lesions which so frequently occur. As yet, however, facts are wanting to shew in what precise manner the composition of blood is affected in scurvy.

Purpura Hemorrhagica and Haemophilia.

In these two diseases no constant alteration of the blood has been discovered.

It was maintained by Becquerel and Rodier that in these diseases as well as in scurvy, the proportion of fibrin was diminished, but such is not actually the case. The colourless cells of the blood too have in some cases been stated to be increased; in others, on the other hand, to be unaffected. We can scarcely doubt that these are affections in which primarily the blood-vessels are more at fault than the blood.

Gout.

There are few diseases in which so notable a change in the composition of the blood can be observed as in gout. Whatever may be its cause, gout appears to be preeminently a disorder of nutrition, in which there is a great tendency to a peculiar inflammatory process, affecting in the first instance and chiefly (though by no means exclusively) certain joints. In this peculiar inflammation crystalline deposits of sodium urate are formed in the affected parts.

It is the great merit of Dr Garrod[1] to have discovered and demonstrated that during the attack of acute gout, as well as in the course of chronic gout, the blood *invariably* contains an excess of uric acid, so that from a comparatively small quantity of blood serum, a characteristic microscopic crystallization of uric acid can be obtained. This accumulation of uric acid in the blood is probably in some measure due to its non-elimination by the kidneys, seeing that whilst the attack of gout is at its height, though the relative proportion of uric acid in the urine may be high, and an actual spontaneous separation from it often occurs after emission, the actual amount excreted is usually below the normal.

The non-elimination is doubtless connected with the kidney affection which is an almost invariable concomitant of gout.

Garrod shewed, moreover, that in gout the blood contains oxalic acid[2], and it is very probable that it contains an excess of urea; upon this point, however, further research is greatly needed.

It appears to be indisputably proved that chronic lead poisoning renders those affected with it specially liable to attacks of gout, also that the ingestion of minimal doses of lead compounds will sometimes induce in the gouty an acute attack of the disease.

[1] Garrod, "Observations on certain pathological conditions of the blood and urine in gout, rheumatism and Bright's disease." *Medico-Chirurgical Transactions*, Vol. xxxi. p. 83, (1848). See also *A treatise on Gout and Rheumatic Gout*, 1876; and Article "Gout," Reynolds's *System of Medicine*, 3rd ed. London, Vol. i. p. 810.

[2] *Medico-Chirurgical Transactions*, Vol. xxxvii. (1854), p. 54.

Articular Rheumatism and Rheumatoid Arthritis.

In acute rheumatism there occurs a specific inflammation of the serous membrane of the joints, usually accompanied by considerable effusion, but scarcely ever ending in suppuration. There is at the same time a very great tendency to inflammatory changes of the pericardium and endocardium.

Clearly separated from gout by the absence of an excess of uric acid in the blood, and in the large majority of cases by that of lesions of the kidney, acute articular rheumatism is a type of a disease in which local inflammatory changes are accompanied by a high temperature.

As in all other diseases in which a high temperature accompanies local inflammatory changes, the most marked modification in the chemical composition of the blood consists in an augmentation of the fibrin which separates on coagulation.

The blood is usually buffed and cupped; the amount of fibrin is said to bear some relation to the intensity of the febrile disturbance.

At first the increase of fibrin is the only change which chemical analysis reveals; in the course of the disease, however, anaemia supervenes, and is evidenced by the aspect of the patient, no less than by a diminution in the number of the corpuscles. The changes, if any, which occur in the anatomical characters of the corpuscles or in the proportion of haemoglobin which they contain, have not yet been made out.

According to Becquerel and Rodier, in acute rheumatism, as well as in all other acute inflammations, there occur (1) an increase in the amount of fibrin; (2) a diminution in the amount of blood-corpuscles; (3) a diminution in the serum-albumin; (4) an increase in the fatty matters; (5) a diminution in the soluble salts of the blood. With the exception of the increase in the amount of fibrin, the other changes are in all probability chiefly to be referred to the disturbance between the balance of income and expenditure which occurs in all acute diseases, and which leads to rapid loss of weight and anaemia; these are, however, like the changes revealed by analysis, but the *effects* of a morbid process of which the exact starting point, no less indeed than the exact seat, is hidden from us.

In subacute rheumatism and in rheumatoid arthritis there are no constant changes in the composition of the blood. It is, however, of great importance, as constituting the widest difference between the so-called rheumatic gout (rheumatoid arthritis) and true gout, whether acute or chronic, that in the first-named disease there is no tendency whatever to accumulation of uric acid in the blood, and to its subsequent deposition in the tissues of the body.

Rickets and Osteomalacia.

In these two diseases, in which grave disorders of nutrition exist, which must necessarily affect the composition of the blood, no systematic investigation, so far as we know, has been attempted.

B. The Blood in Fevers.

Febricula or Ephemeral Fever.

Becquerel and Rodier[1] have made many analyses of the blood in febricula, which led them to the conclusion that none of the constituents of the blood undergo any constant or perceptible change in amount.

Typhus Fever.

So far as we are aware the only analyses of the blood of typhus fever are those made by Dr Guennau de Mussy and M. Rodier when sent in 1847 by the French government to report upon an outbreak of typhus fever in Ireland. The observations published by other continental writers, and which have been supposed to refer to typhus, have, for the most part at least, referred to typhoid[2]. The observations of Guennau de Mussy and Rodier shewed that in typhus there is, in general, a diminution in the density of the blood, no augmentation in the amount of fibrin, and a lowering of the specific gravity of the serum.

Typhoid Fever.

Very thorough analyses of the blood in typhoid were performed by both Andral and Gavarret, and Becquerel and Rodier. From their researches the latter observers arrived at the following conclusions. (1) That in typhoid fever, in its early stages, there is usually no change in the composition of the blood. Occasionally, however, in very acute cases accompanied by great prostration and by hemorrhages, there occurs a diminution of the chief elements of the blood, and particularly of fibrin. (2) That in the later stages of the disease, the corpuscles and serum-albumin diminish, under the influence of the deficient nourishment and of the losses of liquid which the patient is subject to. The amount of fibrin remains normal, or is diminished as the disease advances or becomes more grave.

Relapsing Fever.

Though there can hardly be any doubt that all zymotic diseases are produced by certain germs or elementary organisms, yet there are only two diseases in which the presence of such germs in the blood has been clearly demonstrated. These two diseases are: Relapsing fever, and Splenic fever.

Obermeier[3] was the first to demonstrate in the blood taken from

[1] Becquerel et Rodier, *Traité de Chimie pathologique*, p. 133.

[2] This remark applies to the observations of Lehmann (*Phys. Chem.*, Vol. II. pp. 265 and 266), which are referred to by Dr Buchanan in his Article on "Typhus Fever" in Reynolds's *System of Med.*, Vol. I. p. 549.

[3] *Centralbl. f. d. med. Wissensch.*, 1873, p. 145.

patients suffering from relapsing fever the presence of small organisms, which consisted of slender spiral filaments in a continual state of lively motion. Obermeier further observed that these organisms, which belong to the class of Schizomycetes and which resemble spermatozoa or spirilla, were absent in the period of apyrexia which intervened between the first and second period of fever, and that they reappeared again with the onset of the second period of fever. Obermeier's observations have been verified by all subsequent observers, notably by Weigert, Lebert and Heydenreich[2]. According to these observations it is now clearly made out, that these organisms have exactly the same appearance as the Spirochaeta described by Ehrenberg and found in stagnant waters. They consist of slender spiral filaments 0·001 mm. in diameter, 0·15—0·2 mm. in length; they are homogeneous in structure; and they are continually moving, their motion being both rotatory and progressive; they occur either singly or in large groups, attached to the red and white corpuscles of the blood (fig. 27).

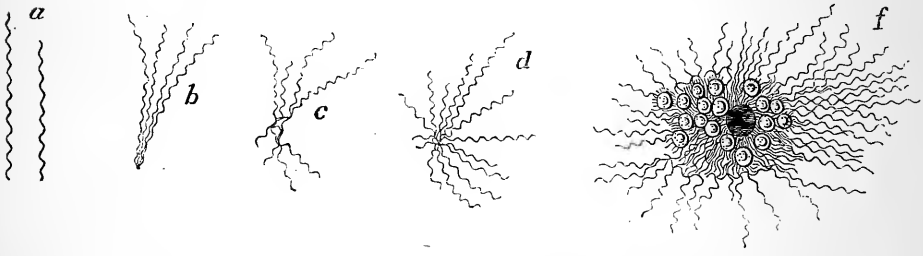


FIG. 27. THE 'SPIRILLUM' OF RELAPSING FEVER. (Heydenreich.)
a, *b*, *c*, *d* shew various conditions of aggregation. At *f* groups of filaments are seen to entangle a colourless and several coloured corpuscles.

They are only found in the blood of patients suffering from relapsing fever, and have not been found in any of the organs of such patients after death. They are found several hours after the onset of the febrile attack, and increase in quantity up to the termination of the first pyrexial period; they are absent in the interval and reappear again in the blood several hours after the second pyretic period has set in. When examined under the microscope their movements continue for several hours at the ordinary temperature, but a low or a high temperature and several reagents (alkalies, acids, etc.) stop their movements at once.

About their life-history nothing definite is known[1], nor have observers been able to propagate them in cultivating fluids. In several cases, however, inoculation with a small quantity of blood taken from patients suffering from Relapsing fever has induced the disease.

[1] *Klinische und mikroscopische Untersuchungen über die Parasiten des Rückfalltyphus*, 1877.

[2] See an interesting paper "On the life-history of Spirillum," by Patrick Geddes and J. Cossar-Ewart, *Proceedings of the Royal Society*, 1878, No. 188, p. 481, which treats of the spirillum of stagnant water.

Besides the presence of spirillum, other changes have been noticed in the blood in cases of relapsing fever, such as the disproportion between white and red blood corpuscles and the presence of large masses of protoplasm and of degenerated endothelial cells; these changes are however found in many pathological conditions, especially in fevers.

Splenic Fever (of Cattle).

Bacillus Anthracis.

Splenic fever is a disease not uncommon amongst cattle, and is both enzootic and contagious, *i.e.* the disease occurs chiefly in certain districts where it is enzootic, but is also propagated by contact. In the blood of animals suffering from splenic disease, Davaine long ago found certain bacilli or rod-like bacteria, which he believed to be the cause of splenic fever. It was however found that, though inoculation with blood containing these bacilli produced splenic disease, the blood of the animals thus inoculated did not contain any bacilli, though highly active when inoculated.

Koch[1] and after him Cossar-Ewart[2] have studied the life-history of these organisms and have cleared up the discrepancy. Koch found that in the blood and juices of the living body these rod-like bacteria multiply by fission; after the death of the animal, or when brought into cultivating fluids, these rods grow into long filaments or threads which produce spores—provided always the surrounding temperature remains moderately high; eventually the threads disappear and nothing but spores remains behind. These spores, which withstand decomposition for a long time (while the bacilli are very perishable), have been found to develop again into bacilli, when in contact with the air and at a moderate temperature.

The contagious character is due to the bacilli, but in the blood of the slaughtered or dead animals the bacilli develop under favourable circumstances into spores, and these being much less perishable and easily wafted about by currents of air are the cause of the epizootic character of the disease.

The observations on the presence of germs in the blood in other infectious and contagious diseases have as yet led to no perfectly definite results, though bacteria and micrococci have been seen in the blood in Pyaemia, Septicaemia, Diphtheria and Scarlatina[3].

[1] Cohn's *Beiträge zur Biologie der Pflanzen*, Band 1, Heft 3, p. 277. "Die Aetiologie der Milzbrand-Krankheit, begründet auf die Entwicklungsgeschichte des *Bacillus Anthracis*." Cohn's *Beiträge zur Biologie der Pflanzen*, Vol. II. 1876.

[2] Cossar-Ewart, "On the life-history of *Bacillus Anthracis*." *Quarterly Journal of Microscopic Science*, Vol. XVIII. (New Ser.), p. 161.

[3] While speaking of living organisms in the blood we may just mention the presence of some more highly developed parasites found occasionally in the blood, thus: The *Bilharzia Haematobia* (Cobbold), or *Distoma Haematobium* (Bilharz) inhabits the branches of the portal system, but most commonly the small veins of the ureter, bladder and pelvis of the kidney. It is endemic in Egypt where Griesmeyer found it in nearly one-third of all autopsies. The full-grown animal, bisexual and belonging to the trematodes, measures from 5—8 mm., and is soft-skinned.

This disease is recognized during life by the presence of the characteristic ova and embryos in the urine, which invariably also contains some blood. The eggs are

Intermittent Fevers.

There is primarily no chemical change in the blood in these fevers, as is shewn by the observations of Andral and Gavarret and of MM. Leonard and Foley, who studied intermittent fevers in Algeria[1]. According to the latter observers, especially, whatever the type of the intermittent, there is primarily no alteration in the blood, and no perceptible difference between the condition of the blood taken during an ague fit and that taken in the period of intermission. As the disease advances, however, the patient becomes anaemic, the blood-corpuscles and the solid matters of the serum diminishing in quantity.

Bacillus Malariae. Klebs and Tommasi-Crudeli[2] have made the remarkable discovery that the soil in malarial districts contains a bacillus to which they have given the name of *Bacillus Malariae*, which when introduced into the system of rabbits induces fever of an intermittent type. They have succeeded in cultivating this organism, which by cultivation loses none of its specific power. In the animals in which intermittent fever was thus artificially induced, the spleen became enlarged and in its juice, as also in the blood, were usually found the spores of *B. Malariae*, and sometimes the rod-like forms of the mature organism.

Since the above observations were first published, Marchiafava[3] has in three fatal cases of pernicious malarial fever succeeded in discovering in the splenic pulp, in the blood, and in the marrow of the bones, spores and filaments of *B. Malariae*.

Pigment in the blood. In severe forms of intermittent fever particles of black pigment have been found in the blood, and accumulations of such masses have been seen after death in and around the smaller blood-vessels and capillaries in the brain, liver, and spleen, and, according to Arnstein[4], in the medulla of the bones.

The pigment occurs in the form of smaller or larger granules, and is found either free in the blood or contained in the white blood-corpuscles.

oval, boat-shaped, bodies with a spiny projection at the anterior end. The embryo (as seen in the urine occasionally) is ciliated and flask-shaped.

Filaria sanguinis hominis (Lewis), a minute nematoid entozoon, is found in the urine and also in the blood of patients suffering from chyluria. The disease is endemic in India, but some cases have also been observed by Dr Bancroft in Australia.

The filaria is a long narrow worm about the breadth of a red blood-corpuscle and about 0·033 mm. long, it has a hyaline tubular envelope closed at both ends; under a high power transverse striae and granular masses are seen. When examined in the fresh state it shews active snake-like movements.

[1] Leonard et Foley, quoted by Bequerel et Rodier, *Op. cit.*, p. 132.

[2] Edwin Klebs e Corrado Tommasi-Crudeli, "Studi sulla natura della Malaria." *Reale Accademia dei Lincei*, Roma, 1879. (Memorie della classe di scienze fisiche, matematiche e naturali. Serie 3ª. Vol. IVº. Seduta del 1 giugno 1879.)

[3] Dr Ettore Marchiafava, quoted by Klebs and Tommasi-Crudeli, *op. cit.* p. 61.

[4] Arnstein, "Bemerkungen über Melanämie und Melanose." Virchow's *Arch.* Vol. LXI. p. 494.

The pigment is considered to be altered haematin, and, according to the older view (Frerichs, Virchow), its presence in the blood is due to a breaking up of the red blood-corpuscles in the spleen and liver. According to Arnstein, however, the pigment originates in the blood itself, from the destruction of the red blood-corpuscles during the attack of fever, and penetrating into the leucocytes of the circulating blood becomes deposited along with them in the spleen, liver and medulla of bone[1].

The formation of black pigment has lately been observed going on within the coloured corpuscles by Marchiafava[2], whose observations have been confirmed by Klebs and Tommasi-Crudeli; in the process, iron is set free from the state in which it exists in haemoglobin, so that when the corpuscles which have become black are treated with dilute hydrochloric acid and potassium ferrocyanide they acquire a blue colour.

Scarlet Fever, Measles, Small-pox, Erysipelas.

In the first of the above diseases there is primarily no alteration in the proportion of blood constituents. In scarlet fever, however, when kidney complications set in, there is a tendency to accumulation of urea in the blood, which often attains a high degree, and subsequently a great diminution of the proteids of the blood occurs.

In small-pox, coincident with the inflammatory changes in the skin, there occurs a moderate increase in the amount of fibrin.

In erysipelas, the blood at first presents in a characteristic manner the appearances which it always assumes when an acute inflammatory process involves an organ of any magnitude, or implicates any considerable extent of one of the tissues; the blood, in consequence, yields much fibrin. There is, however, no other constant alteration, unless there be any truth in the very doubtful statement of Schönlein[3] that the serum which separates from the blood, in erysipelas, is always tinged yellow by the colouring matter of bile.

The Blood in Cholera.

A very elaborate investigation into the changes which blood undergoes in cholera was made by Professor Carl Schmidt[4] during the epidemic of that disease which ravaged Dorpat in the summer and autumn of 1848.

In consequence of the very great transudation of water and albumin from the alimentary canal, the blood in cholera becomes excessively poor in water and relatively rich in solid constituents, so

[1] Virch. *Arch.* Vol. LXI. p. 494.

[2] Marchiafava, "Commentario clinico di Pisa. Fascicolo del gennaio 1879." Quoted by Klebs and Tommasi-Crudeli, *op. cit.* p. 57.

[3] Schönlein, quoted by Simon, *Animal Chemistry*, Vol. I., p. 278.

[4] Carl Schmidt, "Charakteristik der epidemischen Cholera gegenüber verwandten Transudationsanomalien." Leipzig und Mitau, 1850.

as to assume a viscid consistency. There thus appears to be an increase in the number of the coloured blood-corpuscles and even of the serum-albumin. Whilst the proper salts of the serum may fall to one half, the blood-corpuscles also are robbed of their mineral constituents, their potassium and phosphoric acid diminishing.

C. Schmidt pointed out that the blood in cholera also contains urea; his method was however obviously not calculated to obtain very accurate information on this point. Voit found as much as 2·43 grammes of urea in 1000 parts of the blood of a cholera patient, and Chalvet as much as 3·60 grms in 1000[1].

C. The Blood in Diseases of the Heart.

Mode in which heart diseases influence the composition of the blood.

Through the interference which is brought about in the functions of other organs, especially of the lungs, the liver, and the kidneys, diseases of the heart often lead in the end to marked alterations in the quality of the blood. We have only to cite the cyanosis which accompanies a patent foramen ovale or persistent ductus arteriosus to remind the reader how a mechanical lesion of the vascular system may interfere with the respiratory exchanges of the blood so markedly as to require no elaborate investigation of the gases of the blood to reveal it.

In lesions of the mitral valve particularly, conditions are established which, by rendering the pulmonary circulation difficult, bring about changes in the lungs which soon lead to deficient oxygenation of the blood and to its concomitant symptoms; especially is this the case in *mitral stenosis*. The difficulty which the left auricle encounters in completely emptying itself of blood, leads first of all to a rise of the blood-pressure in the pulmonary capillaries, and this in its turn prevents the propulsion of more than a small amount of blood from the right ventricle, which in its turn reacts upon the right auricle and through it upon the whole venous system. The increased pressure in the latter opposing a greater resistance than normal to the passage of blood from the arteries back to the heart, there is set up an engorgement of vessels which soon reveals itself by congested liver and kidneys, and by functional disorders of the alimentary canal, and by increased transudations, which give rise to anasarca and to dropsy of the serous sacs.

Diseases of the heart lead therefore *secondarily* to changes in various organs, which in their turn react upon the composition of the blood, and the extent to which they do so depends upon the manner or extent in which each organ is affected.

Thus any great impediment to the pulmonary circulation will lead directly to non-elimination of carbonic acid, and a diminution of the oxygen taken up, evidenced by the blue cyanotic appearance of the lips and face. A congested liver will be accompanied by the passage

[1] Quoted by Gautier, *Chimie appliquée*, &c., Vol. II., p. 337.

of biliary ingredients into the blood; congested kidneys will secrete a urine more or less charged with albumin and probably deficient in urea, and secondarily there may be set up the condition of uraemia.

So multifarious are the ways in which heart disease may modify the condition of the blood, that it would be useless to attempt to classify all the changes which are thus brought about.

The Anaemia of Heart disease.

Amongst the most interesting of the phenomena induced by heart disease is the condition of anaemia. Cases of anaemia in connection with heart disease may be arranged in two classes. In the first, the anaemia follows very closely upon the establishment of the cardiac lesion, and is obviously dependent upon the disturbance in the blood-pressure, which has not yet been compensated for, as it subsequently is, by changes in the circulatory apparatus. The establishment of anaemia in these cases is clearly explained. A certain difference between the arterial and venous pressure, and more than that, a certain value of the arterial pressure, is absolutely necessary in order that the nutrition of each organ of the body shall be efficiently maintained; when the conditions for effecting this do not exist, an alteration of the blood will be amongst the first evidences of impaired nutrition.

Thus, then, we explain the anaemia which occurs so often a few months after the setting up of an organic lesion of the heart by the endocarditis of rheumatism. In this class of cases, however, the disorder of nutrition is often only transitory, as the disturbances in the circulation which followed the sudden establishment of the cardiac lesion are compensated, more or less completely and more or less durably, by changes brought about, somewhat gradually, in the circulatory apparatus. The compensating hypertrophy of the left ventricle in aortic regurgitation, and of the left auricle in mitral stenosis, are the best illustrations of the changes referred to.

In a second class of cases, however, anaemia occurs, and is more intense than in those already referred to; these are cases in which anaemia occurs in the last stages of heart disease. After existing for many years in an apparently dormant condition, without influencing in any very obvious manner the health of the individual subject to them, certain heart affections reveal themselves apparently with great suddenness. The shortness of breath, the puffy swelling of the feet, which had been scarcely noticed for a long time, are followed by symptoms which indicate how profoundly the circulation is interfered with. We have no longer evidences of a mere deficient blood-supply to important organs, depressing their functional activity, but the sure signs of a disturbance of the proper relations between arterial and venous pressure, which no compensating changes can overcome. Congestions of important organs occur, dropsical accumulations in the serous sacs or the areolar tissue and, of necessity, if the patient live long enough, anaemia.

This anaemia is not difficult to explain. It is dependent partly upon the alimentary canal being unfit to digest enough food to make

up for the waste of the body; partly upon the presence within the blood of products of waste which the organs formerly charged with their excretion can no longer get rid of; but doubtless in great measure to the disturbance in the nutrition of all tissues and organs consequent upon the altered relation between arterial and venous pressure; the arteries are never as full of blood as they normally should be, and, as a mere consequence of this, nutrition must suffer, even were all other conditions to remain normal. The increased pressure in the venous system may, in addition, hinder, in some measure, the discharge of lymph and chyle into the blood.

Becquerel and Rodier's classification of cases of Anaemia in Heart disease.

Becquerel and Rodier made a very complete investigation into the changes which the blood undergoes in heart disease; they classified these cases into three categories, in the first of which whilst a definite lesion existed it had not made itself manifest by any pronounced symptoms. In the second category the general health had become impaired; there was some anaemia, breathlessness, and palpitation, and oedema had supervened; whilst the cases in the third category were accompanied by great dyspnoea, by abundant dropsy and by a markedly cachectic pallid skin.

In heart diseases belonging to the first category, according to Becquerel and Rodier, there is a slight increase in the water of the blood, and a tendency to diminution of both blood-corpuscles and albumin. In the second stage the corpuscles and the serum-albumin continue to decrease, and consequently the mean density of both blood and serum falls. The fibrin of the blood often increases in quantity though there be no localized inflammatory lesion. The fall in the number of the blood-corpuscles is evidenced by the anaemic look of the patient, and the diminution in the amount of serum-albumin by the dropsy which supervenes.

The following table exhibits the mean composition of the blood, with the maxima and minima in 24 cases of heart disease in the third stage. Of the 24, 16 were cases of auriculo-ventricular (presumedly mitral) stenosis; in 10 cases anaemia existed, and in 11 dropsy.

Analysis of 1000 parts of blood:

	Mean.	Maxima.	Minima.
Density of the blood	1052·54	1066·86	1040·88
Water	801·96		
Blood-corpuscles	117·05	149·42	54·00
Solids of the Serum	77·53	99·52	61·74
Fibrin	3·46	6·46	1·25
Density of Serum	1027·60	1035·10	1020·10

In the third stage, whilst the water of the blood increases, and the corpuscles undergo a further diminution, the chief changes are perceived in the serum, in which, whilst the water increases, the amount of albumin, fats and salts diminishes more and more.

The following table exhibits the mean composition of the blood, with the maxima and minima, in 31 cases of heart disease in the third stage.

Of these 20 were cases of mitral stenosis, 4 of mitral regurgitation, 2 of aortic stenosis, and 5 of aortic regurgitation. 29 of the 31 were affected with dropsy, and 27 had a cachectic appearance, the skin being pallid, yellowish and generally discoloured.

Analysis of 1000 parts of blood :

	Mean.	Maxima.	Minima.
Density of the blood	1050·19		
Water	815·82		
Blood-corpuscles	110·03	148·55	73·50
Solids of the Serum	71·60	81·10	52·40
Fibrin	2·55	4·47	1·30
Density of the Serum	1025·02	1032·50	1022·30

D. The Blood in Diseases of the Lungs.

In acute inflammatory affections of these organs, the blood exhibits in a highly characteristic manner the properties which it acquires whenever a sufficiently extensive and acute inflammatory change occurs.

In pneumonia, especially, the blood becomes 'buffed and cupped' and from it very much larger quantities of fibrin may be obtained than from normal blood.

The following are the mean results of the determination of the fibrin in several forms of acute pulmonary affections made by Becquerel and Rodier:—

		Fibrin.
In acute bronchitis 1000 parts of blood yielded		4·8 grms.
„ acute pleurisy	„ „ „ „ „	6·1 „
„ acute pneumonia	first blood-letting „	7·4 „
	second blood-letting „	6·8 „

In chronic lung diseases, especially in phthisis pulmonalis, there is invariably a considerable degree of anaemia, with marked diminution in the number of coloured blood-corpuscles. It is alleged that in phthisis, the amount of fibrin is frequently very much increased.

E. The Blood in Diseases of the Liver.

In affections of the liver in which, through an obstruction to the flow of bile, jaundice occurs, there is always an accumulation of bilirubin in the blood, and from their passage into the urine in some cases, we may doubtless assume the frequent concomitant presence of salts of the bile acids. In cases of acute yellow atrophy the blood contains leucine and tyrosine, and doubtless (though the fact has not been directly ascertained) the amount of urea in the blood is diminished.

A diminution of the red blood-corpuscles occurs in the course of organic diseases of the liver, as is evidenced by the pale sallow face of patients affected with cirrhosis.

F. The Blood in Diabetes Mellitus.

The constant and characteristic feature of the blood in this disease is the presence of an excess of glucose.

There are not wanting facts which point to other less investigated changes in the blood which supervene in the course of the disease, such as for instance a large increase of the fatty matter of the blood and the formation of acetone.

Increase in the glucose of the Blood.

Whilst in health, the amount of glucose amounts on an average to about 0·9 parts per 1000 p.c. (Pavy), in diabetes it may, according to the gravity of the case, be several times as great. In those cases where the urine contains a large percentage of sugar, the blood likewise is very rich in that constituent, as will be seen by the accompanying table taken from Dr Pavy[1]:—

COMPARATIVE STATE OF BLOOD AND URINE IN DIABETES.

	Urine.				Blood.
	Quantity per 24 hour.	Specific gravity.	Sugar per 1000 parts.	Sugar excreted in 24 hours.	Sugar per 1000 parts, mean of two analyses.
Case 1.					
Jan. 5, mixed diet.	6608 c.c.	1040	109·91	751·6 grms.	5·763
Case 2.					
Jan. 8, mixed diet.	6474 c.c.	1041	94·08	633·0 grms.	5·545
Jan. 28, restricted diet.	3407 c.c.	1031	61·34	245·2 grms.	2·625
Case 3.					
June 8, mixed diet.	5878 c.c.	1036	93·39	567·7 grms.	4·970
July 20, restricted diet.	2470 c.c.	1033	45·49	115·8 grms.	2·789
Case 4.					
March 9, partially restricted diet.	1704 c.c.	1036	48·11	21·81 grms.	1·848
June 28, partially restricted diet.	852 c.c.	1034	31·76	14·40 grms.	1·543

Formation of Acetone in Blood. Acetonaemia.

In the course of diabetes mellitus there is apt to occur a peculiar group of symptoms (first alluded to by Prout, though only carefully studied of late years by Kussmaul, Petters, Kaulich, Sanders and Hamilton),

[1] The Croonian Lectures on certain points connected with Diabetes delivered at the Royal College of Physicians, London, 1878.

which are included under the term diabetic coma. In reality, the first symptoms are those of a very remarkable dyspnoea, in which there is equal exaggeration of inspiratory and expiratory movements; usually it is only after this has existed for some hours that the patient, who has been becoming more and more prostrate, sinks into a state of coma and dies.

The peculiar ethereal smell exhaled by the breath of diabetics had long been noticed, but it was Petters who first pointed out that in certain cases of diabetic coma, the apartment in which the patient is confined acquires a peculiar odour, and that on distilling the urine and even the blood of the patient, there is obtained a distillate which contains traces of acetone.

Petters based on these facts the theory that the phenomena of diabetic coma depend upon a disengagement of acetone in the living blood, that they are the symptoms, indeed, of a poisoning by acetone, *Acetonaemia.*

That the blood in these cases does evolve acetone in small quantities is proved by the concurrent testimony of several observers, though it is very probable that the body does not exist free in the blood, but is derived from the splitting up of ethyl-diacetic acid[1].

There are however some serious objections to accepting the acetonaemic theory of diabetic coma. In the first place, diabetics sometimes evolve the most marked acetone (?) smell, without any of the symptoms of diabetic coma being present; in the second place, the administration of very large doses of acetone is required in order to produce any marked physiological symptoms, which even when produced, are by no means identical with those of diabetic coma.

It has been averred that when acetone is added to blood, there is produced a white creamy appearance exactly similar to that which has been observed, especially of late, in certain fatal cases of diabetic coma. As has been shewn by Sanders and Hamilton, this statement is erroneous. Acetone dissolved in alcohol and added in very small quantities to blood, leads to no morphological change; if added in larger quantities it produces a coagulation of the proteids of the serum and a solution of the coloured corpuscles, as has been shewn by Rupstein.

In a case of diabetes, which ultimately ended by coma, which came under the Author's notice, the patient for some time evolved an intense ethereal smell, which attracted the attention of patients in the ward; during the diabetic coma which preceded death, the acetone (?) smell had diminished and the blood had only a faint smell of acetone.

[1] $\underbrace{C_6H_9NaO_3}_{\text{Sodium ethyl-diacetate}} + \underbrace{2H_2O}_{\text{Water}} = \underbrace{C_3H_6O}_{\text{Acetone}} + \underbrace{C_2H_6O}_{\text{Alcohol}} + \underbrace{NaHCO_3}_{\text{Sodium hydric Carbonate.}}$

This is the equation by which Rupstein (*Centralblatt*, 1874, No. 55) explains the formation of acetone in the system from ethyl-diacetic acid.

In support of this theory is the fact that the urine of diabetics gives with Fe_2Cl_6 a reddish brown colouration which disappears on the addition of HCl, or on boiling—properties which are possessed by ethyl-diacetic acid.

This subject will be discussed again in connection with the urine in diabetes.

Whatever may be the part played by the acetone-like body in the production of the phenomena of diabetic coma, we may safely assert that when a diabetic exhales large quantities of that body, the prognosis is peculiarly grave, the probability of a rapid fatal termination being considerable. It is to be noted that the symptoms of diabetic coma may set in and afterwards subside—a statement which the Author bases upon a case observed and recorded by Quincke[1], and upon a second case observed by his friend Dr Grahame Steell in the Manchester Royal Infirmary.

Lipaemic condition of the Blood in Diabetes.

It has been stated (p. 59) that in a perfectly physiological condition, the serum of blood often presents a milky appearance which is due to the suspension of fatty matters. Some of the older writers noticed that the blood in diabetes is specially characterized by this lactescent appearance; the observations of Dr Babbington on this matter being very precise[2]. The fact was, however, lost sight of for a long time, or explained on the theory that diabetics consume large quantities of food, and that as a result, their blood presents the appearance which is usual whilst a full meal is being digested[3]. Recently investigated cases[4] have directed attention afresh to this lipaemic condition of the blood. It was observed by Dr Balthazar Foster[5] that the blood in certain fatal cases of diabetes presented a milky appearance, and he averred that this was similar to the appearance produced on adding acetone to blood, ether having no solvent action on the fat-like matter. Professor Sanders and Dr Hamilton, in cases which they observed, noticed that the blood had a pink colour, and that there separated from it milk- or cream-like serum; they however quite correctly remarked that the milk-like appearance "proved to be due to oil, both by microscopic examination, and by the removal of the milky appearance by the action of ether, as well as by staining with perosmic acid. Nothing identical with this can be produced by adding acetone to blood."

The interest attached to this lipaemic condition of the blood depends upon the fact that Sanders and Hamilton discovered, in one case where death had resulted from diabetic coma and where the blood was intensely lipaemic, *fat emboli* in the vessels of the lungs and kidney, the appearances being exactly similar to those observed in cases of fat embolism from fractured bone. The resemblance of the symptoms which were observed by Czerny in a case of fat embolism due to this cause to those of diabetic dyspnoea and coma led Sanders and Hamilton to advance the theory that "the peculiar terminal dyspnoea and coma of diabetes are due to lipaemia and fat embolism, rather than to acetonaemia."

[1] Quincke, "Ueber Coma diabeticum." *Berliner klinische Wochenschrift*, 1880, No. 1.
[2] See Article "Blood" by Babbington in Todd's *Cyclopaedia of Anatomy and Physiology*, Vol. I. p. 422.
[3] Pavy, *Researches on the Nature and Treatment of Diabetes*, London, 1862, p. 111.
[4] Sanders and Hamilton, "Lipaemia and Fat Embolism in the fatal Dyspnoea and Coma of Diabetes." *Edinburgh Medical Journal*, July 1879, p. 47.
[5] Foster, "Diabetic coma. Acetonaemia." *British Med. Journal*, 1878, Vol. I. p. 78.

Two cases which the Author has had the opportunity of studying do not, however, support this fascinating theory. In one of these cases the amount of fat in the blood was exceedingly great, yet a most scrupulous investigation of the lungs, the kidneys and the brain, conducted by Dr Dreschfeld, led to the conclusion that no emboli were present. In the second case the amount of fat in the blood (or rather the amount of matters soluble in ether) was not larger than usual, and in this case also a most painstaking search shewed the absence of emboli.

Quincke, who has rejected the 'Acetonaemia theory' of diabetic coma, is inclined to consider it as a condition which, like uraemia, is probably induced by a combination of circumstances, and by the toxic action of more than one product of tissue metabolism, amongst which may be the body which is excreted in the urine, and is coloured red by perchloride of iron. Whatever may be the toxic agent or agents, it is difficult to see cases of diabetic coma without coming to the conclusion that the condition is one due to a toxic action and not to a suddenly-developed nervous lesion. As bearing upon this question it is worthy of mention that in the first of the two cases of which the notes are given below, the liver was found, after death, to be the seat of intense fatty infiltration, similar to that observed in cases of poisoning by phosphorus.

The following are brief notes of two cases previously referred to, in which the Author has had the opportunity of examining the blood of patients suffering from diabetic dyspnoea and coma.

I. X, a man of about 35 years of age, a patient in the Manchester Royal Infirmary, under the care of Dr Roberts, F.R.S., had been suffering from diabetes of two and a half years duration. Since his admission into the hospital his urine had amounted to 300 ounces per diem with a specific gravity of 1030-1035. The patient exhaled a peculiar ethereal (acetone-like?) odour which pervaded the whole ward and attracted the attention of the other patients. On 9th July, 1879, after returning from a walk, the patient was seized with intense dyspnoea; the exaggeration of inspiratory and expiratory movements was equally marked; there was no evidence of venosity of blood; the exaggerated respiratory movements continued uniformly without any rhythmical variation in intensity. At first the patient was conscious, but he subsequently became comatose, and died 21 hours after the commencement of the attack.

During the attack of coma the blood was examined microscopically without any deviation from the normal appearance being noticed. Some blood was drawn from the arm by venesection; it coagulated normally, and there separated from it a serum distinctly milky, though not more so than is compatible with a physiological condition. At the post-mortem examination a considerable quantity of blood was collected from the cavity of the chest. The broken-up clot mixed with serum was placed in a bottle. After some hours a creamy layer had floated to the surface of the liquid, this layer being about one-sixth of the total volume of the liquid. The milkiness was found to depend upon oil globules of various sizes.

An analysis of the blood drawn during life and of that collected after death gave the following results :—

		Blood drawn during life.	Blood collected after death.	
Water in 1000 parts		744·6	757·7	
Total solids „		255·4	242·3	
Ethereal Extract.	Neutral Fats	10·8	9·86	13·55
	Lecithin		1·55	
	Cholesterin	1·96	2·14	

II. J. T., a man 32 years of age, was admitted into the Royal Infirmary on 3rd Nov. 1879, suffering from diabetes. He appeared very ill and exhaled a very intense ethereal odour. At this time there were no symptoms of dyspnoea or coma. The urine contained sugar and was of high specific gravity. On the night after his admission he was seized with purging, and in the morning he appeared very ill; his breathing then became laboured and had the characters of diabetic dyspnoea. In the course of the day he became unconscious, and he died shortly after midnight on the 5th Nov. The urine which was passed on the 4th contained, besides sugar, some albumin and many hyaline casts.

At the post-mortem examination the blood was found to possess the acetone (?) like odour. Some was collected and analysed with the following results :—

Ethereal extract of 1000 parts of Blood	1·88	parts.
Cholesterin contained in ethereal extract	0·642	„

The amount of the ethereal extract obtained from the blood in case I. was much larger than has been found in any published analyses of human blood. Thus the mean amount of fat (including under this term all the constituents of the ethereal extract of blood, viz. neutral fats, cholesterin and lecithin) found by Becquerel and Rodier in their numerous analyses was 1·6 parts, the maximum being 3·25 and the minimum 1·00 per 1000 parts of blood.

In published analyses of the blood of diabetics by C. Schmidt, the amount of fat was respectively 1·82 and 2·13 per 1000; in these cases however, there was no diabetic coma. Hoppe-Seyler [1] also mentions that he found the proportion of fat materially increased in the blood in four cases of diabetes.

G. The Blood in Diseases of the Kidney.

There is probably no class of diseases in which a change in the chemical composition of the blood is so soon induced as in Bright's disease, or exerts a more marked influence upon the exchanges of the matters of the organism.

The fundamental knowledge which we possess on this subject was mainly acquired by the classical investigations of Christison [2] which

[1] Hoppe-Seyler, *Physiologische Chemie*, p. 482.

[2] Christison, "Observations on the variety of Dropsy which depends on diseased kidney." *Edinburgh Med. and Surg. Journal*, Vol. 32 (1829), p. 262.

Christison, "On granular degeneration of the kidneys, and its connection with Dropsy, Inflammation and other diseases." Edinburgh, 1839.

followed very closely the masterly memoir in which Dr Bright[1] had first announced the connexion between albuminuria and morbid changes in the kidney.

Christison pointed out that in the early stages of kidney disease the blood presents the following characters; the density of the serum is low (1020 or even 1019), the proportion of albumin diminishes, the fibrin of the blood may be increased, the proportion of blood-colouring matter is unaffected, but, above all, the serum frequently contains urea. He shewed that as the disease became chronic some of these changes in the blood became less distinct, *e.g.* the diminution in the amount of albumin, and the presence of urea, but that a very constant and considerable diminution of the blood-colouring matter was a characteristic occurrence.

Subsequent researches have thoroughly confirmed the statements of Christison as to the excess of urea which is present in the blood of Bright's disease. Although there is no longer any difference of opinion as to the accumulation of urea in the blood in cases of Bright's disease in which there is a marked suppression of urine or a very obvious deficiency in the elimination of urea, facts have hitherto been wanting to decide whether after the establishment of any of the chief lesions of the kidney there is a permanent impairment of the normal power which the kidneys possess of eliminating urea. This question has occupied the attention of the Author, and he is inclined to believe that in most cases of chronic Bright's disease, even whilst the patient is in the apparent enjoyment of fair health, there is a persistent excess of urea in the blood.

The convulsions and coma which are apt to supervene when the elimination of urea is defective have been designated as 'uraemic' or as evidences of 'uraemic' poisoning. At first it was held as certain that these nervous phenomena were occasioned by the accumulation of urea in the blood acting as a poison on the great nerve centres. There can be no doubt, however, that this simple explanation is not sufficient; the condition of uraemia is one which depends upon many factors. It must not be forgotten, that before the condition of uraemia is induced, the blood has usually become rich in water, poor in albumin, poor in corpuscles, and that in addition to an accumulation of urea and uric acid it probably contains an excess of other proximate principles which may exert a specially poisonous action.

It was suggested by Frerichs[2] that uraemic phenomena are due to the conversion of urea into ammonium carbonate in the blood, but there is no ground for believing that such a conversion actually does occur during life.

[1] Bright, "Cases illustrative of some of the appearances observable on the examination of diseases terminating in dropsical effusion,—and first of the kidney." Bright's Reports. London, 1827.

[2] Frerichs, *Archiv f. phys. Heilk.*, 1852, Vol. xi. p. 88.

CHAPTER IV.

THE BLOOD (*continued*).

Description of Certain Methods of Research.

Determination of the Specific Gravity of the Blood.

Except by operating with extreme expedition and at temperatures below 0° C. it would be quite impossible to determine the specific gravity of the uncoagulated blood. It is obvious, however, that the specific gravity of defibrinated blood can only differ very slightly from that of blood which has not yet coagulated. It is therefore usual to take the specific gravity of defibrinated blood as sufficiently representing that of the blood.

In the case of blood, this is best done with the aid of a specific gravity bottle, of which two forms are represented in the annexed woodcuts (Figs. 28 and 29).

The flask is first weighed when empty; then when filled with distilled water at a known temperature; the distilled water being then poured out and the flask dried, the bottle is filled with defibrinated blood at the same temperature as the water, and again weighed. By these operations we ascertain the weight of the water and of the blood respectively which at a given temperature are contained in the bottle.

Let a be the weight of the water contained in the flask, and b that of the defibrinated blood; then the specific gravity of the latter, designated by d, will be

$$d = \frac{b}{a}.$$

It is however very inconvenient to be obliged to weigh liquids at one particular temperature, and if we have at our disposal a specific gravity bottle such as is represented in fig. 29, and a table of the density of water at various temperatures, we can readily ascertain the specific gravity of the blood, though its temperature be not exactly the same as that of the water

which was weighed. Let us assume that we have found our bottle to hold 25·6515 grams of water at 15°, and 27·270 grams of defibrinated blood at 7° C.; we must first calculate the weight of water which would be con-

Fig. 28. Specific Gravity Bottle, consisting of a light flask with a well-ground perforated stopper.

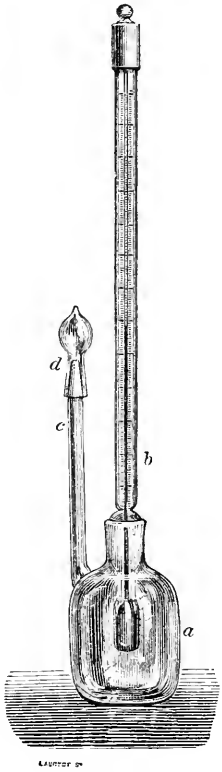


Fig. 29. Geissler's Specific Gravity Bottle.

a is a light flask. *b* a very accurately graduated thermometer. *c* is a tube connected with *a*, through which fluid escapes when the thermometer is inserted in the bottle. *d* a cap which fits perfectly the top of *c* and which is applied to it after it is filled.

tained if the temperature had been 7° and not 15°. We find on looking at the subjoined table of the specific gravities of water that whilst the specific gravity of water at 15° is 0·99915, at 7° it is 0·99994, and we can get the weight of water which our bottle would hold at 7° by the following proportion:

$$0{\cdot}99915 : 0{\cdot}99994 :: 25{\cdot}6515 : x$$

$$x = \frac{0{\cdot}99994 \times 25{\cdot}6515}{0{\cdot}99915} = 25{\cdot}67.$$

Knowing the weight of equal volumes of water and blood at the same temperature we can at once get the density as before by dividing the latter by the former:

$$d = \frac{27{\cdot}27}{25{\cdot}67} = 1{\cdot}0623.$$

TABLE OF THE DENSITY OF WATER AT TEMPERATURES BETWEEN 0° AND 30°C.

Temp.	Density	Temp.	Density
0°	0·99988	16°	0·99900
1°	0·99993	17°	0·99884
2°	0·99997	18°	0·99866
3°	0·99999	19°	0·99847
4°	1·00000	20°	0·99827
5°	0·99999	21°	0·99806
6°	0·99997	22°	0·99785
7°	0·99994	23°	0·99762
8°	0·99988	24°	0·99738
9°	0·99982	25°	0·99714
10°	0·99974	26°	0·99689
11°	0·99965	27°	0·99662
12°	0·99955	28°	0·99635
13°	0·99943	29°	0·99607
14°	0·99930	30°	0·99579
15°	0·99915		

Determination of the Reaction of Blood.

As was stated at p. 26, the reaction of blood cannot be accurately ascertained by immersing into it ordinary test-papers, but by following one of the methods suggested by Kühne, Zuntz, or Liebreich. With the aid of one of these methods the amount of a standard acid required to neutralize a given volume of blood may be determined. It is essential, however, to employ a carefully prepared solution of litmus, free from alkali. With this object 16 grammes of commercial litmus are finely pulverized, and the powder is mixed in a beaker with 120 c.c. of water and frequently stirred. After 24 hours the solution, which contains nearly all the free alkali of the litmus, is thrown away and the residual litmus is again treated with 120 c.c. of water for 24 hours. The solution thus obtained is divided into two equal portions; the one is carefully treated with a little very dilute acid, added by means of a glass rod, until a red tint just appears, and then to this is added some of the other portion, until a little of the fluid, when much diluted, presents a blue-violet colour.

If a plaster of Paris slab (see p. 26) be imbued with such a solution of litmus, a drop of blood or of blood-serum will be surrounded at its edges by a distinct blue ring. In order to determine the degree of alkalinity, a standard solution of tartaric acid may be made by dissolving 7·5 grammes of crystallized tartaric acid in 1000 c.c. of water; one cubic centimetre of this solution should exactly neutralize 0·004 grm. of NaHO. The acid solution is added from a burette to 50 or 100 c.c. of the serum or blood, a drop of the mixture being placed from time to time upon the slab coloured with litmus; the addition of acid is continued until the reaction is faintly acid. The alkalinity of the blood may then be expressed as corresponding to x milligrammes of sodium hydrate per 100 c.c. of blood.

Determination of the Water, Total Solids and Ash of the Blood.

A Berlin porcelain crucible, furnished with a cover and having a capacity of about 20 c.c., is dried and then accurately weighed. From 2·5 to 5 grammes of defibrinated blood are carefully weighed out in the crucible, which is then placed in a hot-water oven heated to 100° C. (Fig. 30), until an apparently dry residue is left; the crucible is then heated in a hot-air oven furnished with a regulator (Fig. 31), and kept at a temperature of 110° C. After some time the crucible is transferred to an exsiccator (Fig. 32 or 33), where it is allowed to remain for a few minutes to cool,

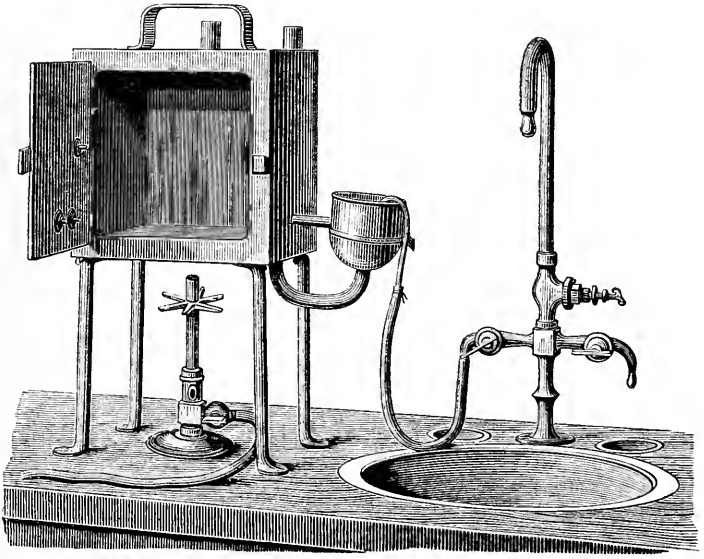


FIG. 30. HOT-WATER OVEN WITH ARRANGEMENT FOR KEEPING THE WATER AT A CONSTANT LEVEL.

in dry air, it is then placed on the balance and weighed. The weight having been noted, the crucible is again heated to 110° for some

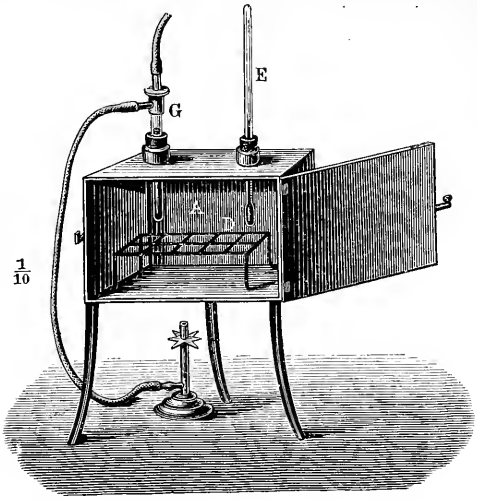


FIG. 31. HOT-AIR OVEN WITH BUNSEN'S REGULATOR.

time, and again weighed as before, the process being repeated until two successive weighings give the same result.

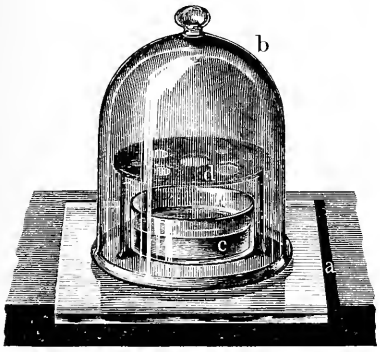


FIG. 32. AN EXSICCATOR.

A bell-jar *b*, with ground rim, fits air-tight over the plate *a*. *c* is a vessel containing sulphuric acid or phosphoric anhydride. *d* is a tray with circular holes for crucibles and capsules.

FIG. 33. A SMALL EXSICCATOR, SUITABLE FOR A CRUCIBLE.

The crucible is now uncovered and placed, upon a triangle, over a Bunsen flame, so as to char its contents. At first the application of heat is conducted with much caution, the flame being at a considerable distance from the bottom of the crucible; if this precaution be not taken the contents are very apt to froth up and to be partially lost. Soon, however, the heat may be increased by placing the crucible so that it is surrounded by the flame, and it will prove advantageous to place the crucible in the tilted position indicated

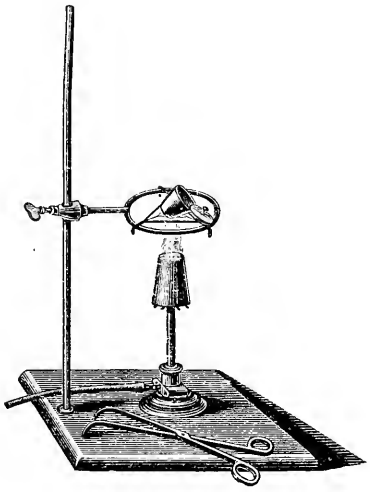


FIG. 34. ARRANGEMENT EMPLOYED IN DETERMINING THE AMOUNT OF ASH IN BLOOD.

in the annexed figure (Fig. 34). If conducted in a porcelain crucible the process is a very slow one. When the carbon has been entirely burnt away, the ash presenting a reddish-white colour without intermixture of black, the crucible is cooled in the exsiccator and weighed. This method of determining the ash does not possess great value, for the reasons already referred to at considerable length (see p. 66). The following process should be followed when it is desired to attain as great accuracy as is compatible with the method of incineration.

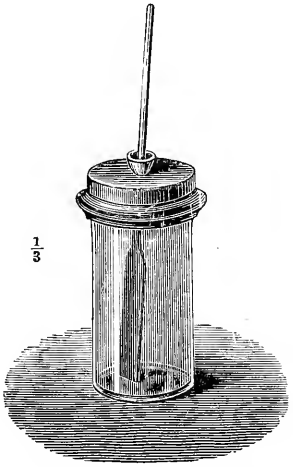
Rose's method. The dried residue of the blood is heated over a Bunsen flame until it is thoroughly carbonized; care is however taken that the crucible does not become even faintly red. Having been allowed to cool, the contents of the crucible are treated with boiling distilled water and heated for some time; the aqueous solution is filtered through a small filter of Swedish filter paper, and kept. The carbonized residue is treated again and again with hot distilled water, to make sure of dissolving all soluble salts.

The insoluble matters together with the small filter previously referred to, are now dried in the hot-air oven and then ignited at a red heat; when the whole of the carbon has been burned away the crucible is cooled and the solution of the soluble salts added to it; the contents are first evaporated to dryness in the water and air ovens and then ignited at a barely perceptible red heat; the crucible is then cooled and weighed, and thus the total amount of ash found. Or, by weighing in separate crucibles the aqueous solution and the ignited insoluble residue, the amount of soluble and insoluble constituents of the ash is ascertained [1].

Determination of the amount of Fibrin yielded by the Blood.

If it be desired to determine the amount of fibrin which will separate from the blood, the best method is the following:

Hoppe-Seyler's method. A beaker, of a capacity between 100 and 150 c.c., is fitted with a caoutchouc cap, provided with a single opening in the centre through which is thrust a rod of vulcanite, somewhat spatula-shaped at its lower end (Fig. 35). The weight of



$\frac{1}{3}$

Fig. 35. Hoppe-Seyler's Apparatus for separating Fibrin from the Blood.

[1] The most valuable determinations of the salts of the serum have been made by the method of direct precipitation. It has been shewn that sulphuric and phosphoric acids, calcium, and magnesium may be precipitated from serum, as from aqueous solutions; the precipitates are separated by subjecting the liquid to rapid rotation in the centrifugal machine; with the aid of the latter they may be efficiently and rapidly subjected to the process of 'washing by decantation', and then treated according to the ordinary methods. The reader who wishes to pursue researches in this direction should consult the account given of these methods by Pribram and Gerlach (see p. 66).

the apparatus is determined when empty. The caoutchouc cap having been momentarily withdrawn, 30 or 40 c.c. of uncoagulated blood are allowed to flow into the beaker; the cap is replaced and the blood is stirred with the little spatula, until the fibrin has separated. The apparatus is then weighed. This operation being completed, the caoutchouc cap is removed, the beaker filled with distilled water, and the contents stirred by the aid of the spatula which is left *in situ*. When the fibrin has subsided the red supernatant liquid is decanted. The beaker is then filled up again with a 1—3 per cent. solution of common salt, the contents again stirred and allowed to subside. These operations are repeated until the fibrin is almost colourless; the beaker is then filled up once or twice with distilled water, the water decanted, and then the fibrin is transferred to a small weighed filter, washed with boiling alcohol, and then dried in the hot-water oven at 100° C., or preferably in the hot-air oven at 110° C.; the filter and its contents are then placed between two, weighed, ground

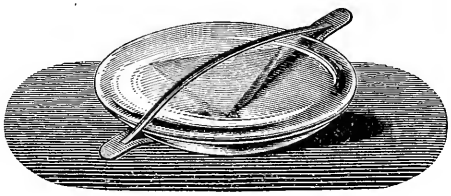


FIG. 36. WATCH-GLASSES, WITH CLIP, IN WHICH FILTERS ARE ALLOWED TO COOL AND WEIGHED.

watch-glasses (Fig. 36) held together by a clip, and weighed. On subtracting the weight of the watch-glasses, clip, and filter-paper from the total weight found, the weight of the fibrin contained in the amount of blood analysed is ascertained.

Determination in Coagulated Blood.

If it be required to determine the amount of fibrin in blood which has already coagulated, the total weight of the blood having been determined by weighing, the whole is thrown upon a filter made of well-washed calico. When the serum has drained through, the filter is tied so as to enclose the clot in a little bag. This is then kneaded between the fingers, whilst a stream of water is allowed to play upon it continuously. After long-continued washing, the whole, or nearly the whole, of the coloured corpuscles having been removed from the clot, the cloth is opened, when it is found to contain filaments of fibrin, which are more or less completely decolorized; these are collected by means of a pair of forceps, and transferred to a small beaker, washed with weak salt solution, and afterwards with water, and treated as in Hoppe-Seyler's method.

Determination of Haemoglobin in the Blood.

1. Hoppe-Seyler's method. This method consists in comparing the tint of the blood diluted with a known volume of distilled water, with the tint of a solution of pure haemoglobin of known strength, and then adding water to the first until it assumes exactly the colour of the second.

This method necessitates in the first place a solution of pure Oxy-haemoglobin. Oxy-haemoglobin, prepared from the blood of the dog and at least twice crystallized, is dissolved in water at 0° C., and the saturated solution is filtered. 50 c.c. are measured out in a capsule and evaporated to dryness, first over a water-bath and then over sulphuric acid, *in vacuo*. In this way the strength of the solution is determined.

In addition to this solution there are needed two haematinometers[1] (see Fig. 16, p. 92), and an accurate burette divided into tenths of a cubic centimetre.

The two haematinometers being placed side by side, with a sheet of white paper beneath them, and in such a position as to be illuminated in exactly the same manner, 10 c.c. of the standard solution, diluted with from 10 to 60 c.c. of water, are placed in the one haematinometer.

A solution, of known strength, of the blood to be investigated is now made, e.g. by diluting 5 grammes of blood to 100 c.c.; 10 c.c. of the solution are placed in the second haematinometer.

The solution of blood will now be seen to be very much deeper in tint than the solution of haemoglobin. Water is added to the former, from a burette, until there is no perceptible difference in tint between it and the standard solution. When this result has been obtained, the amount of haemoglobin in the two solutions must be equal.

The method and the calculation required will be understood from the following experiment quoted from Hoppe-Seyler:—20·186 grms. of defibrinated blood were diluted with water to a volume of 400 c.c. 10 c.c. of this solution were placed in a haematinometer and 38 c.c. of water had to be added so as to produce a solution of the same shade as the standard solution of haemoglobin which had been placed in a second haematinometer. The volume of water which the whole quantity of the solution of blood would have required to bring it to the standard tint is found by the proportion

$$10 : 38 :: 400 : x$$
$$x = 1520 \text{ c.c.}$$

[1] Haematinometers similar to the one represented in Fig. 16 are constructed by the opticians Schmidt u. Haensch, Stallschreiber-Strasse 4, Berlin; they are sold in pairs, at 30 marks (£1. 10*s*.) the pair.

By adding then 1520 c.c. of water to 400 c.c. of the diluted blood we should obtain 1920 c.c. of a solution equal in shade and intensity of colour to the standard solution of haemoglobin. But on analysis the latter was found to contain in 100 c.c. 0·145 grms. of haemoglobin; we now have the data for determining the total quantity of haemoglobin in the diluted blood:

$$100 : 0{\cdot}145 :: 1920 : x$$

$$x = 2{\cdot}784 \text{ grms.}$$

But as this quantity of haemoglobin was derived from 20·186 grms. of blood, the amount contained in 100 parts is found thus:

$$20{\cdot}186 : 2{\cdot}784 :: 100 : x$$

$$x = 13{\cdot}79 \text{ grms.}$$

This method gives most accurate results; its use was formerly deprecated, inasmuch as it requires solution of pure oxy-haemoglobin, which cannot easily be prepared except in the depth of winter, and which when prepared will not keep more than a few days in the open air. Hoppe-Seyler has however shewn that solutions of pure haemoglobin in sealed glass tubes may be kept indefinitely without the haemoglobin undergoing decomposition. Nothing can therefore be easier than to make a stock of pure solution of oxy-haemoglobin in winter and store it in a large number of sealed glass tubes for use during the succeeding year. The oxy-haemoglobin is soon reduced to haemoglobin, but, after that, resists all further change. When the tubes are opened the solution rapidly absorbs oxygen and a solution of oxy-haemoglobin is obtained.

Instead of employing a standard solution of oxy-haemoglobin, we may, as suggested by Rajewsky[1], use, as a standard, a solution of picro-carminate of ammonia, corresponding in tint to a solution of haemoglobin of known strength. A solution of picro-carminate, if perfectly neutral, may be preserved in stoppered bottles for long periods; according to Malassez[2] it may be kept indefinitely.

The following is the method of preparing a solution of picro-carminate of ammonia.

Take 100 c.c. of a saturated solution of picric acid. Prepare an ammoniacal solution of carmine by dissolving 1 grm. in a few c.c. of water, with the aid of an excess of ammonia and heat. Boil the picric acid, and when boiling add the carmine solution. Evaporate the mixture to dryness, and dissolve the residue in 100 c.c. of water, and filter. A clear solution ought to be obtained; if not, add some more ammonia, evaporate, and then dissolve as before[3]. This solution is now added, little by little, to a mixture of equal parts of water holding a little phenol in solution, and of glycerine, until the tint, observed in a haematinometer, is exactly similar to

[1] A. Rajewsky, "Zur Frage über die quantitative Bestimmung des Hämoglobingehaltes im Blut." Pflüger's *Archiv*, Vol. XII., p. 70.

[2] Malassez, "Sur les diverses méthodes de dosage de l'hémoglobine et sur un nouveau colorimètre." *Archives de Physiologie*, 1877, pp. 1—43.

[3] Rutherford, *Outlines of Practical Histology*, p. 173.

that of a standard solution of haemoglobin. As the picro-carminate has a yellower shade than blood, it is advisable to add to it a small quantity of a perfectly neutral solution of carmine.

2. Preyer's method. This method requires (1) at least one haematinometer, (2) a spectroscope, (3) a steady light, (4) a standard solution of haemoglobin, (5) a finely-divided burette.

The haematinometer being placed between the luminous source and the spectroscope, a strong solution of crystallized oxy-haemoglobin in water is poured into it. If the solution be very strong, as was mentioned at p. 97, only the red rays will pass. Water is now added very gradually from the burette, until the first gleam of green between E and F, and close to *b*, is perceived. In order to make the appreciation of this more easy, the experiment is conducted in a darkened room, and the lamp is furnished with a shade (as in Fig. 18) which only allows the rays of light to proceed in the direction of the spectroscope. The amount of haemoglobin in the standard solution is now ascertained as in Hoppe-Seyler's method, by evaporating a known volume to dryness. (Preyer has found that when examined in a haematinometer of which the sides are 1 centimetre apart, a solution of haemoglobin containing 0·8 grms. of the substance per cent. just allows a narrow band of green close to *b* to appear.)

About 0·5 c.c. of the blood, of which the amount of haemoglobin is to be determined, is now poured into a haematinometer, and water added to it from a burette until, when examined under exactly the same circumstances as the standard solution, the green close to *b* just appears. The amount of water added must be very precisely measured. The amount of haemoglobin contained in the blood is then found by the following equation:

$$x = \frac{Hb\,(e+s)}{s},$$

where *Hb* is the weight of haemoglobin contained in 100 c.c. of the standard solution, *e* the volume of water added to the blood analyzed, and *s* the volume of the latter.

Dr Gowers' apparatus for the clinical estimation of Haemoglobin[1]. The tint of the dilution of a given volume of blood with distilled water is taken as the index to the amount of haemoglobin. The distilled water rapidly dissolves out all the haemoglobin, as is shewn by the fact that the tint of the dilution undergoes no change on standing. The colour of a dilution of average normal blood one hundred times is taken as the standard. The quantity of haemoglobin is indicated by the amount of distilled water needed to obtain the tint with the same volume of blood under examination as was taken of the standard. On account of the instability of a standard dilution of blood, tinted glycerine-jelly is employed instead. This is perfectly stable, and by means of carmine and picro-carmine the exact tint of diluted blood can be obtained.

[1] See "Report of the Meeting of the Clinical Society," the *Lancet* II. 1878, p. 822.

The apparatus consists of two glass tubes of exactly the same size. One contains a standard of the tint of a dilution of 20 cubic mm. of blood in 2 cubic centimetres of water (1 in 100).

The second tube is graduated, 100 degrees = two centimetres (100 times twenty cubic millimetres).

The twenty cubic millimetres of blood are measured by a capillary pipette (similar to, but larger than that used for the haemacytometer). The quantity of the blood to be tested is ejected into the bottom of the tube, a few drops of distilled water being first placed in the latter. The mixture is rapidly agitated to prevent the coagulation of the blood. The distilled water is then added drop by drop (from the pipette-stopper of a bottle supplied for that purpose) until the tint of the dilution is the same as that of the standard, and the amount of water which has been added (*i.e.* the degrees of dilution) indicates the amount of haemoglobin.

Since average normal blood yields the tint of the standard at 100 degrees of dilution, the number of degrees of dilution necessary to obtain the same tint with a given specimen of blood is the percentage proportion of the haemoglobin contained in it, compared to the normal.

For instance, the twenty cubic millimetres of blood from a patient with anaemia gave the standard tint at 30 degrees of dilution. Hence it contained only thirty per cent. of the normal quantity of haemoglobin.

By ascertaining with the haemacytometer (p. 77) the corpuscular richness of the blood, we are able to compare the relation between the number of corpuscles and the amount of haemoglobin. A fraction, of which the numerator is the percentage of haemoglobin, and the denominator the percentage of corpuscles, gives at once the average value per corpuscle. Thus the blood mentioned above, containing thirty per cent. of haemoglobin, contained sixty per cent. of corpuscles; hence the average value of each corpuscle was $\frac{30}{60}$ or $\frac{1}{2}$ of the normal. Variations in the amount of haemoglobin may be recorded on the same chart as that employed for the corpuscles.

In using the instrument the tint may be estimated by holding the tubes between the eye and a window, or by placing a piece of white paper behind the tubes; the former is perhaps the best. Care must be taken that the tubes are always held in the line of light, not below it. In the latter case some light is reflected from the suspended corpuscles from which the haemoglobin has been dissolved. If the value of the corpuscles is small, then a perceptibly paler tint is seen when the tubes are held below the line of illumination. If all the light is transmitted directly through the tubes the corpuscles do not interfere with the tint.

In using the instrument it will be found that between six or eight degrees of dilution it is difficult to distinguish a difference between the tint of the tubes. It is therefore necessary to note the

degree at which the colour of the dilution ceases to be deeper than the standard, and also that at which it is distinctly paler. The degree midway between these two will represent the haemoglobin percentage.

The instrument is only expected to yield approximate results, accurate within two or three per cent. It has however been found of much utility in clinical observations[1].

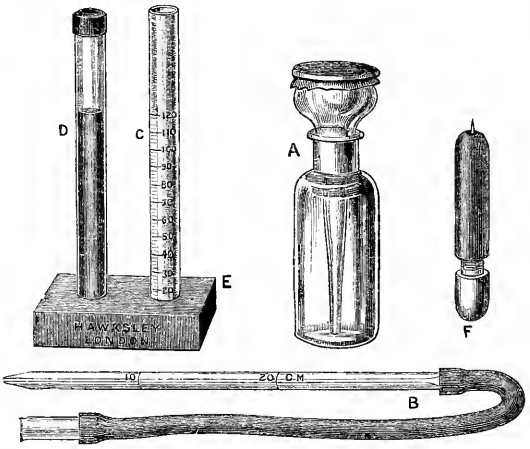


FIG. 37. DR GOWERS' APPARATUS FOR THE CLINICAL ESTIMATION OF HAEMOGLOBIN.

E, block of wood with two holes, to serve as a stand for the tubes C and D.
D, tube containing glycerine-jelly treated with picro-carmine.
C, graduated tube in which the blood is diluted with water.
B, capillary pipette marked so as to allow of 20 cubic millimetres of blood being measured.
F, lancet-shaped needle for puncturing the finger. The point of the needle may be protruded to a greater or less extent, so as to produce a more or less deep puncture.
A, bottle for distilled water.

3. By the determination of iron contained in the ash of the blood.

As it is known that pure haemoglobin contains 0·43 per cent. of Fe, and as all the iron in the blood exists in haemoglobin, we may calculate the amount of this constituent if we know how much iron is contained in the ashes of a known volume or weight of blood.

With this object about 100 grammes of blood are evaporated to dryness in a platinum basin, and then ignited, care being taken not to lose any portion of substance by incautiously heating, which would cause the burning organic matter to froth over. The ash is cooled and boiled with 10 or 20 c.c. of pure hydrochloric acid diluted with its own volume of water, and the extraction is repeated. The solution of ferric chloride in then reduced by the action of metallic zinc, and

[1] Dr Gowers' apparatus is sold, under the name of Haemoglobinometer, by Mr Hawkesley, Surgical Instrument Maker, 300, Oxford Street, London, W.

the amount of iron determined with the aid of a standard solution of potassium permanganate. By multiplying the amount of metallic iron in 100 parts of blood by 100 and dividing by 0·43, we obtain the percentage of haemoglobin.

For the details of the method of determining the iron volumetrically we refer the reader to any systematic work on Quantitative Analysis.

Determination of Cholesterin, Lecithin, and Fats in Blood (Hoppe-Seyler).

20 to 50 c.c. of blood are treated with 3 or 4 times their volume of absolute alcohol, set aside for a few hours, and then filtered. The insoluble matter is washed, first with pure alcohol and then with alcohol holding ether in solution.

The mixed alcoholic and ethereal solutions are evaporated to dryness on the water-bath. The residue is dissolved in ether, filtered, evaporated to dryness, and weighed.

In this way is obtained the combined weight of the cholesterin, lecithin and neutral fats.

The residue, after being weighed, is then treated with alcohol, and a little alcoholic solution of caustic potash added, and heated, in a silver dish, for some hours in the water-bath, until the whole of the alcohol is expelled. The residue contains cholesterin, soaps, glycerin, caustic potash and products of the decomposition of lecithin, viz. glycerin-phosphoric acid, neurin, &c.

The residue is then mixed with water and agitated repeatedly with ether. The ethereal solution is evaporated to dryness, and dissolved in absolute ether, which dissolves the cholesterin alone, leaving undissolved traces of soaps, which were mixed with it. The ethereal solution is evaporated at a low temperature, then dried below 80° C. and weighed.

The watery solution, from which the cholesterin has been removed by ether, is evaporated to dryness in a silver dish and fused with sodium hydrate and pure nitre. The fused mass is dissolved in water and treated with an excess of nitric acid.

The phosphoric acid is then precipitated by means of an acid solution of ammonium molybdate. The precipitate, which has separated after 12 hours, is dissolved in ammonia and the solution precipitated by magnesia mixture, the precipitate being washed, dried, ignited and weighed as magnesium pyrophosphate.

100 parts of magnesium pyrophosphate correspond to 764·5 parts of lecithin.

For full directions how to estimate the phosphoric acid in the above case, the reader is referred to works on quantitative analysis.

Determination of Water, Total Solids, and Salts of the Serum.

As the processes are exactly similar to those mentioned at page 177, they require no further notice here.

Determination of the total amount of Proteids contained in the Serum, and of the Serum-albumin.

Precipitation by heat. 50 to 100 c.c. of water are boiled, and an accurately weighed amount of serum (about 15 or 20 grammes) poured in. The fluid is boiled for some minutes, a drop or two of very dilute acetic acid cautiously added with a glass rod, until the precipitate separates in flakes from a perfectly clear (i.e. not opalescent) liquid; the precipitate is collected on a weighed filter, washed with water, then with boiling alcohol, dried at 110° C. and weighed. By this process all the proteids of the serum are precipitated together; by subtracting the weight of paraglobulin as determined by other methods, that of serum-albumin is found.

Precipitation by alcohol. *a.* Hoppe-Seyler's method[1].

An accurately measured or weighed quantity of serum, say 20 grammes, is mixed in a beaker with three or four times its volume of spirits of wine, and set aside at the ordinary temperature for some hours; the precipitate is then collected on a weighed filter free from ash, and washed, first of all with spirits, then with hot absolute alcohol, then with ether, and lastly with warm water.

There are thus left on the filter only proteids and insoluble salts. The filter is washed with spirit so as to displace the water, and is then dried at 120°, allowed to cool in an exsiccator, weighed, &c. The filter with its contents is then ignited, and the weight of ash deducted from that of the proteids. This method is of universal application to albuminous fluids, and is useful as it enables one to obtain in one operation, not only the amount of proteids, but alcoholic and ethereal extracts, in which other constituents may be determined.

b. Schmidt's method[2].

A weighed portion of serum is neutralized with acetic acid, mixed with ten times its volume of strong alcohol, set aside for 24 hours, and

[1] Hoppe-Seyler, *Handbuch der physiologisch- und pathologisch-chemischen Analyse*, 3e Aufl., p. 312.

[2] A. Schmidt, "Weitere Untersuchungen des Blutserums, &c." Pflüger's *Archiv*, Vol. xi. p. 10 (1876). This method has lately been subjected to examination by Prof. F. A. Hoffmann of Dorpat, who found it reliable. (Virchow's *Archiv*, Nov. 1879, p. 255.)

boiled. The flocculent precipitate is then collected on a filter and washed with a mixture of 10 parts of alcohol and 1 part of water, then with absolute alcohol and ether. It is then treated as the precipitate obtained by process *a*.

Determination by means of polarimeter[1].

This can only be done in the case of the serum being absolutely colourless and transparent.

The amount of albumin per gramme of solution will be found by the formula

$$w = \frac{\alpha}{-56^0 \times l}$$

where α indicates the rotation observed and l the length of the tube.

The results are however only approximative.

Determination of the amount of Fibrinogen contained in Liquor Sanguinis. (Frederique's method[2].)

If it be desired to determine the amount of fibrinogen in liquor sanguinis, the liquid, which has been kept from coagulating by exposure to cold, or by the addition of magnesium sulphate, is heated to 60°; the precipitate is allowed to subside, washed by decantation with a $\frac{1}{2}$ per cent. solution of sodium chloride, and then thrown on a small weighed filter. The filter is washed with distilled water, then with alcohol, dried at 110° C., cooled under an exsiccator, and weighed between watch-glasses. The filter and contents may be afterwards ignited and the weight of ash deducted.

Determination of the amount of Serum-globulin in the Serum.

Two methods may be employed, which give however entirely different results; the second is alone reliable.

Schmidt's method.

a. Serum is placed in a dialyser, the water around which is frequently renewed. After about 48 hours a stream of CO_2 is passed through the contents of the dialyser. The precipitate which has formed is collected on a weighed filter, washed, dried, and weighed.

Hammarsten's method[3].

b. 5 c.c. of serum are diluted with 25 c.c. of saturated solution of magnesium sulphate and then treated with powdered magnesium sulphate. The fluid is stirred from time to time and the salt added to saturation. After at least 24 hours, it is filtered; the precipitate is collected on a filter moistened with saturated solution of magnesium sulphate; the

[1] Hoppe-Seyler, "Bestimmung des Eiweissgehaltes an Blutserum verm. Polarisation." Virchow's *Archiv*, Vol. XI. p. 547.

[2] L. Frederique, "Recherches sur la constitution du Plasma sanguin." *Travail du Laboratoire de Physiologie de l'Université de Gand et du Laboratoire de Physiologie de la Faculté des Sciences de Paris*, 1878.

[3] Hammarsten, "Ueber das Paraglobulin." Pflüger's *Archiv*, 1878, p. 413.

substance left on the filter is thoroughly washed with the same solution and the funnel and filter are heated for some hours to 110° C. At the end of that time the serum-globulin has become so insoluble that it can be washed with boiling water without any risk of solution. It is then treated repeatedly with warm alcohol and ether, dried, weighed, incinerated, and the weight of ash deducted.

By Hammarsten's method the amount of serum-globulin found in serum is sometimes greater than that of serum-albumin. (Consult p. 61.)

Determination of the presence and quantity of Urea in the Blood.

Picard's method[1] as modified by Meissner[2] and Gscheidlen[3].

A weighed quantity of blood is diluted with about four times its volume of water, acidulated with sulphuric acid, and boiled so as to free it from proteids.

The clear filtrate is concentrated, and treated with a solution of barium hydrate, which precipitates sulphates and phosphates, and the excess of baryta is removed by the cautious addition of sulphuric acid. The fluid is then evaporated to a syrupy consistence and mixed with absolute alcohol. The alcoholic solution is separated from the precipitate (chiefly composed of inorganic salts) which forms, the alcohol evaporated, and the residue dissolved in water. The solution, which is of a light or deep yellow colour, possesses an acid reaction. Solution of mercuric nitrate is added cautiously; this solution may be obtained by diluting Liebig's mercurial solution for the estimation of urea with an equal volume of water. A copious precipitate falls, the fluid is filtered and the filtrate is rendered alkaline by adding baryta water or a solution of sodium carbonate, and then more mercurial solution is added, until a drop of the mixture brought in contact with solution of sodium carbonate gives a yellow precipitate.

Proceeding in this manner a white precipitate is obtained, which is well washed, then diffused in water and subjected to a stream of sulphuretted hydrogen, which precipitates the mercury as sulphide.

The filtrate from the precipitate of sulphide of mercury is concentrated and treated with a little concentrated and perfectly colourless nitric acid. After some time crystals of nitrate of urea separate, which are collected on a filter, dried over sulphuric acid and weighed.

Whilst acknowledging the value of this method as demonstrating the presence of urea in the blood in the most conclusive manner,

[1] J. Picard, "De la présence de l'Urée dans le sang et de sa diffusion dans l'organisme à l'état physiologique et à l'état pathologique." *Thèse de Strasbourg*, 1856.

[2] Meissner, "Beiträge zur Kenntniss des Stoffwechsels im thierischen Organismus. Der Ursprung des Harnstoffs im Harn der Säugethiere." Henle u. Pfeufer's *Zeitschrift f. rat. Medizin.* Dritte Reihe. Vol. xxxi. pp. 234—349.

[3] Gscheidlen, *Studien über den Ursprung des Harnstoffs im Thierkörper.* Leipzig, 1871.

it is perfectly obvious that it must furnish results which must be decidedly too low.

Gréhant's method. A carefully weighed quantity of defibrinated blood is mixed with twice its volume of strong alcohol and set aside from one day to the next. The alcohol is then filtered off, the insoluble coagulum is squeezed in a press and washed with alcohol, the alcoholic liquids are concentrated in a water bath. The residue is dissolved in water and is then introduced into the vacuum of a mercurial pump, where it is subsequently mixed with Millon's reagent (solution of mercurous nitrate in nitric acid). The urea undergoes decomposition, yielding equal volumes of carbonic acid and nitrogen, mixed with much nitric oxide. The gases are collected and analyzed, and from the results the amount of urea is calculated. The author has found this method very troublesome of execution and by no means as accurate as has been maintained by its advocates. The decomposition takes place during a considerable period of time, and in consequence of the disengagement of nitric oxide continuing almost indefinitely the operator is never sure when the process should be considered at an end. Moreover the volumes of carbonic acid and nitrogen evolved are not strictly equal; in the author's experiments the volume of carbonic acid was always somewhat below that of the nitrogen.

By decomposition with sodium hypobromite. This method of estimating urea, which will be described at length under the head of Urine, has been employed by the author in the determination of the amount of urea in the blood.

A weighed quantity of blood, usually about 50 grammes, is mixed with twice its volume of absolute alcohol and set aside in a stoppered bottle for about 24 hours. At the end of this time the precipitate is collected on a linen filter, washed with absolute alcohol and subjected to firm pressure in a screw press. The alcoholic liquid is evaporated to dryness in the water-bath, and the residue is taken up in absolute alcohol, filtered, evaporated to dryness, dissolved in a little water, and the watery solution filtered. The filtrate is now placed in the decomposing bottle of Dupré's apparatus (Fig. 38) and subjected to the action of sodium hypobromite. The nitrogen evolved, instead of being collected in a wide tube such as is shewn in the engraving, is, in the case of urea determinations in blood, collected in a much narrower tube divided into tenths of a cubic centimetre.

From the volume of nitrogen obtained, the urea can readily be calculated[1].

It may be objected to this process that in reality it only furnishes us with the amount of nitrogen given off by the extractive matters of blood when treated with alkaline hypobromites, and that as other

[1] The reader is referred for a full description of the process of estimating urea by solutions of hypobromite to the section on Urine.

nitrogenous proximate principles than urea evolve nitrogen under these circumstances, it is unphilosophical and inaccurate to consider the nitrogen as all derived from urea. There is doubtless much force in this objection; nevertheless as unquestionably urea is

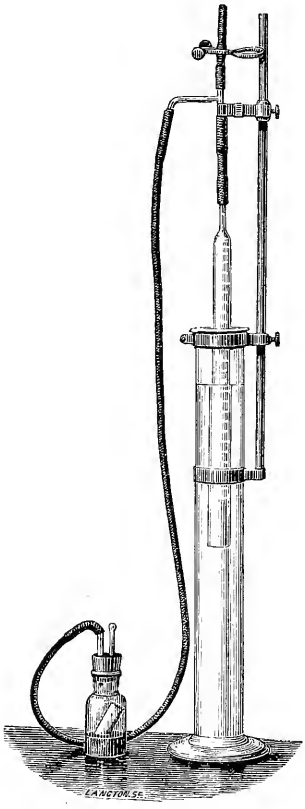


FIG. 38. DUPRÉ'S APPARATUS.

infinitely the most abundant of the nitrogenous extractive matters of the urine and of the blood, and as it is the only body which does yield nearly the whole of its nitrogen to hypobromite, the estimate of urea based upon nitrogen evolved under these circumstances is very near the truth. The method is one, too, which yields most concordant results.

Haycraft's method[1]. This method, which was worked out by John Haycraft, M.B., in Professor Ludwig's Laboratory, appears to be likely to supersede all others, though it has not yet been

[1] Communicated privately to the Author. A short account of this method has already appeared in the *Journ. f. pract. Chemie.*

employed in any series of researches. It may be described as follows:—

Defibrinated blood, varying in quantity between 10 and 20 c.c., is placed in a dialyser, so as to form a layer on the parchment-paper not deeper than 4 mm. The dialyser is placed in a vessel containing a volume of absolute alcohol equal to twice that of the blood.

In a period varying between one and four hours the fluid part of the blood, holding the urea in solution, has passed into the alcohol, leaving a solid mass behind. This is removed from the parchment-paper, mixed with a little water and again placed in the dialyser. The process is repeated three or four times, so as to make sure that all the urea has been extracted. The alcohol is then poured into a shallow porcelain dish, and after acidifying with oxalic acid, so as to convert all urea into oxalate, the fluid is evaporated to dryness. In the residue, crystals of oxalate of urea may be seen with the naked eye, mixed with some fat, colouring matter, and crystals of common salt. The fat and colouring matter are in great part removed by the aid of *petroleum naphtha*, which readily dissolves those substances, whilst it leaves the whole of the oxalate of urea undissolved. This is then dissolved in water and mixed with a little barium carbonate, and the mixture evaporated to dryness. On boiling with alcohol this fluid extracts the urea, leaving traces of proteids and common salt. On evaporating the alcoholic solution, almost pure crystals of urea are left; the amount of this substance is determined by weighing, or by Bunsen's method (see 'Determination of Urea in Urine'), or by means of the hypobromite method.

This method is so precise that Dr Haycraft has never failed, by its aid, in preparing a naked-eye demonstration of urea from ten cubic centimetres of normal blood. He has found that by this method the maximum error does not exceed 6 p.c. of the total weight of urea.

Determination of the amount of Uric Acid in the Blood.

The amount of uric acid in the healthy blood of man is so small as to render its determination, and even its detection, impossible. In the blood of Birds, whose urinary excretion is very rich in uric acid, Meissner[1] succeeded, by a process for which we refer the reader to his original memoir, in determining the amount of uric acid.

The following is the process employed by Dr Garrod in the detection and estimation of uric acid in the blood of gouty patients. "The serum of the blood is first dried over a water-bath, then

[1] Meissner, "Der Ursprung der Harnsäure des Harns der Vögel." *Zeitschr. f. rat. Med.*, Dritte Reihe. Vol. xxxi. p. 144 et seq.

reduced to coarse powder, and treated with hot alcohol; the spirit being removed, the residue is afterwards to be digested for some minutes in distilled water, and raised to the boiling point; the watery solution is then filtered and evaporated to a thin syrupy consistence. A drop or two of the solution, when heated on a piece of porcelain, with nitric acid and ammonia afterwards added, exhibits at once the murexide test. A small portion of the same solution, if acidulated strongly with acetic acid, and allowed to evaporate spontaneously, gives rise to the crystallization of uric acid, the crystals exhibiting its characteristic forms; and lastly, the syrupy solution, if merely allowed to evaporate without the addition of any acid, exhibits upon its surface, after a few hours, small white tufts of acicular crystals of urate of soda; the nature of the base being determined by the examination of the white alkaline ash left after incineration; the acid by the murexide and other tests." In cases where the amount of uric acid which separates on acidulating the aqueous solution in the above process by acetic acid is considerable, it may be collected on a weighed filter, washed, dried, and weighed.

"In the clinical examination of the blood, this process would be too elaborate and tedious; but a method which answers admirably for practical purposes is, to put about two drachms of the serum in a flat glass dish, somewhat larger than a watch-glass, acidulate slightly with acetic acid, and having placed in the fluid an ultimate fibre from a piece of linen cloth (unwashed huckaback answers well) set it aside in a safe place until the evaporation has proceeded sufficiently far to cause it to become of a gelatinous consistence. If there is uric acid in any abnormal quantity in the serum, the fibre becomes studded with crystals of uric acid, which can be at once recognized by placing the glass under the microscope with a low power, or by the use of a small magnifying glass. I have never yet, after very numerous trials, failed to discover uric acid in the blood of gouty patients by this method, and the test has an especial advantage in only requiring the abstraction of a very small quantity of so important a fluid[1]."

Determination of the amount of Sugar in the Blood.

The principle upon which all methods of estimating the amount of sugar in the blood are based is to dilute the blood (sometimes only the serum) and to coagulate the proteid matters and haemoglobin which it contains; thereafter to determine the sugar in the filtrate by one of the methods to be described in detail under the head of Urine. The following method of separating the proteid matters is that which has been followed by Dr Pavy[2] in his researches.

[1] Garrod, Article "Gout," Reynolds's *System of Medicine*, Vol. I. p. 825 and 826. See also Garrod, *Med. Chir. Transactions*, Vol. XXXVII. (1854) p. 826.

[2] Pavy, "The Croonian Lectures, on certain points connected with Diabetes." London, 1878.

"Forty grammes of sulphate of soda in small crystals are weighed out in a beaker of about 200 c.c. capacity. About 20 c.c. of the blood intended for analysis are then poured upon the crystals, and the beaker and its contents again carefully weighed. In this way, the precise weight of the blood taken is ascertained. The blood and crystals are well stirred together with a glass rod, and about 30 c.c. of a hot concentrated solution of sulphate of soda added. The beaker is placed over a flame guarded with wire gauze, and the contents heated until a thoroughly formed coagulum is seen to be suspended in a clear colourless liquid, to attain which actual boiling for a short time is required. The liquid has now to be separated from the coagulum and the latter washed to remove all the sugar. This is done by first pouring off the liquid through a piece of muslin resting in a funnel into another beaker of rather larger capacity. Some of the hot concentrated solution of sulphate of soda is then poured on the coagulum, well stirred up with it, and the whole thrown on the piece of muslin. By squeezing, the liquid is expressed, and to secure that no sugar is left behind, the coagulum is returned to the beaker and the process of washing and squeezing is repeated.

"The liquid thus obtained may be fairly regarded as containing all the sugar that existed in the blood. From the coarse kind of filtration and squeezing employed, it is slightly turbid, and requires to be thoroughly boiled to prepare it for filtration through ordinary filter paper. A perfectly clear liquid runs through, and to complete this part of the operation the beaker that has been used and the filter paper are washed with some of the concentrated solution of sulphate of soda before referred to." In the solution thus obtained the sugar is determined by the amount of cupric oxide which it can reduce, the copper being separated electrolytically.

v. Mering[1] merely dilutes the serum with four or five times its volume of water, boils and adds dilute acetic acid in sufficient quantity to cause thorough separation of the proteids in a flocculent form; the filtrate is then concentrated, and the sugar in it determined either by Fehling's solution or by Sachsse's method (see 'Urine').

Determination of the weight of the Moist Corpuscles contained in the Blood.

Various methods have been suggested for determining the weight of the moist blood corpuscles, all of which are attended with considerable practical difficulties. Fortunately, by a combination of the processes of enumeration of the blood corpuscles and determination of the amount of haemoglobin contained in the blood, information is acquired which possesses as great value to the physician as

[1] v. Mering, "Ueber die Abzugswege des Zuckers aus der Darmhöhle." *Archiv f. Anat. u. Physiol.* 1877, p. 379 et seq.

would attach to a knowledge of the actual weight of the blood corpuscles. Determinations of the weight of the moist corpuscles will probably in the future be rarely attempted. The following very brief description of the one method which is to be recommended above all others will suffice: it is based upon *finding the relative weight of fibrin in the liquor sanguinis and in the blood* (Hoppe-Seyler).

This method, which can only be carried out when all suitable preparations can be made before the blood is removed from the living body, is as follows:

Blood is received in a cylindrical (metallic) vessel which is surrounded with ice[1], and at the same time another portion of 30—50 c.c. of blood is collected and the fibrin determined in it by the proceeding described at page 179.

After an interval of an hour or two, the corpuscles having had time to subside from the liquor sanguinis in the sample of blood first collected, from 30 to 50 c.c. of the clear liquid are drawn off by means of a cooled pipette and placed in a second apparatus for the extraction of fibrin, and the process carried out exactly as in the first case. The amount of fibrin being known, the operator is in possession of the data required to be known.

The calculation will be understood by quoting the following example from v. Gorup-Besanez.

(1) the weight of fibrin in 1000 grammes of blood was found to be . . . . . 3·95 grms.

(2) the weight of fibrin in 1000 grammes of liquor sanguinis was found to be . . . . . 8·07 grms.

If 8·07 grms. of fibrin correspond to 1000 grms. of plasma, to how much plasma will 3·95 grms. of fibrin correspond?

$$8{\cdot}07 : 1000 :: 3{\cdot}95 : x,$$

$$x = \frac{1000 \times 3{\cdot}95}{8{\cdot}07} = 486{\cdot}98.$$

Thus is found the weight of plasma in 1000 parts of blood, and the weight of moist corpuscles is found, by subtraction, to amount to

$$1000 - 486{\cdot}98 = 513{\cdot}02.$$

Separation and Determination of the Gases of the Blood.

The methods now universally adopted and alone to be recommended for the extraction of the gases of the blood consist in introducing an accurately determined volume of blood into the vacuum of a mercurial air-pump, exposing it to a temperature of 40°—45° C., removing the gases *pari passu* with their disengagement, collecting them over mercury, and then subjecting them to analysis. Whilst the principles which guide these operations are very similar,

[1] A vessel constructed on Dr Sanderson's plan (Fig. 9, p. 32) should be used for this experiment.

the actual form of mercurial pump employed and the details of the different operations employed by different experimenters, and, indeed, in different laboratories, vary very much.

We shall here describe (1) methods of collecting blood intended for gas analysis; (2) the various pumps which may be employed with advantage; and (3) the methods of determining the composition of the separated gases.

Collection of Blood for the Determination of its Gases.

In all cases the blood to be investigated must be collected over mercury in such a way as to avoid all access of air.

The apparatus (Fig. 39) is admirably adapted for this purpose

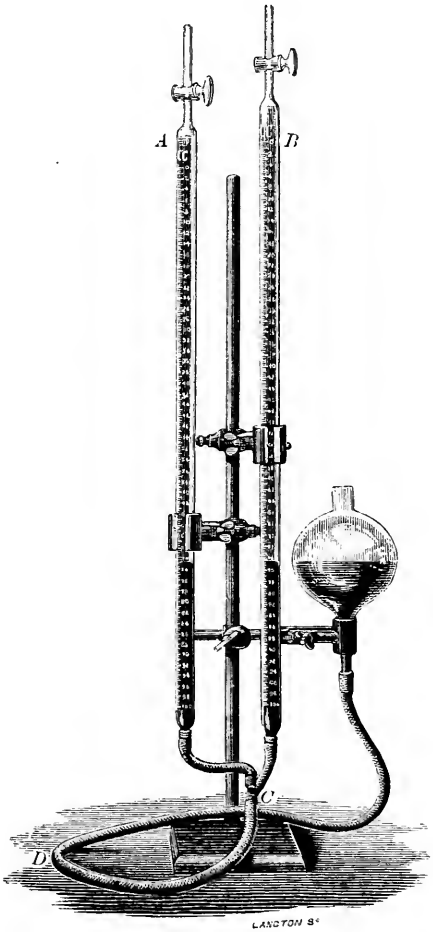


FIG. 39. APPARATUS FOR COLLECTING BLOOD OF WHICH THE GASES ARE TO BE DETERMINED.

One or both the tubes having been filled with mercury and the stop-cocks being shut, a narrow elastic tube leading from the blood-vessel whence the blood is drawn, after being allowed to fill with blood, is slipped over the free tube leading upwards from the stop-cock and which is quite full of mercury. The filling bulb (*R*) being in a suitable position, and the stop-cock opened, blood will flow into the tube displacing the mercury which it previously contained. When enough blood has been collected, the stop-cock is closed, and a clip (Fig. 40) being applied to the india-rubber tube

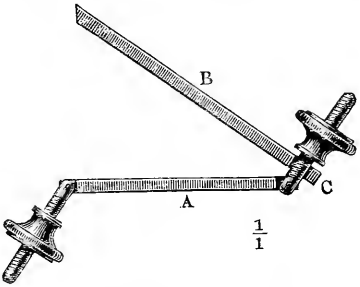


FIG. 40. CLIP FOR COMPRESSING INDIA-RUBBER TUBES.

leading from the graduated tubes to the filling bulb, the tube is released from the clamp which held it and shaken, or rather inverted repeatedly, so as to defibrinate its contents. When fibrin is separated by shaking blood and mercury in this way, it does so in a state of very fine division. A suitable tube being now attached to the constricted part of the graduated tube, in place of that which served to conduct blood into it, and the tube having been again fixed by its clamp to the stand, the mercury bulb is raised, and the graduated tube may be placed in communication with the blood-receptacle of the mercurial pump, and any quantity of the blood which it contains may be allowed to flow into the vacuum. As the tube is graduated, the volume allowed to flow in can be determined. If some time must intervene between the collection of the gas and its analysis, the tube *A* may be removed from its clamp and laid in a trough containing broken ice.

Although other methods of collecting and measuring the blood which is introduced into mercurial pumps have been employed, and will be referred to in describing the various forms of mercurial pump, those here given, which the Author is in the habit of employing in his laboratory, will be found to meet all requirements.

Mercurial Pumps.

Ludwig's pump. The first pump to be described is Ludwig's[1], which was first figured and described in a memoir by his pupil,

[1] The first pump to which the name of 'Ludwig' could be applied was described by his pupil Setschenow (*Zeitschr. f. rat. Med.*, 3rd ser., Vol. x. p. 112). The form described and figured is that at present employed in the Leipzig laboratory.

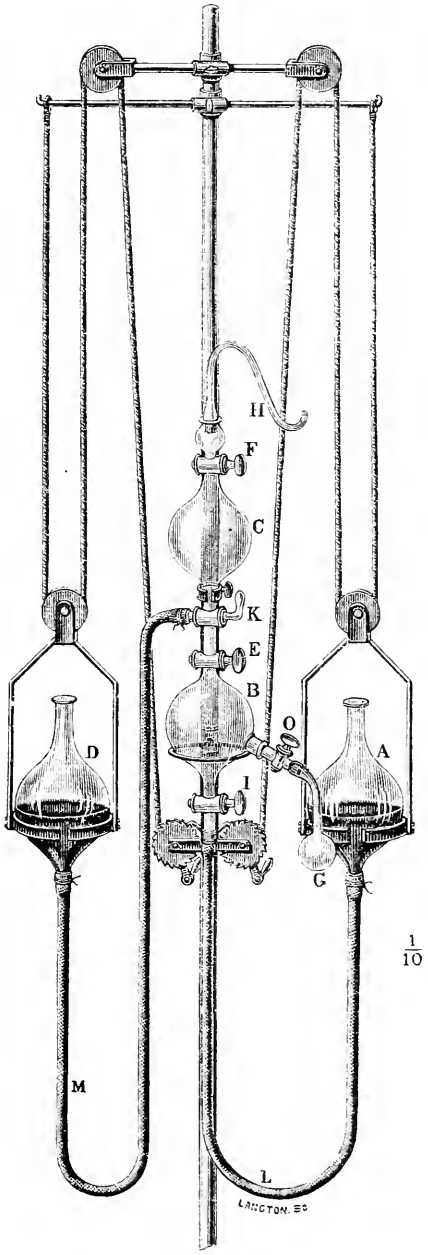


FIG. 41. LUDWIG'S MERCURIAL PUMP.

Alexander Schmidt[1]. It consists in reality of a combination of two mercurial pumps. The vessel containing the blood of which the gases are to be determined, *G*, is connected with the bulb *B*. When a complete vacuum has been made in *B* and in *C*, the stop-cock *E* is closed, *O* is opened and *G* is plunged into hot water. The blood enters into ebullition and its gases pass into *B*, some of the blood also passing into that vessel. By opening *E* and *K*, the gases are collected in *C*, and *K* being then turned so as to shut *C* off from *B* but to place it in communication with *D*, the latter is raised so as to compress the gas in *C*; on now opening the stop-cock *F*, the gas may be made to pass through *H* into a gas jar standing over mercury. This very brief description will be understood by carefully examining the drawing, especially if the reader make himself acquainted with the construction of Pflüger's or Alvergniat's pumps as described in the succeeding paragraphs.

In using Ludwig's pump, the blood is always defibrinated before analysis. The blood to be analysed is introduced, without coming in contact with air, into the receptacle *G*, which has previously been filled with mercury and detached from the pump. The bulb having a known capacity the volume of the blood analysed is known.

Pflüger's pump. The pump, of which one form is represented by Fig. 42, possesses arrangements whereby watery vapour which is disengaged in vacuo is at once absorbed.

G, *C*, *E*, *F*, *D*, represent parts of the pump proper; *C* is the barometric chamber of about two litres capacity, provided at *G* with a three-way cock, which enables the chamber to be shut off or placed in communication either with the chambers to be exhausted, *B*, *A*, or with the open air, or by means of the glass gas delivery tube *H* with a mercurial pneumatic trough. *D* is a bulb larger than *C*, and communicating with it by means of a stout caoutchouc tube covered externally with a stout woven fabric, so as to enable it to resist considerable internal pressure without dilating. *D* is contained in a box which may easily be wound up and down by means of the ratchet-wheel *L*, and the band and pulley connected with it.

Mercury is poured into the filling globe *D*, when the latter is in its lowest position. By winding *D* up until its level is above that of *C*, and placing the stop-cock *G* in such a position that *C* communicates with the external air, the bulb *C* is filled with mercury. The stop-cock *G* is then turned so as to shut off *C* completely from communication above. On now bringing *D* down to its initial position, viz., about a metre below *C*, the mercury in the latter sinks until it stands at the height of the barometer above the mercury in the reservoir *D*. There is then a Torricellian vacuum in *C*. By a suitable turn of the three-way cock *G*, the chamber *C* is now brought into communication with the apparatus to be exhausted. After the gas contained in the latter has diffused

[1] Alex. Schmidt, "Ueber die Kohlensäure in den Blutkörperchen." Erste Abhandlung. *Ber. d. königl. sächs. Gesellsch. d. Wissenschaft. zu Leipzig.* Math.-phys. Classe. Vol. XIX. (1867) S. 33.

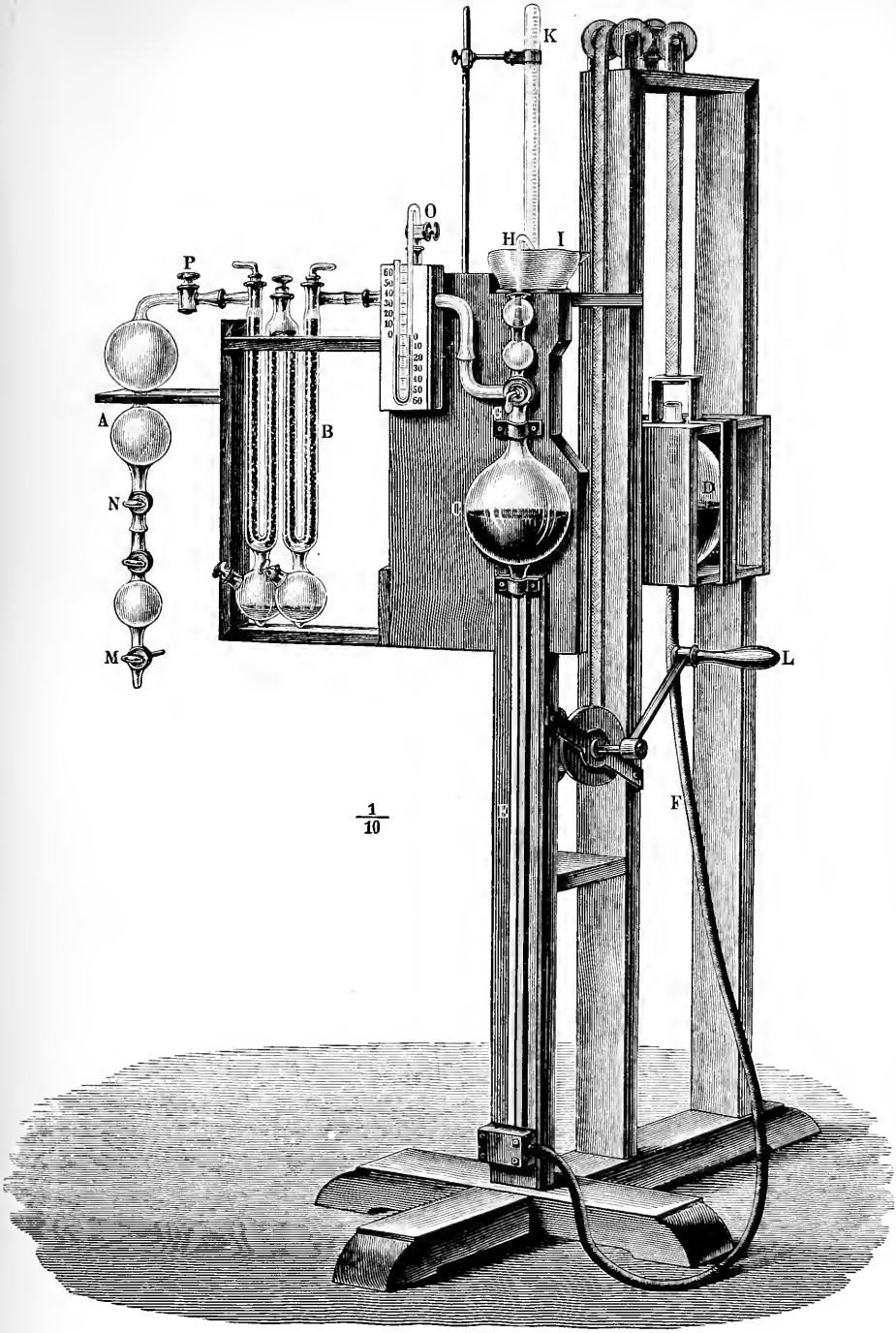


Fig. 42. Pflüger's mercurial air-pump, with the arrangements for separating the gases of the blood (as made by Geissler of Berlin).

into the chamber, the stop-cock *G* is shut, the globe *D* is elevated, and by a suitable movement of the stop-cock the imprisoned gas is allowed to pass either into the air, or is collected through *H* over mercury in the graduated tube *K* standing in the pneumatic trough *I*. By repeating several times the series of operations described the amount of residual gas in the apparatus sinks to an insignificant amount, and, without great labour, a practically perfect vacuum is obtained.

The accessory apparatus shewn in the drawing requires description. *O* is a mercurial gauge, *B* is the drying chamber, composed of four glass tubes communicating below with two small reservoirs. The tubes are filled with pumice-stone or asbestos saturated with sulphuric acid, and the bulbs also contain some of the same acid. The drying-chamber is in communication with two large glass bulbs *A*, which are intended to arrest the froth which arises from the boiling blood. To the 'froth-chamber' is carefully attached a glass bulb *M*, into which the blood is placed. This bulb has at its upper part a single-way stop-cock, but below it is provided with a two-way cock. The plug of the stop-cock is, in the drawing, shewn to be prolonged considerably beyond the socket into which it fits. This plug is perforated in its long diameter by a canal which passes through it obliquely, and is so arranged that the fluid passing through the canal may be directed upwards into the 'blood-bulb,' or downwards and outwards. (Fig. 43.)

A vacuum having been made in the 'drying-chamber,' the 'froth-chamber,' and the 'blood-bulb,' the plug of the lowest stop-cock of the blood-bulb (*a*, Fig. 43) has attached to it, by means of a piece of thoroughly sound black elastic tube, a flexible metallic tube, which is connected peripherally with a glass cannula which is tied into the blood-vessel whence the blood is to be drawn, or preferably with a blood-measuring tube.

Blood is now allowed to flow through the elastic tube until the latter is filled, the plug being placed in such a position that the displaced air and the displacing blood flow at first not into the blood-bulb but outwards. At a given moment the stop-cock is turned (in the position shewn in Fig. 43) so as to open a communication between the blood-bulb and the blood-measuring tube, or the blood-vessel: the blood flows into the vacuous bulb, and immediately enters into ebullition. As soon as enough blood has entered, the lower stop-cock is shut, and the stop-cocks which shut

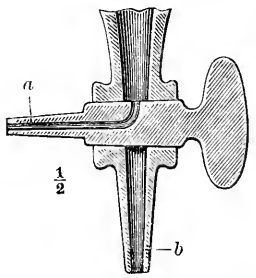


FIG. 43 exhibits the construction of the two-way cock (*M*, Fig. 42) at the lower part of the blood-bulb. When the plug is in the position shewn the tube *a* communicates with the interior of the bulb. When the position is reversed *a* communicates with *b*. In intermediate positions the bulb is shut off and the tubes *a* and *b* do not communicate.

off the upper part of the blood-bulb from the drying-chamber and the barometric chamber of the pump are opened. The blood-bulb *M* is now immersed in a vessel containing water at 40° C., when the blood enters into violent ebullition: if arterial in colour before being introduced into the bulb, it assumes almost instantly the cherry-red colour which is characteristic of reduced haemoglobin; if the reddened walls of the froth-chamber be viewed through a spectroscope the simple broad band of reduced haemoglobin is then seen. After a few minutes the gases which have been given off are collected over mercury in a tube filled with mercury, the vacuum is renewed, and the process of ebullition continued.

Some observers who have used the pump shewn in Fig. 42, have determined the amount of blood analysed, by actually weighing it. With this object, the exhausted and empty blood-bulb is detached from the pump and weighed; thereafter the quantity of blood to be analysed is introduced into it, in the manner previously mentioned; the stop-cock *M* through which blood has flowed is then rinsed, first with water, then with alcohol, and rapidly dried, and the bulb is again weighed. The blood-bulb is then again joined to the bulbs *A* (Fig. 42), of which the stop-cock *N* has been kept closed. The junction having been made, the small quantity of air which intervened between the upper stop-cock of the blood-bulb and *N* having been removed by a few strokes of the pump, the process of boiling the bulb is commenced. This process appears to the Author to be tedious and unsatisfactory in the extreme. It is always better to pass the blood from an apparatus in which it is first measured to the blood-bulb; it is indeed quite practicable to measure the blood and pass it into the bulb, before coagulation has had time to set in.

In the mercurial pumps made by Geissler of Bonn, and which are, the Author believes, identical with those used by Professor Pflüger himself, the arrangement shewn in Fig. 44 is employed.

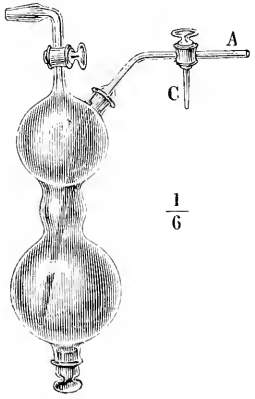


FIG. 44. LARGE RECEIVER OF PFLÜGER'S PUMP, AS MADE BY GEISSLER OF BONN, INTO WHICH BLOOD IS INTRODUCED, AND BOILED FOR THE EXTRACTION OF ITS GASES.

The tube which conveys the blood to be analysed is slipped over *A*. By suitable manipulation of the stop-cock, the blood is first made to expel the air in the tube *A*, outwards through *C*. The blood may then be directed

into the lower of the two large bulbs, which are attached to the pump, and which have been perfectly exhausted.

The quantity of blood analysed is determined after the gases have been extracted by weighing the bulb apparatus (Fig. 44), and the sulphuric acid drying apparatus, and subtracting the weight of the same, as determined *before* the blood was boiled. It is obvious that with this pump also it is easiest to measure the blood before it is introduced into the vacuum.

The special features of the process described above, and which renders it preferable to some others employed for the same purpose, are, (1) the blood may readily be brought directly (if desired) without previous defibrination, from the blood-vessels into the apparatus where its gases are separated: in this respect it differs, for instance, from Ludwig's pump; (2) the blood is at once introduced into a very large vacuous space, so that the O-pressure outside the blood is always very much below the dissociation-tension of the O of the blood, the latter therefore escapes very rapidly; (3) the vacuum is maintained in a dry condition by the sulphuric acid in the drying chamber; this appears to have very great influence in facilitating the removal of the gases from the blood. With such an arrangement it is possible, for instance, in a very brief space of time rapidly to extract all the carbonic acid of the blood without the necessity of adding a dilute acid.

Alvergniat's pump. This pump, constructed by MM. Alvergniat frères of Paris, was first employed in the investigation of the gases of the blood by Gréhant and Bert[1], and has already proved most useful. Being constructed exactly on the principle of Pflüger's pump, it does not require a special description; it will be observed that its barometric chamber is very much smaller than that of the first-named instrument, and that it is not, as sold, provided with any arrangement for absorbing watery vapour which may be given off in vacuo, though such an arrangement can be contrived and connected with it by the operator. The special features of this pump are, 1st, that just above the three-way cock is situated, *permanently*, a most convenient small mercurial trough, 2ndly and chiefly, that the three-way cock is immersed in an iron box which is filled with mercury, so that all risk of leakage is avoided.

In extracting the gases of the blood with this small pump it is usual to connect with it a long glass tube about 2 inches in diameter, with a bulb blown at its lower end having a capacity of about one litre. This bulb is closed by an india-rubber stopper which is perforated by a thermometer tube of narrow bore. The junction between the tube and the pump is made by means of india-rubber tube carefully wired, and is protected by a water-joint. The bulb also is immersed into a tin vessel containing water. The narrow thermometer tube has attached to it a fine glass stop-cock with an almost capillary bore. This stop-cock is also immersed in water. This system of protecting every junction by surrounding it either with water or mercury is un-

[1] Bert, *Leçons sur la Respiration.* Paris, 1870.

doubtedly an admirable one, and relieves the mind of the experimenter from the fear of an accidental leak—an eventuality which, unfortunately, does occur where many stop-cocks are freely surrounded by air.

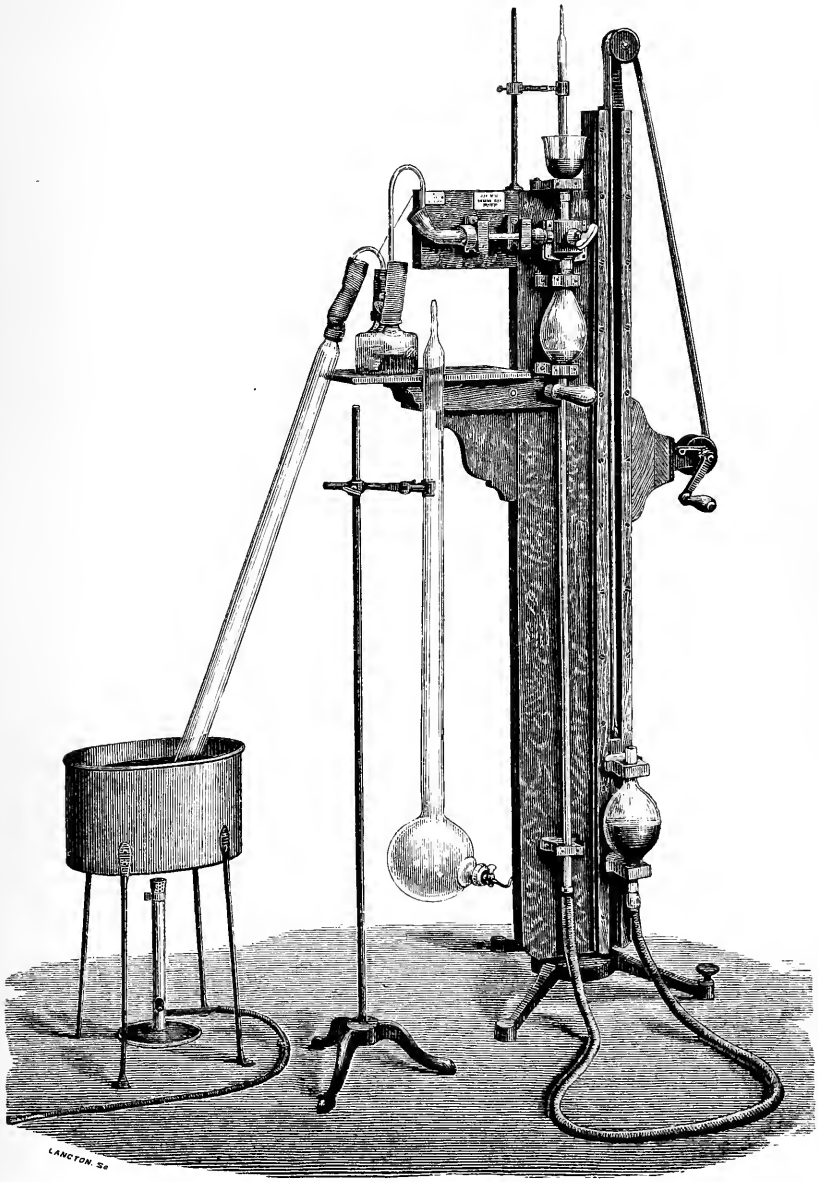


FIG. 45. ALVERGNIAT'S MERCURIAL PUMP, FITTED UP AS IN AN ACTUAL EXPERIMENT.

In Fig. 45 the pump is shewn as fitted up for an actual experiment; between the pump and the blood-receiver a wide glass bottle containing sulphuric acid and asbestos is shewn. All the connections are protected by water or mercury joints. Standing in front of the pump, and held by an iron clamp, is a tube similar to the one which is only partially seen in the drawing of the pump and its connections.

The Author's experiments with Alvergniat's pump have impressed him most favourably. The smallness of the barometric chamber naturally makes the process of exhausting the apparatus connected with the pump a very tedious matter, unless the plan be adopted of exhausting at first by means of an ordinary air-pump or with the aid of a water aspirator, and towards the close of the exhaustion allowing two or three cubic centimetres of boiled-out water to enter the nearly empty bulb and heating the water which surrounds the bulb so as to cause the contained water to boil. The steam which is disengaged, very rapidly and perfectly expels the last traces of air. Without this expedient the experimenter will almost despair to obtain a good vacuum with Alvergniat's pump, when there are connected with it vessels having a capacity of between 1500 and 2000 c.c.

The Author has found it convenient to interpose a small sulphuric acid chamber between the pump and the blood-receptacle, the object being to prevent the passage of water into the former and from it into the tube in which the gas is collected. With this addition he can recommend Alvergniat's pump as adapted for researches on the gases of the blood[1]. By its portableness, it lends itself admirably to demonstrations in the lecture-room.

It will be found convenient to employ about 30 or 35 cubic centimetres of blood for the determination of gases. The temperature at which the process is best carried on is 45° C. By simply heating in vacuo, the whole of the gases which are in a state of solution or feebly combined may be removed; the last portions of carbonic acid are however more rapidly evolved by allowing a small volume (one or two cubic centimetres) of a thoroughly boiled out solution of phosphoric acid to enter the blood-receptacle *near the close of the operation*. As has been shewn, however, by Pflüger and his pupils, the addition of an acid to blood *before* the oxygen has been pumped out leads to a considerable diminution in the volume of oxygen obtained, in consequence doubtless of the gas being used up in processes of oxidation.

ANALYSIS OF THE GASES OF THE BLOOD.

It is not consistent with the object of this work to give detailed descriptions of operations which belong to general chemistry, and

[1] This pump is manufactured by MM. Alvergniat frères, Rue de la Sorbonne, Paris. It costs only 160 francs; the tube with bulb, &c. being sold separately.

which may be learned by reference to systematic works on analysis. Accordingly, the extensive subject of gas analysis will not be treated of with any pretence to completeness, the reader being referred to other sources for information on the subject. The analysis of gases is best carried out either by the methods suggested by Bunsen[1] or with the aid of the most ingenious and accurate methods devised by Professor Frankland.

Description of the methods of Frankland for the analysis of gases.

We quote the whole description of these methods from the excellent account given by Professor Burdon Sanderson[2].

Frankland's smaller apparatus for the analysis of gases by absorptiometric methods.

"With a view to the analysis of the gases of drinking water, Frankland has introduced an apparatus of great simplicity (*see* Fig 46), the working of which will be readily understood by the diagram. It consists of two parts, viz., a laboratory tube (*k*), in which the gas to be analysed is first received, and a measuring apparatus, to which it can be transferred from the laboratory tube, in order that its volume may be determined before and after each absorption. The measuring apparatus consists of two tubes (*a*, *b*), fixed vertically side by side in a stand, surrounded by a chamber containing water (*n*). They communicate below both with each other and (by the long flexible tube) with a mercury-holder (*t*), like that of Alvergniat's pump. One of them can be brought into communication by the arm (*g*) with the laboratory tube; the other (*b*) is open at the top. A scale of millimeters is engraved on it, the zero of which is opposite *o*. A corresponding scale, starting from a zero at the same level, is engraved on the measuring tube. The apparatus is filled with mercury by raising the mercury-holder (*t*) to a sufficient height, the stop-cock (*f*) remaining open; in doing which the surface of the mercury in *t* must not be more than a few millimeters higher than the tap. As soon as mercury appears at *g*, the stop-cock is closed. The next step is to fill the laboratory tube. Having inverted it in the trough, which has been previously raised to the proper height, the operator draws out most of the air by means of a bent tube, the point of which rises to the top of the laboratory tube, and shuts the stop-cock as soon as the mercury rises. The removal of the air is completed by joining *g* and *g'* so as to connect the laboratory tube with the measuring apparatus, and then causing the air contained in the former to pass over into the latter, by depressing *t*. The stop-cock *h* must now be closed and *g* and *g'* disconnected to allow of the expulsion of the air from *a*. This having been accomplished, *g* and *g'* are again brought together and carefully secured. The whole apparatus is now full of mercury; as soon as it has been ascertained that the joint is air-tight at all pressures, it is ready for use. Before proceeding further, however, the measuring tube, which, as already stated, is graduated in millimetres measured from an arbitrary zero line near the bottom, must be calibrated. In other words, it must be ascertained as regards each principal mark of the

[1] Bunsen's *Gasometry*, translated by Roscoe.
[2] *Handbook for the Physiological Laboratory*, pp. 202—207.

graduation, what volume of air or water (as the case may be) the tube contains, when the upper convex surface of the mercury stands exactly level with it. For this purpose the orifice *a* is connected by means of an

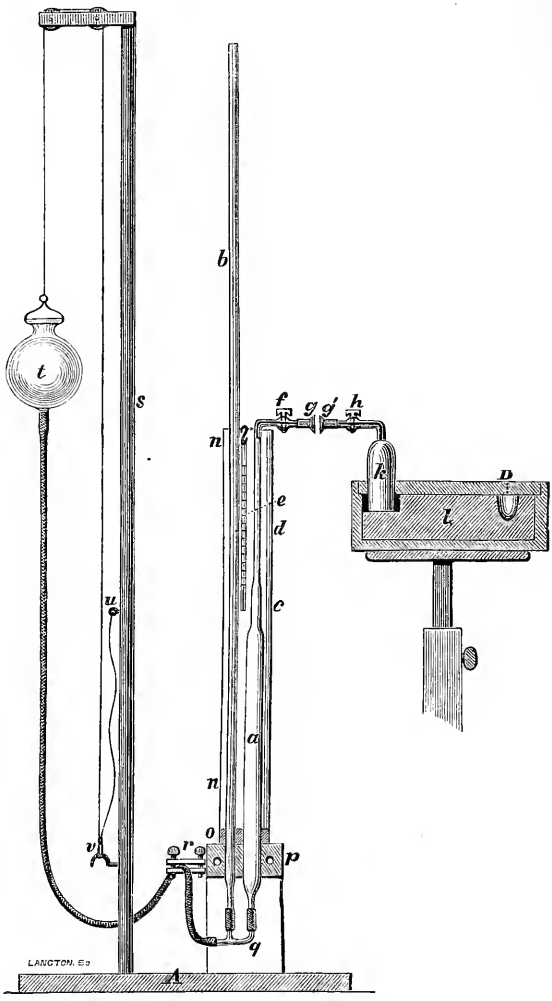


FIG. 46. FRANKLAND'S SMALLER APPARATUS FOR THE ANALYSIS OF GASES BY THE USE OF LIQUID REAGENTS. (From Sutton's *Volumetric Analysis*.)

india-rubber tube with a reservoir (a funnel) containing distilled water. The mercurial column is then allowed to descend until it stands exactly at zero. A weighed beaker having been then placed under *a*, water is expelled till the column stands at a height of fifty millimetres, and the beaker again weighed. In a similar manner the outflow of the water corresponding to a rise

of the mercurial column from fifty to one hundred millimetres is determined, until the capacity which corresponds to each fifty millimetres of the scale is ascertained. To ensure accuracy, the process must be repeated several times. If the results, after correction for difference of temperature, are in close accordance, the means may then be taken as expressing the capacities required. In the upper part of the tube, calibration must be made at shorter intervals. In calibrating, as in all subsequent measurements, the height of the column must be read horizontally through a telescope, so adjusted that its axis is at the same height as the surface of the mercury. The temperature is read by a thermometer suspended in the cylinder of water by which the barometer and measuring tube are surrounded.

"The measuring and laboratory tubes having been brought into connection in the manner described above, and both filled with mercury, the gas to be analysed is introduced into the laboratory tube from the test-tube in which it has been collected. It is then at once transferred to the measuring tube by depressing t until the mercury rises in the laboratory tube as far as the stop-cock g'. This done, the stop-cock g is closed, and t raised or depressed till the column stands at one of the marks of the graduation, in reference to which the capacity of the tube has been determined. The temperature is then observed, and the pressure determined by adding the difference between the height of the column in the measuring tube and that in the pressure tube, to the reading of a barometer which stands by. A few drops of solution of caustic potash having been introduced into the laboratory tube, the gas is returned from the measuring tube. Absorption takes place rapidly. It is accelerated by slightly agitating the trough, and by allowing the mercury to stream into the laboratory tube after the gas has passed. The measurement of the gas after absorption is performed in the same manner as before. About half a centimetre of strong solution of pyrogallic acid is then introduced in the same way as the potash, and the gas again returned. After absorption of the oxygen, what remains is nitrogen. In analysis of blood gases, the proportion of nitrogen is nearly constant, viz. about 2·5 volumes in 100 volumes of blood. If a larger quantity is obtained, the fact indicates that air has entered. Whatever method of analysis is employed, the results must be reduced to 0° temperature and 760 millimetres pressure—*i.e.* they must be expressed as if the measurements had been made under those conditions. A further deduction must be made from each measurement in respect of the aqueous vapour which the gas contains (the measuring tube being always moist). This is accomplished by the following well-known formula:—

$$V = \frac{V'}{1 + t\,0{\cdot}00367} \cdot \frac{H' - f}{760}.$$

V denotes the corrected volume; V' the volume read; t the temperature; H' the observed pressure; and f the tension of aqueous vapour at the temperature t. The values of $1 + t\,0{\cdot}00367$ and f are always obtained from tables. For these and many other important practical details relating to the performance of gas analysis, the reader is referred to Mr Sutton's 'Volumetrical Analysis,' whom I have to thank for two of the woodcuts with which this section is illustrated. To illustrate the application of the method to the analysis of the gases of the blood, I give the following example:—

"ANALYSIS OF GASES OF ARTERIAL BLOOD OF DOG.

	1st Measurement. Total Quantity of Gas Extracted.	2nd Measurement. After Absorption of Carbonic Acid Gas.	3rd Measurement. After Absorption of Oxygen.
Height of Column in Measuring-tube	230·0	270·0	450·0
" " Pressure-tube	312·8	369·0	320·0
Difference	82·8	99·0	−130·0
Reading of Barometer	764·0	764·0	764·0
H′ =	846·8	863·0	634·0
Temperature = 19·8° C. = t			
Tension of Aqueous Vapours from table = f =	17·2	17·2	17·2
H′ − f =	829·6	845·8	616·8
Volume of Gas as measured in cubic centimetres = V′ =	11·822	3·865	0·562

$1 + t\ 0{\cdot}00367$ (from table) $= 1{\cdot}0725$.

Hence from first measurement we have—

$$V = \frac{11{\cdot}822}{1{\cdot}0725} \cdot \frac{829{\cdot}6}{760} = 12{\cdot}030.$$

From second measurement—

$$V = \frac{3{\cdot}865}{1{\cdot}0725} \cdot \frac{845{\cdot}8}{760} = 4{\cdot}010.$$

From third measurement—

$$V = \frac{0{\cdot}562}{1{\cdot}0725} \cdot \frac{616{\cdot}8}{760} = 0{\cdot}425.$$

Thus the total volume of gases obtained as measured at 0° C. and 760 mm. was 12·030 cubic centimetres: of carbonic acid gas was 12·030 − 4·010 = 8·02 c.c.; of oxygen 4·010 − 0·425 = 3·585 c.c.; and of nitrogen 0·425 c.c.

As the volume of blood employed was 20·266 cubic centimetres, we have the following final result:—

In 100 volumes of blood—

Carbonic acid gas	39·585	volumes	$\left(= \frac{8{\cdot}020}{0{\cdot}20266} \text{ vols.}\right)$
Oxygen	17·695	"	$\left(= \frac{3{\cdot}585}{0{\cdot}20266} \text{ vols.}\right)$
Nitrogen	2·09	"	$\left(= \frac{0{\cdot}425}{0{\cdot}20266} \text{ vols.}\right)$
Total	59·370	"	$\left(= \frac{12{\cdot}030}{0{\cdot}20266} \text{ vols.}\right)$

"In the preceding example such variations of temperature and barometric pressure as may occur during the analysis are disregarded. The readings are taken immediately after the absorption of the carbonic acid gas; as the time occupied in the analysis up to this point is very short, the error arising from the variations in question is inconsiderable. As regards the absorption of oxygen, the error might be of more consequence, were it not that the residue of nitrogen is so small. As it is, it can be easily shewn that it would require a difference of pressure amounting to three millimetres, and a difference of a degree of temperature, to make an error of one-hundredth of a percentage in the result as regards nitrogen or oxygen. Within these limits, therefore, the errors arising from this source may be regarded as trivial.

Frankland's larger apparatus for the analysis of gases by eudiometric as well as absorptiometric methods.

"Although determinations of oxygen made by absorption with hydrate of potash and pyrogallic acid are not entirely free from objection on the score of accuracy, the results obtained by the method above described are quite accurate enough for most of the purposes of physiological research, for the small errors are practically inappreciable, as compared with the variations in the proportion of oxygen contained in the blood to be analysed, produced by what might be regarded as very trifling differences in the mode of collecting it. If it is desired to have recourse to explosion with hydrogen, the best methods for the purpose are those of Dr W. Russell and of Frankland and Ward. The following short description of the latter will be readily understood from what has preceded. The apparatus (Fig. 46*a*) consists of two parts, corresponding to the laboratory tube and measuring tube of the instrument previously described. The measuring tube (*F*, Fig. 46*a*) communicates, as in that instrument, with a second tube (*H*, Fig. 46*a*), containing a column of mercury, by the height of which the pressure to which the gas to be measured is subjected can be estimated. The chief difference is that, whereas in the former more simple instrument the pressure tube is open at the top, so that if air is contained in the measuring tube, and the stop-cock by which it communicates with the laboratory tube is closed, the difference between the heights of the two columns indicates the difference between the tension of the gas in the measuring tube and that of the atmosphere—in the instrument now before us the tube is closed and constitutes a barometer, so that the difference expresses the actual tension of the gas in inches of mercury. In the horizontal channel, by which the measuring tube and barometer communicate at the bottom, is a three-way stop-cock (not shewn in the figure), by which they may be brought into communication either with a vertical escape tube, the end of which dips into a receptacle containing mercury several feet below, or with a tube open at the top (*G*, the middle and longest in the figure), called the filling tube. In this way the gas can be expanded or compressed at the will of the operator, and consequently can (in most analyses) be readily brought to the same volume after each successive operation. The convenience of this is very great, for obviously the tensions of different quantities of gas when expanded to the same volume are proportional to the volumes they would assume if they were all under the same pressure, so that the original volume of gas to be analyzed being known, the relation between that volume and the volume of the other quantities to be measured can be readily calculated, the several volumes being proportional to the

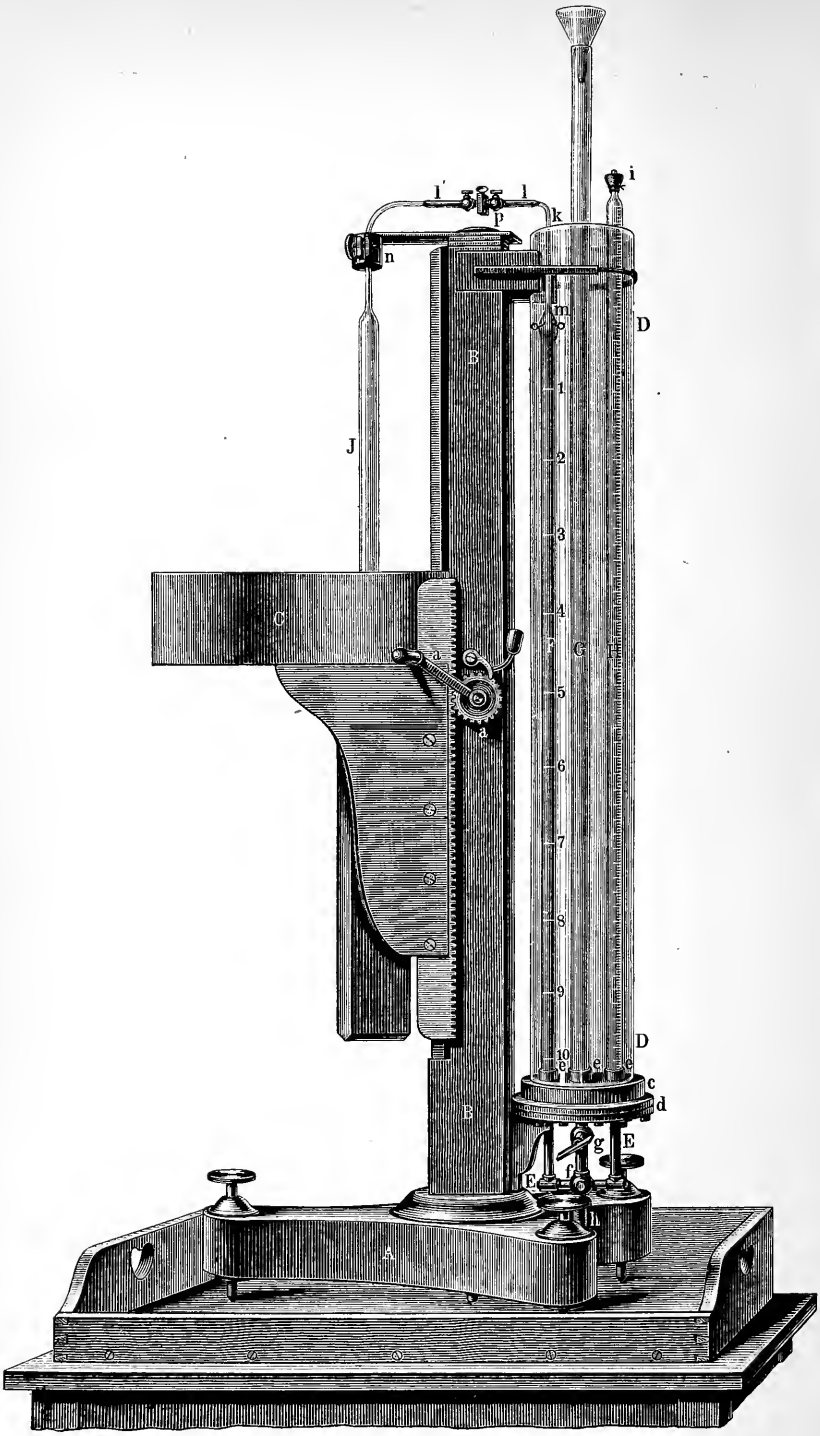


FIG. 46*a*. FRANKLAND'S LARGER APPARATUS FOR THE ANALYSIS OF GASES.

corresponding readings of the barometer. The original volume of gas to be analyzed is measured as before described, with this difference, that the absolute pressure to which it is exposed is known without reference to the barometric pressure outside at the time. The explosion is effected in the eudiometer, into the upper end of which two platinum wires are fixed for the purpose; the arrangement of these wires is the same as in Bunsen's eudiometer. As to the mode of preparing and introducing pure hydrogen and of exploding the mixture, the reader will find sufficient information in Roscoe's translation of Bunsen's Gasometry."

Description of more simple methods of gas analysis.

For purposes of demonstration it is sometimes convenient to employ the following expeditious and far from inaccurate method.

The tubes for collecting the gases which are strongly recommended are represented in Fig. 47. They are about 250 millimetres long, and are

FIG. 47. ABSORPTION TUBE[1], WITH DOUBLE SCALE, AS MADE BY ALVERGNIAT. (Scale about $\frac{1}{2}$.)

FIG. 48. IRON SPOON EMPLOYED IN TRANSFERRING TUBES FROM ONE MERCURIAL TROUGH TO ANOTHER. (Scale about $\frac{1}{5}$.)

[1] These tubes are constructed by MM. Alvergniat frères, 10 et 12 Rue de la Sorbonne, Paris. From personal observation, the Author can testify to their accurate calibration.

provided with two scales placed side by side, one of which indicates volumes in tenths of a cubic centimetre, the other is divided into millimetres. Further, these tubes are very much constricted at their upper part, so that exceedingly minute quantities of gas can be very accurately measured; this device renders the tubes of special value in the determination of the gases of the blood, as the volume of nitrogen which has to be read off is always very small.

We shall suppose, then, that the experimenter has, by employing Alvergniat's pump, collected the gases given off from a known volume of the blood which he is analyzing, in such a graduated tube, the walls of which have been moistened by a drop of water. He now transfers the

FIG. 49. WELL-SHAPED PNEUMATIC TROUGH FOR MERCURY. (Scale about $\frac{1}{12}$.)

FIG. 50. PIPETTE WITH BULB, FOR INTRODUCING LIQUID REAGENTS INTO ABSORPTION TUBES STANDING OVER MERCURY. (Scale about $\frac{1}{2}$.)

tube with its contents to the mercurial trough having the form shewn in Fig. 49; the transference being effected by means of the iron spoon shewn in Fig. 48. The tube is then fixed in a clamp and plunged into the mercury so that the level of the metal inside and outside the tube is exactly the same; it should be left for an hour, and a second observation made to see whether the level is still the same. If any change has occurred the tube is again adjusted and the volume of the gas is read off, either by the unaided eye, or still better by means of a telescope magnifying a few diameters and situated at a distance of a few feet from the tube.

The observer then reads the thermometer and barometer, and thus obtains the data for calculating the total quantity of gas given off by the volume of blood which he has analyzed.

With the aid of a pipette such as is shewn in Fig. 50, the observer now throws up into the tube about half a cubic centimetre of solution

of caustic potash of sp. gr. 1·2, taking care that not a trace of air be introduced; by unclamping the tube and alternately raising and depressing the tube in the mercury the absorption of CO_2 by the caustic potash is much facilitated. After absorption appears to be complete, the tube is again adjusted, so that the level of mercury inside and outside is the same, and the volume of gas determined as before. If we subtract this volume from that of the original gas the amount of carbon dioxide is ascertained.

About half a c.c. of a strong solution of pyrogallic acid (1 part of acid to 8 of water) is now introduced into the tube, and the process of shaking, &c., repeated. After the oxygen is absorbed the absorption tube is transferred to a vessel containing water, and the level of the liquid inside and outside of the tube being the same, the volume of gas is read as before; the gas consists entirely of nitrogen, and by subtracting its amount from that of the gas remaining after the absorption of CO_2, we determine the quantity of oxygen which was present.

It will be apparent to the reader that carried out as above there are certain inherent errors which it is not easy to eliminate; the method may be rendered more accurate, however, by absorbing the carbonic acid by a ball of caustic potash *fused* on platinum wire, then, after determining the volume, absorbing the oxygen by solution of caustic potash and pyrogallic acid, and after complete absorption transferring the tube which contains the residual gas to a trough containing water, and reading the nitrogen over water; or, again, after absorption of the CO_2 by the ball of caustic potash, the oxygen may be absorbed by a bullet of phosphorus left in the gas for 24 hours; in this case, however, before reading off the volume, a bullet of caustic potash must be introduced into the gas and left for some hours, and the residual gas read as dry.

Determination of the total quantity of blood contained in an animal's body.

Welcker's method. A tube is tied into the carotid of the animal whilst yet alive, and a few cubic centimetres of blood collected, defibrinated, measured, and set aside (portion A). The animal is then bled to death, the whole of the blood defibrinated and kept (portion B). The blood-vessels are then washed out with normal salt solution until the washings issue quite colourless; these are added to portion B, and the whole mixed and measured. Some of the red solution thus obtained is placed in a haematinometer, which we shall designate as H.B.; a small quantity of A is then diluted with 10 times its volume of distilled water, and an accurately measured volume (one or two cubic centimetres) is placed in a second haematinometer (H.A.) placed by the side of the first and illuminated in exactly the same manner.

Distilled water is now added from a burette to the contents of H.A. until their tint is exactly equal to the fluid in H.B.; when equality is obtained, the volume of water added is read off, and thus is found the volume of the solution of pure blood which was equal to the previously unknown mixture of portion B and washings. By simple calculations, of which the steps are perfectly obvious, we can

then find the amount of blood which the washings contain, which added to the volume of A gives the total volume of blood contained in the body.

This method may be modified in various ways. Thus the amount of haemoglobin in the fluid may be determined by Preyer's method; or the tissues and organs may be chopped up finely and treated with water, and the fluid thus obtained after being filtered may be added to the washings from the blood-vessels; or carbonic oxide may be passed through the blood and through the mixture of blood and water, so as to secure a fluid of more uniform and more persistent tint.

The following determinations of the relation of volume of blood to weight of body have been made by these methods.

VOLUME OF BLOOD, EXPRESSED AS A FRACTION OF THE BODY WEIGHT, CONTAINED IN THE BODY OF VARIOUS ANIMALS. (GSCHEIDLEN[1].)

	According to				
	Welcker.	Heidenhain.	Gscheidlen.	Panum.	Spiegelberg and Gscheidlen.
Guinea-pig .			$\frac{1}{17}$ to $\frac{1}{22}$		
Rabbit . .		$\frac{1}{15}$ to $\frac{1}{20}$	$\frac{1}{17}$ to $\frac{1}{22}$		
Dog . . .		$\frac{1}{12}$ to $\frac{1}{18}$		$\frac{1}{11}$ to $\frac{1}{12}$	$\frac{1}{11}$ to $\frac{1}{14}$
Cat . . .	$\frac{1}{15}$				

Malassez' method. It is obvious that by the enumeration of blood corpuscles in blood diluted to a known extent, and in the mixture of blood and washings, the amount of blood contained in an animal's body could also be ascertained, though doubtless not so accurately as by Welcker's method.

Malassez[2] has actually attempted to determine the total mass of the blood of a living man by the process of enumeration. The number of blood corpuscles contained in a cubic millimetre of blood obtained from the finger having been determined as exactly as possible, 300 cubic centimetres of blood were removed by venesection. Some hours afterwards, the corpuscles in blood again drawn from the

[1] Gscheidlen, *Physiologisches Methodik*, Dritte Lieferung, p. 337. On this subject consult also

Gscheidlen, "Studien über die Blutmenge." *Untersuchungen aus dem physiolog. Laboratorium zu Würzburg*, Vol. II. p. 153 (1869).

Gscheidlen, "Bemerkungen zu der Welcker'schen Methode der Blutbestimmung und der Blutmenge einiger Säugethiere." Pflüger's *Archiv*, Vol. VII. (1873) p. 544.

Welcker, "Bestimmung der Menge des Körperblutes und der Blutfärbskraft," &c. *Zeitschrift f. rat. Medicin.* 3rd Series, Vol. IV. (1858) p. 147.

[2] Malassez, "Recherches sur quelques variations que présente la masse totale du sang." *Archives de Physiologie normale et pathologique.* 2nd series. Vol. II. (1875). Consult especially pp. 277—280.

finger were counted. By assuming that in the interval which had elapsed between the venesection and the second enumeration the volume of blood had become exactly the same as it had been at the time of the first enumeration, and further that no formation of new corpuscles had taken place in the same period, Malassez obtained data for calculating the total mass of the blood.

It is obvious however that these assumptions are altogether unwarrantable, and if in one case they led to a result not far removed from the truth, such was a mere result of chance.

Medico-Legal Detection of Blood-Stains.

Detection of blood cells by microscope.

It not unfrequently happens that the medical jurist is asked to decide whether a certain stain upon clothes, weapons, floors, &c. is a stain of blood. When recent, the identification of a blood-stain presents no difficulties. By moistening it with diluted glycerine of specific gravity 1025 and, after some time, expressing the liquid, we may obtain microscopic evidence of the presence of blood corpuscles; when such is the case the observer may be able to state positively that the blood was or was not the blood of a mammal, but cannot venture upon any more definite expression of opinion.

Chemical reaction of chief blood constituents in stain.

Whether successful or not in the detection of blood corpuscles, it is always desirable to obtain the chemical proofs of the presence of blood; and with proper treatment this is possible even with blood-stains of considerable antiquity and of small size.

We shall suppose that the observer is examining a cloth stained with blood; having selected the particular stain which he wishes to examine he may, with pencil, draw a circle around it and mark the circle with a letter or number, for purposes of identification and description. He then will proceed to cut out the stain and to pass a thread through it; the blood-stained piece of cloth is then suspended in a very small test-tube containing a few drops of distilled water; the size of the tube must depend upon the estimate which the experimenter forms of the amount of blood in the stain. The piece of stained cloth is left to soak for one or two hours, at the end of which time the water will usually have acquired a more or less distinctly red colouration. By the aid of the thread which had been attached to it the little piece of cloth is now withdrawn from the water and pressed with a small glass rod against the upper part of the test-tube so as to squeeze out the liquid which it had imbibed. A small quantity of the liquid *may be* examined in a small cell with the aid of the microspectroscope; but only when the examination is carried on by a person who has by considerable practice familiarized himself with the use of the instrument and with the various absorption spectra of colouring matters.

In recognizing blood by means of the spectroscope the observer endeavours to obtain a succession of characteristic spectra; even when haemoglobin has been decomposed and the stain contains methaemoglobin or haematin a satisfactory series of spectrum observations may be made[1].

If the quantity of red solution be sufficient, a few drops may be treated with solution of ammonia which will induce no change. The greater part of the liquid, or, if its quantity be small, the whole of it, is now heated to boiling; the red colour will disappear and a turbidity or coagulation will be observed to form, the coagulum having a dirty grey colour; on now adding a drop of a solution of caustic potash to the turbid liquid, this will be instantly cleared and the solution will be observed to be green by transmitted and red by reflected light; on adding a small drop of nitric acid the precipitate will be reproduced.

Guaiacum test. Another test which adds confirmatory evidence to that afforded by other means, and which is of extreme delicacy, rests upon the reaction developed by haemoglobin and its derivatives when brought in contact with guaiacum and hydric peroxide. To try this test it is best to moisten the stain with distilled water, and then to press a piece of white filtering paper firmly against it; a little of the colouring matter will adhere to the filtering paper. Having secured a slight stain on the filtering paper, this is moistened with a drop of tincture of guaiacum, and then with a few drops of an ethereal solution of peroxide of hydrogen. A beautiful blue colour will be developed if the stain is one of blood. It must be borne in memory, however, that this test cannot be relied upon by itself, though the evidence which it affords is valuable when taken in connection with other facts.

The Haemin test. In the case of very old blood-stains it may not only be impossible to obtain blood corpuscles for microscopic examination, but even to obtain a solution containing the colouring matter and proteids of the blood. In such a case the *haemin-test* is of special value. This test is based upon the fact that when haemoglobin or haematin are heated with glacial acetic acid and common salt, a hydrochlorate of haematin is formed, which, on evaporation, is deposited in the form of reddish brown prisms, the so-called *haemin-crystals*. The test is one of great delicacy and the result is remarkably free from fallacy. The blood-stain, having been cut out, is placed with a few drops of *glacial* acetic acid and a very minute (indeed scarcely perceptible) crystal of common salt, in a watch-glass, which is then heated to boiling over a spirit-lamp flame. The liquid will soon assume a brownish red tint; the little piece of cloth may then be squeezed with a rod against the side

[1] Consult Sorby, "On some improvements in the spectrum method of detecting blood." *Monthly Microscopical Journal*, Vol. VI. (1871) p. 9. Also MacMunn, *The Spectroscope in Medicine*. London, Churchill, 1880.

of the watch-glass, and the liquid is evaporated to dryness. The watch-glass is then examined with a magnifying power of about 350 diameters. If no crystals are perceptible, more acetic acid may be added and the process of boiling and evaporation repeated. If present, the crystals present the appearance shewn in Fig. 24 (page 115).

Medico-Legal Detection of Carbonic Oxide in Blood.

As was mentioned at page 105, carbonic oxide expels the oxygen from oxy-haemoglobin and forms a more stable compound, which is not affected by the alkaline reducing solutions which readily reduce oxy-haemoglobin. Blood of animals poisoned with carbonic oxide, if nearly saturated with the gas, presents a remarkably persistent vermilion colouration; if not saturated, the colour may not be very distinctly affected.

The action of a solution of caustic soda of specific gravity 1·3 establishes a very remarkable difference between CO-blood and normal blood[1]. This reagent when added to normal blood converts it into a black, slimy, mass, which when spread in thin layers over a porcelain capsule appears of a greenish brown colour; blood which has absorbed carbonic oxide presents, on the contrary, after treatment with its own volume of the solution of caustic soda, the appearance of a firmly coagulated mass, and, when spread on porcelain, appears of a cinnabar-red colour.

It has been recommended that, instead of employing a simple solution of caustic soda, a mixture of two parts of a solution of caustic soda of sp. gr. 1·3, and $2\frac{1}{2}$ parts of a solution of chloride of calcium in water (1 to 3), should be rubbed up with the blood in a porcelain capsule, fifteen or twenty drops being sufficient for the reaction[2].

A more conclusive proof of the presence of carbonic oxide is obtained with the aid of the spectroscope. The suspected blood is suitably diluted so as to exhibit with perfect distinctness the two absorption bands of O_2-Hb or CO-Hb. Then a small quantity of Stokes's reagent (ammoniacal solution of ferrous tartrate or citrate) is added. In the event of the blood containing carbonic oxide the two bands will not wholly fade, but will persist more or less distinctly. When the blood is saturated with carbonic oxide the spectrum undergoes no perceptible change under the influence of the reducing solution.

[1] Hoppe, Virchow's *Archiv*, Vol. xi. Heft 3 (1857), p. 288.

[2] Eulenberg, *Die Lehre von den schädlichen und giftigen Gasen.* Braunschweig, 1865, p. 48.

CHAPTER V.

THE LYMPH AND CHYLE. THE SO-CALLED TRANSUDATIONS, NORMAL AND PATHOLOGICAL.

Sec. 1. The Lymph (including the Chyle).

Preliminary Observations.

On the nature of the Lymph.

As the blood circulates through the capillaries of the body there is a continual transudation, through their walls, of water holding in solution organic, mineral, and gaseous constituents, which are destined for the nutrition of the elements of the tissues. This nutritive fluid bathes the tissue elements, and is the agent which supplies them directly with the matters which they require for their maintenance and repair, whilst, at the same time, it removes from them soluble effete matters which would, if accumulating, impair the functional activity of the tissues in which they have been formed. The fluid which has transuded from the blood-vessels finds its way into the minutest radicles of the lymphatic system, and is then carried, sooner or later, to lymphatic glands, and through them into larger lymphatics which ultimately empty their contents into the large venous trunks in proximity to the heart. The term *lymph*, although usually applied to the liquid contained in the lymphatic vessels, is also applicable to the fluid which is found in those extra-vascular spaces from which the lymphatics originate, or with which they communicate—to the fluid, for instance, which bathes the lacunae of connective tissue, or which moistens the interior of the great serous sacs. Inasmuch as divers organs take from the fluid transuded by the blood different quantities of organic, saline, and gaseous constituents, according to their wants, and produce different kinds and different quantities of effete products, it follows that the lymph must be a liquid which varies materially in composition, according to the region from which it is derived, and according to the greater or less functional activity of the organs contributing to it.

Chyle is the term applied to the lymph contained in the lymphatics of the small intestine during digestion.

Whilst the lymphatics generally contain a liquid which must be looked upon as a diluted *liquor sanguinis*, deprived of a small fraction of certain of its constituents, and augmented by certain other constituents, such as urea or carbonic acid, which are the effete products of tissue metabolism, certain of the lymphatics—those of the small intestine—contain, during the period of digestion, lymph which is laden with suspended fatty matter in a fine state of division, and which gives to the fluid a milky appearance. The fatty matter has passed from the interior of the alimentary canal through, or between, the cylindrical epithelial cells of the villi into the sub-epithelial connective tissue, whence it has made its way into the commencements of the so-called *lacteals*, as the absorbents of the intestinal villi are called. Chyle is therefore the lymph of the small intestine laden with fat whilst the absorption of that substance is proceeding. The *Chyle* will be considered in detail in relation to the functions of Digestion and Assimilation.

Circumstances which influence the quantity of Lymph and Chyle.

The amount of lymph which is discharged by the lymphatics of a part is much increased by muscular contractions and passive movements of the part. When the arterial pressure is increased the amount of lymph diminishes. When an obstruction to venous circulation exists the amount of lymph increases. Poisoning with curare increases the discharge of lymph. The amount of chyle is materially increased by the digestion of food rich in fatty matters.

Mode of obtaining Lymph.

Small quantities of lymph for microscopic examination may be obtained by puncturing the subcutaneous dorsal lymph-sac of the frog, and aspirating with a capillary pipette.

When large quantities of lymph are required they may be obtained by tying a glass cannula into the thoracic duct of a deeply anaesthetized animal, at the spot where that tube empties itself into the junction of the large veins at the root of the neck[1].

In large animals, such as the horse and ox, a cannula may be tied into one of the large cervical lymphatics accompanying the carotid artery.

For purposes of demonstration small quantities of lymph may be obtained from the thoracic duct of a recently killed animal.

Physical characters of the Lymph.

Colour, and microscopic characters.

When freshly drawn from the thoracic duct of fasting animals the lymph is a transparent liquid, sometimes of a slight yellow colour; when obtained from an animal during the period of digestion, it presents a more or less milky colour owing to the absorption of fatty matters from the alimentary canal.

[1] This method was followed by Dogiel and by Hammarsten, in their researches on the gases of lymph, conducted in the Leipzig Laboratory under the direction of Professor Ludwig. (See 'Gases of Lymph,' p. 225.)

On microscopic examination, the transparent lymph of fasting animals presents colourless corpuscles—*lymph-corpuscles*, identical with the colourless corpuscles of the blood, floating in a clear liquid, *the lymph-plasma;* mixed with these, a few coloured corpuscles are often observed, even though great precautions have been taken to prevent the admixture, with the lymph, of blood from wounded blood-vessels.

It is certain that the lymph corpuscles are comparatively scanty in the radicles of the lymphatic system, and that they are increased in number as the lymph passes through the lymphatic glands. These glands are the chief, though not the exclusive, formers of the lymph cells, for wherever lymphoid or adenoid connective tissue exists, as for instance in the mucous membrane of the stomach and intestines (of which it forms almost the frame-work) or in the follicles of the thymus, of the tonsils, of the spleen (Malpighian bodies), there is doubtless a formation of lymph cells. It is, indeed, the wide distribution of adenoid connective tissue, especially in the alimentary canal, which accounts in great part for the fact, that the lymph of the smallest lymphatics always contains some corpuscles, though some of these are doubtless derived from the blood, and have wandered through the capillary walls into the cell spaces of the connective tissue, and so found their way into the lymphatics.

The lymph of animals in active digestion is milky from admixture with the fatty chyle. It exhibits under the microscope, what has been termed *a molecular basis, i.e.* innumerable very finely divided particles, mainly fatty in nature, which manifest very characteristic Brownian movements.

Reaction. The Lymph has an alkaline reaction, which is, however, less marked than that of the blood.

Taste and Smell. Its taste is saltish, and it has a slight indefinite odour which varies somewhat in different animals.

Specific Gravity. The statements of authors vary in regard to the specific gravity. According to Owen Rees and Marcet the specific gravity varies between 1012 and 1022.

Coagulation of Lymph. In a time which varies between 3 and 20 minutes after it has left the vessels, the lymph undergoes coagulation which is identical with that of liquor sanguinis. A soft trembling jelly is at first formed, and after some time a contracted colourless coagulum floats in a colourless or yellowish liquid, which we may term the *lymph-serum.*

The fibrin which separates from coagulated lymph is identical with that of blood. Very great differences exist in the rate of coagulation of lymph. As a rule lymph which is flowing rapidly coagulates less rapidly than lymph which is flowing slowly; there is no rule to be laid down however. Some lymph does not coagulate at all[1].

[1] Ludwig, quoted by Gorup-Besanez, *Lehrbuch*, &c., p. 378.

The Proteids of the Lymph.

These consist of fibrinogen, of a globulin presumedly identical with serum-globulin, and of serum-albumin.

The amount of fibrin which separates from the lymph varies between 0·4 and 0·8 per 1000, being, therefore, much less in quantity than that which separates from the blood. Accurate data are wanting in reference to the amount of globulin, over and above the fibrinogen, which the lymph contains.

The amount of serum-albumin, found in different specimens of lymph, appears to have varied within wide limits, probably between 21 and 60 parts per 1000.

From certain observations of Wurtz it would appear that lymph yields only about one-fourth of the amount of fibrin which is furnished by the liquor sanguinis, and that it contains rather less than half the amount of serum-albumin contained in that fluid.

The Fats of the Lymph and Chyle.

The amount of fatty matters in the lymph of fasting animals is small. Gubler and Quevenne on one occasion found the lymph obtained from a lymphatic fistula in the leg of a woman, to contain 9·2 parts of fat per 1000, but this perhaps represents the highest limit. In most analyses of lymph, the amount of fat found has been smaller. In the chyle the amount of fat is immensely greater. In his recent researches on the absorption of fat and its passage through the thoracic duct, Zawilski[1] has found that the fluid obtained from the thoracic duct of animals fed upon a purely fatty diet may contain the enormous proportion of 14·6 per cent. of fat, viz. about three times as much fat as average milk. Under the heading of fats are, however, included certain bodies which are not properly fats, viz lecithin and cholesterin. Hoppe-Seyler analysed the ether extract of chyle obtained from a fistula in the human subject and found it to have the following composition:—

In 1000 parts of the ether-extract.

	1st portion.	2nd portion.
Cholesterin . . .	113·2	140·9
Lecithin	75·4	88·4
Olein	381·3	
Palmitin and Stearin	430·1	770·7

The Extractive matters of Lymph.

Like the other constituents of the lymph, the so-called extractive matters vary very greatly in proportion in different specimens. The best known of these extractive matters are sugar and urea, though others, such as lactic acid, leucine and tyrosine have been discovered.

[1] Zawilski, "Dauer und Umfang des Fettstromes durch den Brustgang nach Fettgenuss." Ludwig's *Arbeiten*, Vol. xi. (1876) p. 147—167.

Sugar present in the lymph and chyle.

It has long been known[1] that the lymph contains sugar, and it has lately been shewn by v. Mehring[2] that the amount of sugar in the lymph is approximately the same as in the blood. It had been stated by Bernard that the lymph of the intestinal tract (chyle) does not take up sugar when animals are fed upon a starchy or saccharine diet, and the statement is confirmed by v. Mehring.

Urea present in the lymph.

Urea is a constant ingredient of the lymph and chyle, as was first pointed out by Wurtz[3]. The amount of urea, like that of sugar, appears to be the same in the lymph and blood.

The following are the results obtained by Wurtz; although, owing to the method employed, the amount of urea found was much below the actual amount, the observations are doubtless comparable with each other.

QUANTITY OF UREA FOUND IN 100 PARTS OF BLOOD, LYMPH AND CHYLE.

Animal.	Blood.	Lymph.	Chyle.
Dog	0·009	0·016	
Cow	0·019	0·019	0·019
Horse.....		0 012	
Bull		0·021	0·019

Other extractives present in the lymph and chyle.

According to Lehmann the chyle of the horse contains alkaline lactates, and according to Frerichs and Staedeler, leucine and tyrosine are also present in lymph; no definite information on these subjects is yet available.

The Salts of the Lymph.

Like the other constituents of the lymph, the salts vary considerably in proportion according as the fluid is more or less rich in water.

The salts are relatively much more abundant than the organic solids, so that we may say that in transuding through the walls of the blood-vessels, the liquor sanguinis furnishes to the lymph a small quantity of its fibrinogen, about one-half of its serum-albumin, and a much larger proportion of its salts.

The composition of the salts of the lymph and chyle appears to be the same as that of the salts of the liquor sanguinis, in both cases sodium chloride constituting the overwhelming constituent.

[1] Gubler and Quevenne, *Comptes Rendus*, Vol. XLVI. p. 677.

[2] v. Mehring, "Ueber die Abzugswege des Zuckers aus der Darmhöhle." Ludwig's *Arbeiten*, 1877.

[3] Wurtz, *Comptes Rendus*, July, 1859.

The Gases of the Lymph.

The lymph contains carbonic acid, nitrogen, with traces of oxygen, all removable by the mercurial pump. The composition of the gases of the lymph, especially the proportion and condition of the CO_2 contained in that liquid, has formed the subject of elaborate investigation in the laboratories of Leipzig and Bonn, because of the light which the investigation promised to throw on the seat of the processes of oxidation in the economy. In discussing that question, in another section of this work, we shall again revert to the conclusions which have been drawn from the study of the gases of the lymph, though we think it right to give a systematic account of these in this place.

The first researches were made in the Leipzig laboratory, under Professor Ludwig's direction, by Hammarsten[1]. They shewed that pure lymph, unmixed with blood, contains either no oxygen or mere traces of that gas; that it contains CO_2 in quantity greater than is contained in arterial, but smaller than is contained in venous blood; that it contains about the same quantity of N as is present in the blood. The following are some of the actual results obtained by Hammarsten.

VOLUMES OF GASES (MEASURED AT 0° C. AND 760 MM. PRESSURE) YIELDED BY 100 VOLUMES OF LYMPH, OBTAINED FROM DIFFERENT LYMPHATIC VESSELS OF THE DOG. (HAMMARSTEN.)

		O	CO_2	N
I.	Lymph from the left foreleg, quite free from blood	0·00	41·89	1·12
II.	do.	0·10	47·13	1·58
III.	do.	0·00	44·07	1·22
IV.	Lymph from the thoracic duct	0·10	37·55	1·63
V.	The same lymph as IV. after being kept for 24 hours in ice	0·05	37·50	1·82
VI.	Lymph from the thoracic duct, containing a little haemoglobin	0·04	38·88	1·18

A second observer, Tschiriew[2], pursuing the same subject, under Ludwig's direction, obtained the following results, which shew the simultaneous composition of the gases of lymph, of blood, and of serum of blood, in dogs in an asphyxiated condition.

[1] Hammarsten, "Ueber die Gase der Hundelymphe." Ludwig's *Arbeiten*, 1871.
[2] Tschiriew, "Die Unterschiede der Blut- und Lymphgase des erstickten Thieres." Ludwig's *Arbeiten*, 1875.

VOLUMES OF GASES (MEASURED AT 0° C. AND 760 MM.) YIELDED BY 100 VOLUMES OF LYMPH, BLOOD AND SERUM (TSCHIRIEW).

I. *Dog not under the influence of curare, but asphyxiated.*

	O	CO_2	N
Lymph	0·01	42·06	0·79
Blood	0·04	42·78	1·70
Serum	0·09	48·38	0·56

II. *Same conditions as in* I.

	O	CO_2	N
Lymph	0·01	53·75	0·83
Blood	0·04	58·28	1·38
Serum	0·05	65·83	1·92

III. *Dog poisoned with curare, and asphyxiated.*

	O	CO_2	N
Lymph	0·01	41·25	1·38
Blood	1·11	45·18	1·84
Serum	0·13	50·78	1·50

A third observer, Buchner[1], continuing the observations of Tschiriew, found that in asphyxia, as the quantity of carbonic acid in the blood increased, that in the lymph diminished.

Tension of the CO_2 of Lymph.

From these researches, which do not, it is true, teach us the comparative tension of the gases of the lymph and blood, it was reasonable to come to the conclusion that probably the tension of the CO_2 of the lymph was smaller than that of the blood. Direct experiments made by Pflüger[2] and Strassburg[3] indeed shewed that the carbonic acid of the lymph has a tension, slightly but decidedly, less than that of the blood. According to the views which formerly at least were held by many very eminent physiologists, this result seemed to localize the formation of

[1] Buchner, "Die Kohlensäure in der Lymphe des athmenden und erstickten Thieres." Ludwig's *Arbeiten*, 1876.

[2] Pflüger, "Die Gase der Secrete." *Archiv f. die gesammte Physiologie*, Vol. II. (1869) p. 156.

[3] Strassburg, "Topographie der Gasspannungen im thierischen Organismus." Pflüger's *Archiv*, Vol. VI. pp. 65—96.

carbonic acid within the blood-vessels rather than in the tissues; if, it might be argued, CO_2 is formed in the tissues and passes into the blood, it can only do so in virtue of the CO_2 having a higher tension in the extra-vascular liquids than in the blood. The answer which has been given to this objection may be summarized as follows:— It is conceivable, and indeed most likely, that the tension of the CO_2 at the seats of its formation (in and near the anatomical elements of the tissues) may be much higher than that of the lymph. If instead of analysing the lymph we analyse the normal secretions of the body, such as the urine, bile, saliva, &c., which result more directly from the action of the anatomical elements, we shall be analysing liquids whose gaseous tension will, in all probability, more nearly represent that of the tissues which are the seat of the respiratory combustion. Now the tension of the CO_2 of these liquids is much higher than that of the lymph, and higher even than that of venous blood.

All difficulty in explaining the passage of carbonic acid into the blood has, however, been removed by the last investigation on the gases of the lymph made in the Leipzig laboratory. Gaule[1] has determined the comparative tension of the CO_2, of blood, lymph and serum, and has shewn that whilst the *quantity* of that gas in the serum is greater than in the lymph, the *tension* of the CO_2 is much greater in the lymph than in the serum. The same difference will doubtless hold between the tension of the lymph and the tension of the liquor sanguinis, and as we may consider the exchange of CO_2 to occur in the first place between those two liquids, its passage into the blood is easily accounted for.

The following are the results of one of Gaule's experiments:

PERCENTAGE OF CO_2, AND TENSION OF THE GAS, IN THE BLOOD-SERUM AND LYMPH OF AN ASPHYXIATED DOG.

	CO_2 in 100 vols.	Tension in mm. of Mercury. (Temp. 40° C.)
Blood	24·6	56·7
Serum	34·5	33·4
Lymph	25·5	52·1

This subject will again be referred to at length in discussing the Respiration of the Tissues.

[1] Gaule, "Die Kohlensäurespannung im Blut, im Serum und in der Lymphe." Ludwig's *Arbeiten*, 1878, and *Archiv für Physiologie* of Du Bois-Reymond, 1878, p. 469.

RESULTS OF THE QUANTITATIVE ANALYSES OF LYMPH AND CHYLE MADE BY VARIOUS OBSERVERS.

I. ANALYSES OF THE LYMPH OF MAN.

Constituents in 100 parts.	Gubler and Quevenne.		Marchand and Colberg.	Scherer.	Dähnhardt and Hensen.	Odenius and Lang.
	I.	II.	III.	IV.	V.	VI.
Water	93·99	93·48	96·93	95·76	98·63	94·36
Solid Matters	6·01	6·52	3·07	4·24	1·37	5·64
Fibrin	0·05	0·06	0·52	0·04	0·11	0·16
Albumin	4·27	4·28	0·43	3·47	0·23	2·12
Fat	0·38	0·92	0·26	—	0·15	2·48
Extractive Matters	0·57	0·44	0·31	—		0·16
Salts	0·73	0·82	1·54	0·73	0·88	0·72

II. ANALYSES OF THE LYMPH OBTAINED FROM THE LYMPHATICS OF THE HORSE (C. SCHMIDT).

Constituents in 1000 parts.	I.	II.
Water	963·93	955·36
Solid Matters	36·07	44·64
Fibrin, Albumin, Fats and fatty acids, Other organic matters	28·84	34·99
Inorganic matters	7·22	7·47
NaCl	5·43	5·67
Na_2O	1·50	1·27
K_2O	0·03	0·16
SO_3	0·03	0·09
P_2O_5 combined with alkalies	0·02	0·02
$Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$	0·22	0·26
In the serum from 1000 parts of Lymph Schmidt found:		
Albumin, Fats and fatty acids	23·32	30·59, 1·17
Other organic matters	4·48	1·69

III. ANALYSES OF CHYLE OF THE HORSE, DOG AND MAN[1].

Constituents in 1000 parts.	I. Chyle of Horse.	II. Chyle of Horse.	III. Blood-serum.	IV. Chyle of Dog.	V. Blood-serum of Dog IV.	VI. Chyle of Man.
Water	960·97	956·19	930·75	906·77	936·01	904·80
Solids	39·03	43·81	69·25	96·23	63·99	95·20
Fibrin	2·57	1·27	—	1·11	—	70·8
Albumin	22·60	29·85	56·59	21·05	45·24	
Fats Cholesterin and Lecithin	0·09	0·53	—	64·86	6·81	9·2
Fatty acids in the form of soaps	0·76	0·28	1·57	2·34	2·91	10·8
Other organic matters	5·37	2·24	3·85			
Haematin	0·05	0·06	—	—	—	—
Mineral salts	7·59	7·49	7·14	7·92	8·76	4·4
Loss	—	—	—	—	0·27	—
NaCl	5·76	5·84	5·74			
Na_2O	1·31	1·17	0·87			
K_2O		0·13	0·14			
SO_3	0·07	0·05	0·11			
P_2O_5	0·01	0·05	0·01			
$Ca_3(PO_4)_2$ $Mg_3(PO_4)_2$	0·44	0·25	0·26			
CO_2	1·02	0·82	0·56			

Sec. 2. The Liquids contained in the healthy serous sacs.—Synovia.—The Cerebro-spinal Liquid.

The internal surface of the serous sacs of the body, such as the pericardium, the peritoneum, the pleurae, &c., is, during life, moistened by a small quantity of a liquid which must be looked upon as lymph. These serous sacs are, indeed, in direct communication with lymphatic vessels, and offer the most highly differentiated examples of the lacunar origin of those vessels.

After death it is usual to find in certain of the serous sacs, especially in the pericardium, a small accumulation of the so-called *liquor pericardii;* its presence in them *in quantity* is, however, not to be considered as affording any ground for the belief that such accumulations exist during life, but is rather to be accounted for as due to the changes in the circulation which immediately precede

[1] This Table is extracted from Hoppe-Seyler's *Physiologische Chemie*, pp. 595 and 596. Analyses I., II. and III. are by C. Schmidt; IV. and V. are previously unpublished analyses by Hoppe-Seyler. VI. is the analysis of the chyle of a beheaded person.

death. Probably, in the most healthy condition, the serous sacs are, as was said above, merely moistened with lymph, the excess finding its way, as soon as it is formed, into the open mouths of the lymphatics. Our knowledge of the physical characters and chemical composition of the liquids of serous cavities is, therefore, almost entirely derived from their examination when increased in quantity, and will be fully referred to in the succeeding section of this chapter.

Synovia. The secretion of the synovial sacs of joints requires a special description, as it differs in some important particulars from the contents of the other serous sacs.

Synovia is a transparent, faintly yellow, slimy liquid, of alkaline reaction. It contains a larger proportion of solid matters than the fluid of other serous sacs, and is specially distinguished from them by containing *mucin*.

According to the observations of Frerichs[1] whose analyses of synovia are given below, the joints of animals which have been kept at rest furnished more synovia than those in active exercise; in the latter it is more concentrated.

ANALYSES OF SYNOVIA (FRERICHS).

Constituents in 1000 parts.	I. Synovia of a new-born Calf.	II. Synovia of a stall-fed Ox.	III. Synovia of an Ox at grass.
Water	965·7	969·9	948·5
Solid matters	34·3	30·1	51·5
Mucin	3·2	2·4	5·6
Albumin and Extractives	19·0	15·7	35·1
Fats	0·6	0·6	0·7
Inorganic Salts	10·6	11·3	9·9

Cerebro-spinal Liquid. Although not contained in a serous sac, the so-called cerebro-spinal liquid must be placed by the side of the liquids of serous cavities, inasmuch as it also is essentially identical with lymph. It is a liquid which is contained in the meshes of the sub-arachnoid connective tissue (as that tissue is called which lies between the arachnoid and dura mater) and in the ventricles of the brain, the latter being connected with the sub-arachnoid space by a narrow canal leading into the fourth ventricle, and sometimes termed the *foramen of Magendie*. A certain quantity of cerebro-spinal liquid, which probably never exceeds two ounces, is contained in the sub-arachnoid space during life, and permits of an equalization of intra-cranial pressure under different conditions of fullness of the cerebral blood-vessels.

Cerebro-spinal liquid is alkaline, of low specific gravity (about 1005), and usually does not coagulate distinctly when heated, though

[1] Frerichs, quoted by Gorup-Besanez, *Lehrbuch der phys. Chemie*. 4te Auflage, 1878.

it contains appreciable quantities of globulins. It contains a body which, like glucose, reduces cupric oxide, as was first pointed out by Professor Turner [1]. The cerebro-spinal liquid is occasionally much increased in quantity and the analyses of the liquid made under these circumstances will be considered in the next section.

Sec. 3. The Liquid in Dropsies.

Preliminary remarks on the mode of production of Dropsies.

It has been stated that the lymph consists of the liquid which has transuded from the capillaries and which brings into intimate contact with the anatomical elements of the tissues those elements of the blood which they need for their maintenance and repair.

Under normal circumstances, the composition of the blood, and the differences between the pressure in arteries and veins are so adjusted, that only as much liquid transudes from the blood-vessels as can find its way back to the venous system through the lymphatics. Two sets of circumstances may, however, arise to disturb the normal relation. Firstly, the composition of the blood may be so changed that the transudation from it into the tissues may increase very greatly. This is the case when the relative proportions of the water and proteids of the liquor sanguinis are disturbed, the former increasing and the latter diminishing.

Secondly, the normal difference between the arterial and venous pressure may be disturbed by an actual increase of the latter, as for example by some mechanical obstacle pressing upon large veins and diminishing their *lumen*, or by an obstacle to an easy passage of blood through the heart; or, locally, the normal difference in pressure may be disturbed by vaso-motor changes (as in local inflammations).

Under any of these circumstances, dropsical accumulations may result, *i.e.* accumulations of liquid which has transuded from the capillaries into extra-vascular spaces, and which cannot be carried back to the venous system by the lymphatics—of liquid which must be looked upon as lymph, modified though it is, no doubt, by the circumstances under which it has been formed. The dropsies which are due to a change in the composition of the blood are most apt to be general and to affect, at any rate in the first place, the loose areolar tissue, especially in dependent parts of the body. The most typical example is afforded by the dropsy which occurs in the course of Bright's disease, in which the loss of albumin, by transudation through the renal capillaries into the urine, may in a few days so alter the blood that general anasarca comes on. Another example is afforded by the general dropsy which comes on in some cases of anaemia, which may be due to a derangement of the metabolic processes of the body, and is not necessarily (though it frequently is) dependent upon the draining away of some important blood constituent.

[1] Turner, "Examination of the Cerebro-spinal fluid." *Proceedings of the Royal Society*, VII., 1854—55, p. 89.

Dropsy due to an altered relation between arterial and venous pressure is aptly exemplified by the dropsy in certain cases of heart disease, or which is due to the pressure of an abdominal tumour, or a cirrhosed liver, upon the inferior vena cava. In these cases the dropsy is not general, but only affects the vascular area connected with the obstructed veins.

It was long ago pointed out by C. Schmidt that where dropsical accumulations occur simultaneously in various regions as, for example, in the subcutaneous connective tissue and in several serous sacs, the composition of the extravasated liquid varies in the different situations in consequence of local peculiarities, so that if the liquids were withdrawn and were to accumulate again, (the condition of the blood remaining constant in the interval), the second accumulations would exhibit the same absolute composition and relative differences as the first.

The different dropsical fluids may be arranged in the following order, according to their richness in proteids:

(1) Pleuritic fluid: (2) Peritoneal fluid: (3) Cerebro-spinal fluid: (4) Fluid of subcutaneous oedema.

The quantitative differences in composition presented by fluids removed at the same time from different serous cavities and from the subcutaneous areolar tissue may be illustrated by quoting the two following series of analyses.

I. Composition of various dropsical fluids removed simultaneously from the body of a person who had died of albuminuria (C. Schmidt[1]).

	Fluid from			
	Pleura.	Peritoneum.	Sub-arachnoid.	Oedematous connective tissue of extremities.
Water in 1000 parts	963·95	978·91	983·54	988·70
Solid matters „	36·05	21·09	16·46	11·30
Organic „ „	28·50	11·32	7·98	3·60
Inorganic „ „	7·55	9·77	8·48	7·70

II. Composition of the dropsical liquid removed simultaneously from a patient affected with albuminuria (Hoppe-Seyler[2]).

		Fluid from		
		Pleura.	Peritoneum.	Oedema of feet.
Water in 1000 parts		957·59	967·68	982·17
Solids „ „		42·41	32·32	17·83
Albumin „ „		27·82	16·11	3·64
Ethereal Extract	in 1000 parts.	14·59	5·27	0·50
Alcoholic „				3·71
Aqueous „				1·10
Inorganic salts			10·94	9·00
Loss				0·12

[1] Schmidt, *Zur Charakteristik der epid. Cholera*, p. 116 et seq., quoted by Hoppe-Seyler, *Phys. Chemie*, p. 602.

[2] Hoppe-Seyler, Virchow's *Archiv*, Vol. IX. (1856) p. 257.

The constancy of composition presented by successive dropsical transudations into the same sac is well exemplified by the two following series of analyses[1].

I. Analyses of fluid removed from the pleural and peritoneal cavities on two separate occasions (Scherer).

	Fluid from Pleura.		Fluid from Peritoneum.	
	1st Paracentesis.	2nd Paracentesis.	1st Paracentesis.	2nd Paracentesis.
Water . . .	935·52	936·06	952·99	960·49
Solid matters .	64·48	63·94	47·01	39·51
Fibrin . . .	0·62	0·60	0·32	
Albumin . .	49·77	52·78	34·58	29·73
Ethereal extract	2·14	1·35	1·26	1·63
Alcoholic extract	1·84	1·61	3·02	2·12
Aqueous extract	1·62			
Inorganic salts	7·93	7·40	7·22	5·94

II. Analysis of the fluid removed from the peritoneal sac in a case of Cirrhosis of the Liver (Hoppe-Seyler[2].)

	1st Paracentesis.	2nd Paracentesis.	Removed after death.
Water in 1000 parts	984·50	982·53	983·33
Solid matters ,,	15·50	17·47	16·67
Albumin ,,	6·17	7·73	6·11
Ethereal extract ,,	0·34	0·16	0·25
Alcoholic extract ,,	0·24	0·56	2·16
Aqueous extract ,,	0·67	1·12	0·84
Inorganic salts, soluble	8·30	7·99	8·05
,, ,, insoluble	0·16	0·14	0·19
Errors of analysis	0·38	0·23	0·93
Pressure of liquid in peritoneal cavity	= 23·5 mm. of mercury.	25·25 mm. of mercury.	

General Characters of Dropsical Fluids.

Resemblance to diluted Liquor Sanguinis.

Dropsical fluids always present more or less resemblance to diluted liquor sanguinis. In most cases where a serous sac which contains the liquid is not inflamed, this does not coagulate spontaneously, but does so on the addition of fibrin-ferment. The transudations of an inflamed serous membrane, on the other hand, which are rich in formed elements, yield spontaneous coagula of fibrin.

The Proteids contained in dropsical accumulations.

Whether coagulating spontaneously or not, the transudations which accumulate within serous sacs contain some *fibrinogen*, as evidenced by the formation of a coagulum on the addition of fibrin-ferment, or on heating to 56°—59° C. Serum-globulin and serum-albumin are

[1] These Analyses are transcribed from pages 602 and 603 of Professor Hoppe-Seyler's *Physiologische Chemie*.

[2] Hoppe-Seyler, Virchow's *Archiv*, Vol. IX. (1856) p. 250.

present in addition. Old dropsical accumulations within the serous sacs are richer in proteids than those recently formed.

Saline constituents. The salts of dropsical transudations are similar in character and usually in amount also to those present in the liquor sanguinis. They are most abundant in recent accumulations.

Extractive matters. The extractive matters of the blood, such as urea, uric acid, sugar, occur in the transudations, in much the same proportions as in the liquor sanguinis. In old extravasations cholesterin is occasionally present, and more rarely bilirubin.

Gases. All dropsical extravasations contain gases, CO_2, O, and N, removable by boiling in a Toricellian vacuum.

Of these gases the first is most abundant, the second sometimes absent, and the third is present in about the same proportion as in the blood. The tension of the CO_2 is, in some cases, considerably higher than in the blood (Ewald).

TABLE EXHIBITING THE VOLUMES OF GASES, MEASURED AT 0° C. AND 760 MM. FOUND IN VARIOUS PATHOLOGICAL TRANSUDATIONS BY PLANER, STRASSBURG AND EWALD (HOPPE-SEYLER[1].)

Transudation.	CO_2		Total CO_2	O	N	Authority.
	loosely combined	firmly combined				
Fluid of Peritoneum	9·404	4·866	14·270	0·139	2·107	Planer.
,, Hydrocele	32·49	33·45	64·94	0·16	2·05	Strassburg.
,, Oedema of extremities	22·25	9·15	31·36	traces	traces	Ewald.
,, do. chronic nephritis	21·88	31·18	53·06	,,	,,	,,
,, Pleurisy	39·34	15·59	54·93	0·68	1·33	,,
,, do. in a case of phthisis	18·54	25·99	44·53	0·54	1·87	,,
,, Hydrothorax, in a case of Bright's disease	18·99	34·82	53·81	0·36	1·95	,,
,, Pleurisy after recurrent fever	20·92	38·03	58·95	3·16		,,
,, Pleurisy with pericarditis	18·64	41·16	59·80	?	?	,,
,, Tubercular pleurisy	25·47	46·82	72·29	0·17	1·04	,,
,, Hydrothorax	25·34	48·67	74·01	0·29	0·87	,,
,, do. (of left pleura)	27·70	56·30	84·00	3·24		,,
,, do. (double)	25·71	55·50	81·21	1·01	2·47	,,

[1] Hoppe-Seyler, *Physiologische Chemie*, p. 611.

Characters of particular Transudations.

Having discussed the general characters presented by the transudations which constitute the various forms of dropsy, it is necessary to refer to special facts connected with certain of these liquids.

Pleural transudations. The liquid which accumulates in the pleural cavities in hydrothorax is clear, faintly yellowish, inodorous, and free from viscosity; it is possessed of an alkaline reaction; its specific gravity is low, usually between 1010 and 1015.

In acute pleurisies, the liquid removed by paracentesis soon coagulates, the fibrin which separates amounting to 0·4 or even 0·5 per thousand. Its specific gravity is above 1018. The amount of solid matter exceeds 50 parts per 1000.

In chronic pleurisies fibrin does not usually occur, and the proportion of albumin in the transudation increases.

Several analyses of pleural transudations have been given at pages 232 and 233.

Peritoneal transudations, Ascitic liquid. Possessed of a faint yellow colour, density varying between 1005 and 1024. The liquid does not coagulate spontaneously unless there have existed some peritonitis.

Some analyses are given at pages 232 and 233.

Liquid effused into the Pericardium. Usually is colourless; often spontaneously coagulable. Contains a larger quantity of fibrinogen than other transudations. Contains from 0·879 to 2·468 p. c. of albumin (Kühne).

Liquid of Hydrocele. The density of hydrocele liquid oscillates between 1016 and 1022. Its colour is usually a very faint lemon-yellow, but may be much darker; it sometimes has a greenish tint; sometimes it is slightly viscous.

It contains a large quantity of globulins and serum-albumin, in addition to the fibrinogen which has caused it to be the favourite liquid for experiments on the formation of fibrin. In some cases it contains a large quantity of cholesterin (1—5 p. c.). Succinic acid has sometimes been found in it.

The following is the mean of 17 analyses of hydrocele liquid made by Hammarsten.

Water in 1000 parts	938·85
Solid matters „	61·15
Fibrin (derived from fibrinogen)	0·59
Globulins	13·52
Serum-albumin	35·94
Ethereal extract	4·02
Soluble salts	8·60
Insoluble salts	0·66
NaCl	6·19

In 12 analyses made by Hoppe-Seyler the solid matters of the liquid of hydrocele varied between 41·4 and 80·2 and the proteids between 29·5 and 65 parts per 1000.

Cerebro-spinal Liquid. In cases of spina bifida and chronic hydrocephalus large accumulations of liquid occur, which presents a close resemblance to normal cerebro-spinal liquid. The liquid is clear, has a low specific gravity, and contains usually from 10—13 parts of solid matters per 1000.

Sugar has been described as a normal constituent of cerebro-spinal liquid, or at least a substance having a similar reducing action as sugar upon cupric oxide (see p. 231). According to Hoppe-Seyler, sugar is not a normal constituent of this fluid, and only occurs as a result of irritation or inflammation of the brain or spinal cord.

Cerebro-spinal liquid, when boiled, becomes opalescent, without yielding a flocculent precipitate, which only separates after the addition of acetic acid (Hoppe-Seyler).

Cerebro-spinal liquid differs from other transudations in being usually free from fibrinogen, and therefore not yielding a coagulum of fibrin when treated with fibrin-ferment.

Carl Schmidt found that the cerebro-spinal liquid is remarkably rich in salts of potassium—an observation which is well worthy of being checked by fresh analyses of the liquid obtained by puncturing in cases of spina bifida.

The following are analyses by Hoppe-Seyler of the cerebro-spinal liquid, obtained by puncture, in cases of spina bifida[1].

ANALYSES OF THE CEREBRO-SPINAL LIQUID, OBTAINED BY PUNCTURE, IN TWO CASES OF SPINA BIFIDA (HOPPE-SEYLER).

	I.			II.	
	1st Puncture.	2nd Puncture.	3rd Puncture.	1st Puncture.	2nd Puncture.
Water	987·49	986·88	986·72	989·33	989·80
Solid matters	12·51	13·12	13·28	10·67	10·20
Albumin	1·62	2·64	2·46	0·25	0·55
Extractives	10·27	2·83	2·65	2·30	2·00
Inorganic salts, soluble		7·52	8·21	7·67	7·20
„ „ insoluble	0·25	1·15	0·28	0·45	0·45

Sec. 4. Methods of analysing Lymph, Chyle, and other Transudations Normal and Pathological.

The methods of investigation are precisely similar to those pursued in the analyses of liquor sanguinis and serum (see p. 187 *et seq.*), with the exception of the estimation of fibrinogen.

The amount of fibrin which separates spontaneously may be

[1] Hoppe-Seyler, *Physiologische Chemie*, p. 601.

ascertained by washing the coagulum from a known weight of the transudation and proceeding as stated at page 180.

The fibrinogen may then be determined by one of two methods: firstly, by Frederique's method (see p. 188); secondly, by adding to a weighed quantity of the liquid separated from any coagulum some very active solution of fibrin-ferment (see p. 49), and then placing for 36 hours in an incubator heated to 40° C.; then collecting any coagulum which has separated, washing, and proceeding as directed in the case of blood-fibrin at p. 180. In giving the results of the analyses the amount of fibrin, *corresponding* to fibrinogen, is then stated.

After separating fibrin and fibrinogen, the globulins remaining in solution are estimated by Hammarsten's method (precipitation with magnesium sulphate, see p. 188).

In a fresh portion of the fluid the total proteids are estimated by precipitation with alcohol (see p. 187).

By then subtracting from the result thus obtained the weight of fibrinogen and of globulins, the amount of serum-albumin is ascertained.

The extractive matters, salts and gases, are determined exactly as in the case of blood or serum.

CHAPTER VI.

PUS.

Sec. 1. Introductory remarks on the Physical Properties of Pus and on the Nature of Pus.

Closely connected with the liquids which have been considered in the preceding chapter is one which, unlike these, forms no part of the healthy body, but is invariably the result of a morbid process.

Pus is sometimes found in one of the natural cavities of the body, as, for example, within the interior of a serous sac: sometimes covering an epitheliated surface on the exterior, or opening on the exterior, of the body: most commonly contained within an *abscess*—a cavity whose walls are constituted by inflamed and usually indurated tissues.

Physical characters. Fresh, healthy, *laudable* pus presents the appearance of a somewhat creamy yellow liquid, which unless it have been obtained from the vicinity of the intestines, is destitute of fœtid odour and possesses, at most, a mawkish smell.

Its reaction is usually said to be alkaline, but, according to Ewald, it is often acid. Its specific gravity varies between 1020 and 1040, being on an average 1032. The fluid does not coagulate spontaneously.

Microscopical characters. Under the microscope pus is seen to be composed of a clear liquid—the *pus serum*—in which closely float a large number of cells which, when first formed, resemble, if they are not identical with, the colourless cells of the blood.

These cells are usually more or less spherical: destitute of a cell wall: somewhat granular, and contain one or more (often three, sometimes more) nuclei, which are rendered evident by the action of acetic acid, which causes the protoplasm of the cell to become transparent and indistinct. When very young, pus cells may exhibit amœboid movements, though the opportunity for observing this phenomenon does not often present itself.

The diameter of pus corpuscles usually varies between 8μ and 10μ; by the action of water they swell and become transparent,

allowing their nuclei to be seen, and the latter may then be readily stained with magenta or even with carmine.

As usually obtained, pus corpuscles resemble dead rather than living colourless blood-cells, as evidenced by the absence of contractility.

Pus corpuscles are liable to undergo certain changes, of which the most common is fatty degeneration; the cells then contain a number of highly refracting, obviously fatty, granulations; at a more advanced stage, the cells break down and the fatty granulations thus set free float in the pus-serum.

Nature of Pus and origin of Pus Cells.

The liquid portion of pus—*pus serum*—resembles the liquor sanguinis and the normal transudations very closely, and, doubtless, is in great part, in the first instance, a transudation from the blood. With regard to the pus corpuscles, they are, for the most part, either colourless cells of the blood which have wandered through the capillary walls into the extra-vascular spaces, or the offspring of such emigrated cells; in some cases, however, it is possible that the pus cells are derived from the normal cells of the tissues amongst which they are found, especially from epithelial and endothelial cells.

Sec. 2. The Pus Serum.

The liquid in which the pus corpuscles are suspended may be separated in an unmixed condition by filtration; the process is, however, a tedious one; it may be obtained more readily, by mixing pus with an equal volume of a solution of one part of sodium sulphate in 9 parts of water and then filtering; the liquid which passes through the filter is then a mixture of pus serum and solution of sodium sulphate. Doubtless the separation would, in either case, be much facilitated by the use of the centrifugal apparatus.

Pure pus serum is a turbid liquid which has a brownish tint when examined by reflected light, whilst by transmitted light thin layers appear of a yellow colour. Its reaction is usually alkaline.

Proteid matters of pus serum.

Pus serum contains substantially the same proteid matters as blood serum, viz. serum-globulin and serum-albumin; the former is partly precipitated by CO_2, but may, as in the case of blood serum, be completely precipitated by saturating with magnesium sulphate.

Extractive matters soluble in ether.

These consist of a mixture of neutral fats, cholesterin, and of a derivative of *glycerin-phosphoric acid*. This derivative is, according to Hoppe-Seyler, probably the same as lecithin, the phosphorized proximate principle of the yolk of egg; according to Fischer[1] it is protagon. The matter is yet

[1] Fischer, *Centralblatt f. d. med. Wissenschaften*, 1865, p. 225.

altogether unsettled. In the analyses quoted below the phosphorus in organic combination is supposed to be present in lecithin.

Extractive matters soluble in alcohol and water.

Hoppe Seyler has found leucine and tyrosine in perfectly fresh pus; urea and sugar may also be present. It has been alleged that gelatin and chondrin occasionally occur in pus, but these bodies, though sought for by Hoppe-Seyler, have never been found by him.

The saline constituents of pus serum.

Of these the chief is sodium chloride. The other saline constituents supposed to be present by Hoppe-Seyler will be learned by referring to the next paragraph.

Results of Hoppe-Seyler's analyses of pus serum.

Probably the only analyses of the serum of pus which can be looked upon as really trustworthy are those made by Hoppe-Seyler, of which the results are appended. The pus was in each case obtained from an acute abscess.

ANALYSIS OF THE SERUM OF PUS (HOPPE-SEYLER[1]).

		I.	II.
Proteids	in 1000 parts	63·23	77·21
Lecithin	„ „	1·50	0·56
Fats	„ „	0·26	0·29
Cholesterin	„ „	0·53	0·87
Alcohol-extractives	„	1·52	0·73
Water-extractives	„	11·53	6·92
Inorganic matters	„	7·73	7·77
Solid matters	„ „	86·30	94·35
Water	„ „	913·70	905·65
		1000·00	1000·00

ANALYSIS OF THE ASH OBTAINED BY INCINERATING THE PUS-SERUM EMPLOYED IN THE ABOVE ANALYSES.

Quantity of various Saline Constituents in 1000 parts of Pus Serum.

	I.	II.
NaCl . . .	5·22	5·39
Na_2SO_4 . .	0·40	0·31
Na_2HPO_4 . .	0·98	0·46
Na_2CO_3 . .	0·49	1·13
$Ca_3(PO_4)_2$ . .	0·49	0·31
$Mg_3(PO_4)_2$ . .	0 19	0·12
PO_4 found in excess		0·05
	7·77	7·77

[1] Hoppe-Seyler, "Ueber die quantitative Zusammensetzung des Eiters." *Med.-chem. Untersuchung*, p. 490.

Sec. 3. Pus Corpuscles.

As has been mentioned in a preceding section, these corpuscles may be obtained by mixing fresh pus with solution of sodium sulphate and filtering; the corpuscles left on the filter may be freed from adhering pus serum by washing with an additional quantity of the solution of sodium sulphate.

Action of NaCl on the proteids of the cell protoplasm.

Solution of common salt cannot be employed in the place of solution of sodium sulphate in the separation of pus cells, for, under the influence of sodium chloride, the cells are converted into a slimy, opaque, jelly, precipitable by water.

The Proteids present in the Cell-protoplasm.

It was formerly supposed that the pus corpuscles contained a considerable quantity of a proteid identical with myosin. The most careful investigation yet made (by Miescher[1]) of the constituents of pus cells failed to detect myosin.

According to this author three proteids soluble in water can be obtained from the protoplasm of the pus cells, viz. (1) alkaline albuminate, partially precipitated by CO_2, and more completely precipitated by acetic acid, insoluble in solution of sodium chloride, and soluble in very dilute hydrochloric acid (1 to 1000 of water): (2) a proteid coagulable at 48°—49°; the flakes which separate are insoluble in dilute HCl and in solution of NaCl: (3) a proteid which coagulates at the same temperature as serum-albumin. In addition to these, two proteids insoluble in water are also present, in preponderating quantity, viz. (1) a body insoluble in water, swelling up in solution of NaCl, soluble in very dilute hydrochloric acid (1 to 1000) giving rise to acid-albumin: this is the body, formerly considered to be identical with myosin, which occasions the peculiar phenomenon observed when pus is mixed with solution of common salt; (2) a body unacted upon by water and by solution of NaCl, and attacked with difficulty by dilute hydrochloric acid (1 to 1000).

The matter of the Nuclei. Nuclein (?).

When pus corpuscles are subjected to the repeated action of weak hydrochloric acid, it occasionally happens that a considerable number of free nuclei are obtained; the greater number, however, have some remains of the cell protoplasm yet adhering to them.

By digesting pus cells in artificial gastric juice (made by digesting the mucous membrane of pig's stomach in water containing 10 c.c. of fuming HCl in 1 litre) the nuclei of the pus cells are

[1] Miescher, "Ueber die chemische Zusammensetzung der Eiterzellen." Hoppe-Seyler, *Med.-chem. Untersuchungen*, p. 441 et seq.

isolated in large quantities. In order to obtain them uncontaminated with organic phosphorus compounds soluble in alcohol and ether, it is advisable to treat the pus cells with hot alcohol before digestion.

The nuclei, isolated by the above method, form a grey mass, insoluble in very dilute HCl, but soluble in very weak solutions of sodium hydrate. From this solution, acids added in excess precipitate an insoluble body, which, according to Miescher[1], consists of a definite organic body containing phosphorus, to which he ascribes the name of Nuclein. This body is, according to Miescher, found in the nuclei of the segmentation spheres of the yolk; according to Plosz[2] it is the principal constituent of the nuclei of the coloured blood corpuscles of Birds; and according to Hoppe-Seyler[3] it is found in yeast. It is said also to be present in brain and liver. Indeed, wherever nuclei are found, *nuclein* has been surmised to exist.

Elementary composition of Nuclein.

Miescher[4] has investigated the nuclein obtained from salmon-melt more closely than that obtained from pus-cells, and has come to the conclusion that nuclein is a tetra-basic acid, having the formula $C_{29}H_{49}N_9P_3O_{22}$.

The following is the composition of nuclein according to Miescher.

	Calculated.	Found.
C_{29}	35·95	36·11
H_{49}	5·01	5·15
N_9	13·02	13·09
P_3	9·61	9·59
O_{22}	36·41	36·06

Hoppe-Seyler prepared and analysed nuclein from pus and obtained numbers which differed entirely from those of Miescher.

The following numbers shew the wide discrepancy between the analyses of Hoppe-Seyler and Miescher.

ANALYSES OF NUCLEIN.

	I. (From pus.) (Hoppe-Seyler.)	II. (From spermatozoa of the Salmon.) (Miescher.)
C	49·58	36·11
H	7·10	5·15
N	15·02	13·09
P	2·28	9·59

[1] Miescher, *Op. cit.*

[2] Plosz, "Ueber das chemische Verhalten der Kerne der Vogel- und Schlangenblutkörperchen." Hoppe-Seyler's *Med.-chem. Untersuchungen*, p. 461.

[3] Hoppe-Seyler, "Ueber die chemische Zusammensetzung des Eiters." *Med.-chem. Untersuchungen*, p. 500.

[4] Miescher, "Die Spermatozoen einiger Wirbelthiere," (Protamin, Nuclein). *Separatabrduck aus den Verhandlungen der naturforschenden Gesellschaft in Basel*, Vol. VI., 1874. Abstracted in Maly's *Jahresbericht*, Vol. IV. p. 337 et seq.

Does a definite body Nuclein exist?

Whether the body obtained by Miescher from spermatozoa be a definite body or not, there can be no question that as yet, all proof is wanting to establish the proposition, that the substance composing cell nuclei generally is a definite chemical individual, possessed of constant composition. On the contrary, the evidence of most trustworthy observers shews, that by following the processes which have been recommended for the preparation of nuclein, substances of widely differing composition are obtained.

In different samples of the nuclein of yolk of egg, Worm Müller[1] found 2·2, 2·68, and 7·9 p.c. of Phosphorus. In nuclein from the same source Miescher found 6·7 and 7·1 p.c. In nuclein prepared from casein Lubavin[2] found 4·6 p.c. of P. In nuclein from pus Hoppe-Seyler found 2·28 p.c. of P.; in that prepared from an epithelial tumour he found 3·35 p.c. In the nuclein from pus Miescher found 2·6 p.c. of P.

The statements as to the state in which the P. exists in the so-called nuclein are also discrepant.

We must therefore agree with the conclusions of Worm Müller, and deny the existence of a definite body, Nuclein. Probably, as this author surmises, the different nucleins are mixtures of organic phosphorus compounds with varying quantities of proteid bodies.

The Extractive Matters of Pus Cells soluble in water.

Alleged presence of Gelatin and Chondrin.

It was asserted by Boedecker[3] that pus occasionally contains, besides proteid matters proper, gelatin and chondrin. Miescher examined the aqueous extract of pus cells for these bodies with entirely negative results.

Boedecker's chlorrhodinic acid.

By the name of chlorrhodinic acid Boedecker described a crystalline acid which he obtained from pus by the following method:—The liquid is evaporated to dryness, and the powdered residue is successively treated with ether, alcohol, and then with water. The aqueous extract is precipitated with lead acetate, and the precipitate decomposed by means of sulphuretted hydrogen and boiled in alcohol. On evaporation, the alcoholic fluid deposits groups of microscopic needles, mixed with some crystals of sodium chloride. The former are composed of the acid, which, according to Boedecker, contains nitrogen. Iodine colours it yellow, and chlorine water of a rose colour, or of a dark red tint, according to the amount of acid present. These facts suggest a re-examination of the subject.

[1] Worm Müller, "Zur Kenntniss der Nucleine." Pflüger's *Archiv*, Vol. VIII. (1873), p. 190.

[2] Lubavin, "Ueber die künstliche Pepsin-Verdauung des Caseins." Hoppe-Seyler's *Med.-chem. Untersuchungen*, p. 477.

[3] Boedecker, *Zeitschrift f. rat. Med.*, N. F., Vol. VI.

Presence of Glycogen in pus.

It has been shewn by Salomon[1] that pus generally contains very appreciable quantities of glycogen, and this fact agrees with certain histological observations of Ranvier[2]. Thus a distinction which Hoppe-Seyler[3] sought to establish between the colourless cells of the blood and their descendants, the pus corpuscles, can no longer be maintained.

The Extractive Matters of Pus Cells soluble in alcohol and ether.

The chief of these are cholesterin, fats and derivatives of glycerin-phosphoric acid; free fatty acids may be likewise present in old pus and form crystalline deposits.

The Mineral Matters of Pus Cells.

These consist of a small quantity of sodium chloride and of earthy phosphates. Their amounts, as found by Hoppe-Seyler, will be learned in the next paragraph.

Results of Hoppe-Seyler's analysis of Pus Corpuscles.

The following are the results of the analyses made by Hoppe-Seyler of two samples of pus cells isolated by sodium sulphate.

a. Organic constituents in 100 parts of dried pus contained

	(1)		(2)
Proteids	13·762		
Nuclein	34·257	68·585	67·369
Insoluble matters	20·566		
Lecithin	14·383		7·564
Fats			7·500
Cholesterin	7·400		7·283
Cerebrin	5·199		10·284
Extractive matters	4·433		
	100·000		100·000

b. Mineral constituents

100 parts of dried corpuscles contain

NaCl	0·435 parts
$Ca_3(PO_4)_2$	0·205 „
$Mg_3(PO_4)_2$	0·113 „
$Fe_2(PO_4)_2$	0·106 „
PO_4	0·916 „
Na	0·068 „
K	traces.

[1] Salomon, "Untersuchungen betreffend das Vorkommen von Glycogen im Eiter und Blut." *Deutsche med. Wochenschr.* 1877, No. 35. Abstracted in Maly's *Jahresbericht*, Vol. VII., p. 130. Salomon, "Ueber das Vorkommen von Glycogen im Eiter." *Verhandlungen der physiol. Gesellsch. zu Berlin.* Jahrg. 1877-78, No. 19.

[2] Ranvier, *Progrès Méd.* 1877, p. 422.

[3] Hoppe-Seyler, "Ueber die chemische Zusammensetzung des Eiters." *Med. Chem. Untersuch.*, p. 497.

Sec. 4. Colouring matters found in Pus.

Pus is sometimes coloured with bilirubin. At other times it presents a brownish colouration very similar to that due to bilirubin, but without giving the reactions of this body. More rarely it has a blue or green colour.

Pyocyanin.

It has long ago been known that the pus of old sores sometimes presents a blue or green colouration, especially the former.

Fordos[1] shewed that under these circumstances a blue colouring matter is formed to which he gave the definite name of *pyocyanin*.

Mode of preparation of Pyocyanin.

The material employed by Fordos consisted of bandages stained with blue pus. These are steeped in a weak solution of ammonia, which dissolves the colouring matter and acquires a blueish or greenish tint. The ammoniacal solution is shaken with chloroform, which dissolves pyocyanin, fat, and a yellow colouring matter.

The chloroformic solution is shaken with water holding a little sulphuric acid in solution, when the colouring matter assumes a red instead of a blue or blueish-green colour, and, leaving the chloroform, is dissolved by the acid solution. The supernatant red liquid is then separated, mixed with chloroform, and a little solution of caustic baryta added, which causes the red colouring matter to become blue again and to be taken up by the chloroform. On now evaporating this liquid, pyocyanin is obtained, in the form of blue needles or of rectangular plates. These are soluble in water, alcohol, and chloroform. Pyocyanin possesses, as will have been gathered by the reader from the above description of the mode of preparation, some of the properties of a blue vegetable colouring matter.

Properties of Pyocyanin.

Pyocyanin is soluble in water, alcohol, chloroform, and ether. It is decolourized by chlorine, concentrated nitric acid, and ozone: it is blue in the presence of alkalies, red in that of acids. When blue pus is kept from contact with air the colour disappears, to reappear again when the liquid is shaken with air.

Pyocyanin has not hitherto been analysed.

Pyocyanin a vegetable colouring matter.

It has long been known that by being placed in proximity to a wound of which the purulent discharges are blue, suppurating surfaces which produced yellow pus are apt to furnish a liquid which is also blue or green. Lücke[2] thought he had discovered that the blue colour is due to a blue *vibrio* developing in the pus. The more recent investigations of Fitz[3]

[1] Fordos, "Recherches sur la matière colorante des suppurations bleues: Pyocyanin." Paris, *Comptes Rendus*, LI., 1860, p. 215.

[2] Lücke, "Die sogenannte blaue Eiterung u. ihre Ursachen." *Archiv f. Klinische Chirurgie*, III., 1862, p. 135.

[3] Fitz, see an abstract entitled 'Recent Researches on Bacteria,' *Quarterly Journal of Microscopic Science*, Jan. 1880, p. 106, from which the above account of Fitz's researches is taken almost *verbatim*.

have shewn that the generator of pyocyanin is a species of *bacterium*, which has the form of a *bacillus*, and which possesses in addition the property of decomposing glycerin in the presence of calcic carbonate, with the formation of hydrogen, carbonic acid, butyl-alcohol and butyric acid. Fitz cultivated the pus-bacillus in a solution of calcium lactate and ammonium chloride, and obtained, in the solution, a colourless reduction-product of the colouring matter, which was only blue on the surface, but which when shaken with air assumed throughout a deep blue colour similar to that of a solution of copper sulphate. These properties of the blue colouring matter agree exactly with those described by Fordos and Lücke. The bacteria which produce it, and which multiply luxuriantly in the cultivating liquid, are small elliptic bodies, from one to one and a half micromillimetres in length, and generally occur in couples.

It has been asserted by Herapath that indigo-blue occasionally occurs in blue pus.

Pyoxanthose.

The above term was applied by Fordos[1] to a greenish-yellow colouring matter, already referred to in the description of the preparation of pyocyanin, accompanying that body, and like it soluble in chloroform. It may be separated from pyocyanin by ether, in which it is more soluble than the latter body. It crystallizes in yellow prisms. It is soluble with difficulty in water, but readily soluble in alcohol, ether, chloroform, bisulphide of carbon and benzol. It is coloured red by acids, and violet by alkalies.

Sec. 5. The Gases of Pus.

When pus is subjected to the process employed for the extraction of the gases of the blood—viz. heated in a Toricellian vacuum—it gives off a mixture of carbon dioxide, oxygen and nitrogen, in which the first-named is much the most abundant constituent.

Conclusions to be drawn from Ewald's researches.

Ewald[2], to whom we owe the greater part of our knowledge of the gases of pus, has arrived at conclusions which may be stated briefly as follows:

1. Fresh pus yields to the Toricellian vacuum only carbon dioxide, oxygen and nitrogen, the two latter gases being present in very small quantities. It never evolves hydrogen (as had been asserted by Mathieu and Urbain[3]), or sulphuretted or carburetted hydrogen.

[1] Fordos, "Recherches sur la matière colorante des suppurations bleues: Pyocyanin et Pyoxanthose." Paris, *Comptes Rendus*, LVI., 1863, p. 1128.

[2] Ewald, "Untersuchungen zur Gasometrie der Transudate des Menschen." *Archiv für Anatomie und Physiologie*, 1873, pp. 663—698. An exceedingly full and clear abstract of this most valuable memoir is to be found in Maly's *Jahresbericht*, Vol. IV., pp. 421—431.

[3] Mathieu et Urbain, *Gazette hébdomadaire*, 1871, No. 24, and 1872, No. 21 (quoted by Gautier).

2. The amount of CO_2 contained in a purulent or sero-purulent exudation increases with the age of the exudation.

3. The amount of CO_2 contained in a purulent exudation is small in proportion as the exudation approaches pure pus in its characters. This is not only true of the total CO_2, but particularly of

Results of Ewald's analyses.

COMPOSITION OF THE GASES EVOLVED BY PUS HEATED IN VACUO. (EWALD.)

Nature of the purulent liquid.	Specific gravity.	Reaction.	CO_2			Total. CO_2	N	O
			Firmly combined.		Loosely combined or free.			
Acute purulent exudation from pleura; of 28 days standing.	1029	weakly acid	1·68	+	70·17	= 71·85	1·14	
Do. do.; 10 days standing.	1014	do.	2·77	+	15·73	= 18·50	traces	
Do. do.; do.	1024	do.	?	+	14·76	= 14·76	traces	
Do. do.; do.	1020	do.	0·0	+	21·46	= 21·46	2·9	0·77
Pure pus from an abscess of 21 days duration.	1009	do.	0·0	+	8·05	= 8·05	1·35	0·43
Do. do.	1016	neutral (?)	0·0	+	7·92	= 7·92	not determined.	

the combined CO_2 which is given off on the addition of an acid; indeed pure pus contains only free CO_2[1].

4. Pus corpuscles,—as doubtless also the colourless cells of the blood,—possess the property of decomposing sodium carbonate (Na_2CO_3) and evolving from it CO_2.

5. Pus corpuscles and the colourless cells of the blood are either altogether free from oxygen or contain mere traces of this gas.

Sec. 6. Directions for the quantitative analysis of Pus.

1. Determine the specific gravity by means of the bottle (see p. 174).

2. Ascertain the reaction.

3. Determine the total solids, water and salts, as in the case of blood (see p. 177).

4. Evaporate a known weight of the fluid, say 25 grm., to dryness. Extract with ether and determine the amount of the ether extract. If wished, determine in the latter the amount of cholesterin, lecithin and fats, as in the case of blood (see p. 187).

5. Treat the residue after extraction with ether, with boiling absolute alcohol, filter, evaporate the solution to dryness; weigh, then ignite and weigh again. By subtracting the second from the first weight the amount of the alcoholic extractive matters is found.

6. Mix a weighed quantity of pus with ten times its volume of alcohol, set aside for 24 hours and proceed exactly as in Schmidt's method for determining the total amount of proteids in the serum (p. 188). In this way will be found the combined weight of the proteids of the pus serum and of the corpuscles, together with nuclein. The residue may then be boiled in water, and, after cooling, subjected to artificial peptic digestion in the incubator for 24 hours. The insoluble matters may be treated with a fresh portion of artificial gastric juice and the process continued for a second period of 24 hours. The insoluble residue is then collected on a weighed filter, washed successively with boiling water, alcohol, and ether, and then dried; thus will be found the weight of the dry nuclei (nuclein?).

7. A portion of the pus may be filtered and the solids, salts, extractives, &c. of the pus serum be determined, by following precisely the methods recommended in the case of blood serum.

8. If it be required to determine the presence of urea, sugar, or any other extractive matter, the methods recommended in the case of blood may be followed.

9. For the separation and estimation of the gases of pus the same proceedings are adopted as with blood.

[1] The liquid contained in the pleural cavity, if not purulent and of old standing, always yields a larger proportion of firmly combined than of loosely combined or free CO_2.

CHAPTER VII.

THE CONNECTIVE TISSUES.

Introduction.

Under the term of 'the connective tissues,' histologists have grouped together several tissues which at first would appear to have few points in common—to wit: connective tissue proper, including the white connective tissues and yellow or elastic tissue: cartilage: bone: and dentine.

When we enquire into the grounds of this classification we find that they are the following:—The tissues above named are derived from the same embryonic layer[1] (*mesoblast*); they all perform similar, subordinate, functions of support or connection; they all contain cells which develop a matrix or ground substance, which has various characters in the various tissues; they shade off, as it were, into one another, and represent each other in different species of animals. "In one and the same organism typical development brings with it a substitution of one member of the connective-substance group for another. There, for instance, where in the embryonic state gelatinous tissue existed, the latter is found transformed into connective tissue or fat at a later epoch; cartilage with its derivatives takes on the form of bony substance. Finally we encounter every kind of this substitution in the richest abundance, brought about by the formative activity of a system modified by disease. Almost every member of the group of connective tissues may be replaced by very nearly any other, firstly by immediate metamorphosis, then again more particularly by reconstruction from the offspring of the original tissue[2]."

[1] This is not strictly true. The *neuroglia*, or connective tissue of the great nerve centres and of the retina, is *epiblastic* in its origin; chemically, however, this tissue differs from connective tissue, so that it is really true that true (collagenous) connective tissues are derived from the *mesoblast*.

[2] Frey: *The Histology and Histochemistry of Man*, translated from the fourth German edition by Arthur E. J. Barker, London, 1874, p. 167.

Sect. 1. Connective Tissue proper.

Structural Elements of Connective Tissue.

By this term may be designated a tissue which presents many very important modifications in different situations, and whose function it is to connect together contiguous organs or parts of the body, or actually to bind together the different anatomical elements which enter into the composition of each organ.

Typical connective tissue presents for examination:

(1) Certain cells, which are especially abundant in the early stages of development of the tissue, and which are termed *connective tissue* cells or corpuscles.

(2) Bundles of fine fibres of a white colour, arranged in parallel rows; or crossing one another, so as to leave spaces between them; or so interwoven as to give rise to tough fibrous membranes. These fibres swell up and become so transparent as almost to disappear from view when the tissue is treated with acetic acid.

(3) Other fibres, usually much less numerous than the white, presenting dark outlines, often intercommunicating by processes, having when seen in large numbers a yellowish tint, and exhibiting altogether distinct chemical reactions; they are unacted upon by acetic acid. These are the *yellow*, or *elastic* fibres of connective tissue.

(4) A *ground substance* or matrix in which the other elements are imbedded and which serves to connect them together, so that we apply to it indifferently the name of *ground substance*, or *connecting substance*, or *cement*.

By the preponderance of certain of these elements over others or by the peculiar forms which certain of these elements may present, the different varieties of connective tissue are distinguished. Thus, for example, in 'white fibrous tissue,' of which tendons and ligaments are formed, the white fibrillae preponderate over the other elements, so that on superficial examination of the fully developed structures neither cells nor yellow elastic elements are seen, and the structures might be likened to cords formed of dense bundles of white fibrillae firmly agglutinated together. Again in the yellow elastic ligaments, such as the *ligamentum nuchae* of large herbivores, or the *ligamenta subflava* of the human vertebral column, there is such a preponderance of the yellow elastic over the white fibres, that the former confer upon the structures their peculiar physical properties.

In the *adenoid* or *retiform* connective tissue, by a peculiar modification of the connective tissue cells, which give off branching processes which join together, a network of fine fibres is established, radiating at many points from connective tissue cells—a network admirably adapted to afford support to other structures.

In the *gelatinous* connective tissue, the matrix or ground substance in which cells and fibres are imbedded is abundant and has a gelatinous consistence.

Connective Tissue Cells.

These are the anatomical elements which are alone present in the earliest stages of the development of connective tissue; and it is probably by the differentiation of their protoplasm that the intercellular structures are ultimately formed which give to the different varieties their peculiar characters.

We must refer the reader to works on Histology for a full description of the various forms of connective tissue cells. We shall in this place confine ourselves to the following categorical statements.

(1) Connective tissue cells consist invariably of a more or less finely granular and contractile protoplasm, in which lies imbedded a nucleus (sometimes more than one), usually of a vesicular nature. The connective tissue cell, whilst it is in its state of typical activity, is destitute of a cell wall, though occasionally one may be developed (as in the fat cell) by the differentiation of the peripheral portion of the cell-protoplasm. In certain cases branching processes are given off from the protoplasm of the connective tissue cells and may serve to connect adjoining cells together.

(2) In certain regions connective tissue cells are found (*e.g.* in the cornea) imbedded in cavities in the ground substance; these cavities sometimes communicate by minute canals, so that there is established a canal system through which liquids may permeate (Saftcanälchensystem). Such spaces or cavities are in certain situations doubtless continuous with the smallest lymphatic vessels.

(3) In other situations the cells are discontinuous and resemble rows of cells laid against the bundles of white fibres, one row to each small bundle: being connected to them, and supported, by the ground substance or matrix.

(4) In some situations pigment is deposited in the protoplasm of the connective tissue cells (*e.g.* in the outer layer of the choroid); in others, fat is formed at the expense of the protoplasm.

(5) Lastly, there occur in the connective tissues certain cells, which are in all respects similar to the colourless cells of the blood, and which wander through the connective tissue spaces, in virtue of the amœboid movements with which they are endowed. These are, doubtless, either colourless cells of the blood which have passed through the capillary walls, or they are the offspring of cells which have thus emigrated.

Micro-chemical reactions of the connective tissue cells.

Our information in reference to the chemistry of the connective tissue cell is, necessarily, of the most limited character and is almost confined to a knowledge that the protoplasm is proteid in nature and that the nucleus shares the characters of nuclei elsewhere and has probably the same composition.

It may be convenient however to summarize the effects of certain reagents upon these cells.

(1) The connective tissue cells are unaffected by iodized serum which constitutes, therefore, the best neutral liquid for their examination.

Iodized serum is a reagent of very great value to histologists[1]. It is best made by dissolving iodine in the amniotic liquid of the cow; this fluid is placed *in a thin layer* in a bottle containing fragments of iodine, with which it is frequently shaken. The iodine gradually dissolves, conferring upon the solution a yellowish tint; in the course of time iodates are formed which increase the solvent action of the serum on iodine, so that after one or two months a dark brown iodized serum is obtained; it is when of this colour that it is most serviceable (Ranvier[2]).

(2) Solution of perosmic acid (1 to 100) fixes the cells in the form which they present during life, and permits of their being subsequently stained with picrocarminate of ammonia.

(3) Silver nitrate (from 0·25 to 0·5 per cent.) is of great use in examining fresh connective tissue. Solutions of this salt acting on the tissue fix the cells in the form which they possess whilst alive, but without colouring them. It is however absorbed by the ground substance and on subsequent exposure to light reduction takes place, so that the unstained cells stand out on a stained background. The treatment with silver does not prevent the subsequent action of certain colouring matters (ammoniacal carmine solution, solution of picrocarmine).

(4) Solution of gold chloride (1 to 100) is of great use in demonstrating the arrangement of the connective tissue cells of the cornea. It is absorbed by the cells, which it helps to preserve in their natural condition; the absorbed salt is afterwards reduced and confers upon the cell a reddish violet colour.

(5) Acetic acid causes the protoplasm to become very transparent, whilst it brings out the nucleus very distinctly.

The White Fibres of Connective Tissue.—Collagen and Gelatin.

The most abundantly distributed forms of adult connective tissue contain as their principal anatomical element bundles of white fibres, which, as was previously stated, are rendered so transparent by the action of acetic acid, as to be almost invisible. The fibres of which the bundles are made up are connected together by an agglutinating substance, which is soluble in dilute solutions of caustic baryta, or lime. The substance of which the fibrils are composed has received the name of Collagen, from the fact that when subjected to the action of boiling water it is converted into gelatin or glue (*κόλλα*).

Preparation of Collagen. Rollett's process.

Tendons, being composed almost entirely of the white fibrils of connective tissue, are best employed in the preparation of collagen. They are cut into thin slices and are then soaked in water until all matters

[1] Max Schultze: "Die Anwendung mit Iod conservirter thierischer Flüssigkeiten als macerirendes und conservirendes Mittel bei histologischen Untersuchungen." Virchow's *Archiv*, Vol. xxx. (1864), p. 263.

[2] Ranvier: *Traité technique d'Histologie*, Vol. I., p. 76.

soluble in water are removed. The watery extract contains a little alkaline albuminate, but very little coagulable albumin. The fragments of tendon are then soaked for some days in very weak solution of baryta or lime, by the action of which the connecting substance is dissolved so that the individual fibrils fall asunder. The insoluble matter is then washed, first in water and afterwards in weak acetic acid, finally again in water. The residue consists almost entirely of the substance of the white fibrils (collagen), mixed however with small quantities of yellow elastic tissue and cell nuclei.

When placed in very diluted acids and alkalies, the fibrils swell up and become transparent, their original appearance being restored if the acid is exactly neutralized. When digested in very dilute acetic acid at ordinary temperatures for some days, the fibres gradually dissolve, yielding a solution which contains gelatin, and also a little acid-albumin, produced by the action of the acid upon the residual matter of connective tissue cells.

Gelatin. When the white fibrils are subjected to long-continued boiling in water at the ordinary pressure of the atmosphere, or, still better, to the action of water heated under pressure (as in Papin's digester), they dissolve, and the solution is found to contain a substance termed *Gelatin*. The same body is produced at the temperature of the animal body by the prolonged action of very diluted acids on collagen.

Preparation of pure Gelatin. It is most convenient to employ commercial gelatin for the preparation of the pure substance. The finest commercial gelatin is allowed to soak for some days in large quantities of distilled water, which is frequently changed; in this way the soluble salts of the gelatin diffuse out. The swollen gelatin is now dissolved in distilled water by the heat of a water bath; after allowing insoluble matters to subside the solution is filtered, with the aid of a hot-water funnel, directly into 90° per cent. alcohol. The gelatin separates in the form of white, thready masses, which are collected, reduced to a fine state of division by cutting, and allowed to dry, first of all in the air, and then in a water oven. Gelatin thus prepared contains about 0·6 per cent. of ash[1].

Gelatin is a body which is insoluble in cold water; the sole action of cold water is to cause it to swell up. On adding boiling water to the swollen solid, it dissolves with readiness, and a clear limpid solution is obtained, which when it is cooled sets as a more or less firm jelly—*gelatinizes*. This property is possessed by solutions which contain only 1 per cent. of the solid substance.

The power of gelatinizing is gradually lost when solutions of gelatin are subjected to prolonged heating, and instantly lost when they are heated to 140° in sealed tubes.

[1] Hofmeister: "Ueber die chemische Structur des Collagens." *Zeitschr. f. phys. Chem.* Vol. II. (1878), p. 315.

Gelatin is insoluble in alcohol, ether and chloroform. It is dissolved with the aid of heat in glycerin, and on cooling a jelly (*glycerin jelly*) is obtained.

Aqueous solutions of gelatin are powerfully *laevo-rotatory*, the rotatory power being very much influenced by temperature and by the reaction of the solution (Hoppe-Seyler). In aqueous solutions at 30° $(a)\ j = -130°$.

Gelatin is not precipitated from its solutions by acetic acid and ferrocyanide of potassium—a character which distinguishes it from any proteid substance. It is not precipitated by acetic acid—a character which distinguishes it from the closely allied body *Chondrin*.

Tannic acid precipitates gelatin even when its solutions are very dilute. Solutions of mercuric chloride also precipitate it.

On the other hand gelatin is not precipitated by solutions of lead acetate (which precipitates chondrin) nor by the majority of metallic salts which do precipitate the proteids.

Composition of Gelatin, and its relation to Collagen.

The ultimate analyses made of collagen and of gelatin drew the attention of observers to the fact that the composition of these two bodies is very similar if not identical. They contain carbon, hydrogen, oxygen, nitrogen, and, according to some authors, sulphur.

The following analyses indicate the composition of these bodies.

	Substance of tendons.	Gelatin from tendons.
Carbon	50·9	50·2
Hydrogen	7·2	6·7
Nitrogen	18·3	17·9
O and S	23·5	25·0

According to Schützenberger and Bourgeois, sulphur is not an essential constituent; these authors ascribe to gelatin the formula $C_{76}H_{124}N_{24}O_{29}$.

The relations of gelatin to collagen have been made the subject of a very interesting study by Hofmeister. This author has found that by heating gelatin for some time at 130°, it loses about 0·755 per cent. of water and becomes converted into a body in all respects identical with collagen. Collagen is, therefore, probably an anhydride of gelatin.

The following are the mean results of the analyses of collagen (Hofmeister).

Carbon	50·75
Hydrogen	6·47
Nitrogen	17·86
Oxygen	24·92

To gelatin Hofmeister ascribes the formula $C_{102}H_{151}N_{31}O_{39}$, and collagen is probably related to it as shewn in the following equation:

$$\underbrace{C_{102}H_{151}N_{31}O_{39}}_{\text{Gelatin}} - H_2O = \underbrace{C_{102}H_{149}N_{31}O_{38}}_{\text{Collagen}}$$

Products of decomposition of gelatin.

a. Action of boiling water. When solutions of gelatin are subjected to the action of boiling water for a longer period than 25 hours they lose the property of gelatinizing, and are found to contain a mixture of two peptone-like bodies, to which Hofmeister has given the name of Semiglutin and Hemicollin.

Semiglutin is very little soluble, whereas hemicollin is soluble, in 70—80 per cent. alcohol.

The former substance is precipitated by platinum tetrachloride, the latter is not. Both bodies furnish glycine and leucine when treated with boiling hydrochloric acid and stannous chloride. To semiglutin Hofmeister ascribes the formula $C_{55}H_{85}N_{17}O_{22}$ and to hemicollin $C_{47}H_{70}N_{14}O_{19}$. In the process of decomposition into these bodies collagen takes up water, increasing 2·22 per cent. in weight. The following equation exhibits the relationship of these bodies, according to Hofmeister,

$$\underbrace{C_{102}H_{149}N_{31}O_{38}}_{\text{Collagen}} + 3H_2O = \underbrace{C_{55}H_{85}N_{17}O_{22}}_{\text{Semiglutin}} + \underbrace{C_{47}H_{70}N_{14}O_{19}}_{\text{Hemicollin}}$$

b. Action of boiling sulphuric acid. When subjected to the action of boiling sulphuric acid, collagen and gelatin yield, amongst other products, *ammonia, leucine, glycine,* and, perhaps, *aspartic acid.*

c. Action of caustic baryta in heated sealed tubes. When heated with solution of caustic baryta in sealed tubes, *ammonia, carbon dioxide, acetic* and *oxalic acids,* and a mixture of *amido-acids* (containing *glycine* and *alanine, amido-butyric acid,* traces of *glutamine,* &c.) are obtained.

d. Action of pancreatic ferments. When subjected to the combined action of the pancreatic ferment and putrefaction gelatin yields *gelatin-peptones, leucine, glycine, volatile fatty acids, ammonia* and *carbon dioxide.* Amongst the fatty acids are *acetic, butyric* and *valerianic acids* (Nencki[1]).

The Elastic Fibres of Connective Tissue.—Elastin.

When ordinary connective tissue is treated with acetic acid, the white fibres swell up and become transparent, whilst the elastic fibres remain unaltered and are therefore very distinctly seen. When the same connective tissue is subjected to prolonged boiling in water, the collagen of the white fibres undergoes solution and there is left a network of elastic fibres. These fibres are composed of an elastic substance which offers remarkable resistance to the action of chemical reagents, and to which the term Elastin has been applied.

[1] Nencki: *Ueber die Zersetzung der Gelatine und des Eiweisses bei der Fäulniss mit Pancreas.* Bern, 1876. Abstracted in Maly's *Jahresbericht,* Vol. VI., p. 31.

Preparation of Elastin. The *ligamentum nuchae* of the ox, horse, or still better of the giraffe, is cut into thin slices, which are boiled in ether and in alcohol, and then for at least 36 hours in water. The two first of these reagents free the tissue from fatty matters, whilst the prolonged action of boiling water converts all the collagen into gelatin which passes into solution. The insoluble matter is boiled in strong acetic acid for a long time: then after removal of the acid by water it is boiled in concentrated solution of caustic soda until the fibres begin to swell. The tissue is then successively heated with dilute acetic acid and with water, and lastly placed for 24 hours in moderately concentrated hydrochloric acid. The substance remaining is washed with water until all acid is removed. It is then found to retain all the original characters of fresh elastic tissue.

Composition of Elastin. Müller[1] analysed elastin which he had prepared by following the previously described process and found it to have the following composition.

	(1)	(2)	(3)	(4)
Carbon	55·47	55·72	55·55	55·09
Hydrogen	7·54	7·67	7·11	7·33
Nitrogen	16·09	15·71	16·52	16·43
Oxygen	20·90	20·70	20·82	21·15

Solubility. So far as is known elastin is not soluble in any liquid which does not decompose it. It is soluble in boiling solution of caustic potash, in cold concentrated suphuric acid, and in concentrated nitric acid.

It is gradually dissolved when digested with pepsin and with trypsin, the former ferment being more active than the latter[2].

Products of decomposition. When boiled with sulphuric acid elastin is said to yield leucine but no tyrosine.

Connecting or Ground substance of Connective Tissue.

Absorption of silver salts by the connecting substance, and subsequent reduction. When perfectly fresh connective tissue is immersed for a few minutes in a solution of silver nitrate (0·25 to 1·0 p.c.), then freed from excess of silver by washing with distilled water, and afterwards exposed to light, a deposition of metallic silver occurs which appears to be deposited in the connecting substance which is interposed between the various tissue elements. The protoplasmic elements of the tissue are left perfectly unstained, so that the silver treatment furnishes the histologist with one of the best methods of studying their arrangement. It has been held by many that the absorption of silver, which is afterwards reduced, is a characteristic

[1] *Zeitschrift f. rat. Med.* Third Series. Vol. x., part 2.
[2] A. Ewald und W. Kühne, *Die Verdauung als histologische Methode.*

property of the substance, but this view is probably incorrect, the absorption of the silver salt being merely due to the physical conditions of the connecting substance rather than to any peculiar chemical property which it possesses[1].

Solubility of the connecting substance in solutions of the Alkalies.

When any of the forms of connective tissue proper are macerated for some days in baryta- or lime-water, the various tissue elements fall asunder, in consequence of the solution of the connecting substance. If the alkaline solution thus obtained be treated with a dilute acid, a precipitate insoluble in excess of the reagent is obtained, which, after careful washing with water, is found to be insoluble in alcohol and ether, and when burned on platinum leaves no perceptible amount of ash. This body is now looked upon as identical with a substance which is pretty widely distributed, and which will be conveniently described in this place, viz. *Mucin*.

Mucin.

Distribution.

This body, besides forming apparently a small proportion of all connective tissue proper, is present in specially large quantities in embryonic connective tissue, and in that form of the tissue occasionally met with in the adult animal and which is termed *Gelatinous connective tissue*.

It is found in the epidermis, where it connects together the epithelial cells. It is found in considerable proportions in *synovia*.

It is a frequent product of the activity of certain epithelial cells and is the chief constituent in the viscid tenacious liquid called Mucus, which often covers epitheliated surfaces.

Mucus is a colourless, viscid, semi-liquid substance varying very greatly in consistence. It is sometimes transparent, but often turbid from the presence of epithelial cells or foreign matters. It contains, besides mucin, which is its chief organic constituent, small quantities of proteid substances, and salts, amongst which common salt preponderates.

Mucin constitutes the chief ingredient of the tissues of certain invertebrates, and indeed much of our knowledge of mucin is derived from Eichwald's investigations of this body obtained from *Helix pomatia*. It is not however certain that mucin from this source is identical with that of the mucous membranes and tissues of man.

Modes of preparation of Mucin.

(*a*) *From connective tissue* (*Rollett's method*[2]). Tendons are finely divided and treated with large quantities of distilled water, with the object of removing albuminous and saline matters soluble in that liquid. They are then digested for many days in large quantities of lime- (or baryta-) water. The solution is precipitated by acetic acid, which throws down a pre-

[1] Consult Robinsky, "Die Kittsubstanz auf Reaction des Argentum nitricum." *Archiv f. Anat. u. Physiol.*, 1871, p. 184.

[2] Rollett, *Sitzungsberichte der Wiener Akademie*, Bd. 39, p. 308: Stricker's *Handbook*, Vol. I. p. 72.

cipitate which at first appears granular, but afterwards flocculent. The precipitated body may be collected on a filter, and washed with water or dilute alcohol.

(*b*) *From bile.* As bile often contains very large quantities of mucin, it may serve as raw material for its preparation. The bile is treated with its own volume of 80 p.c. alcohol, which throws down a precipitate composed of mucin mixed with epithelium, proteids, &c. The precipitate is separated by decantation and washed with fresh alcohol, it is then suspended in a large quantity of lime-water; after some days the solution is decanted and precipitated with acetic acid, the precipitate is washed successively with water, alcohol and ether[1]. This method may with slight modifications be employed to separate mucin from *sputum*, or other liquids containing the body. In the case of sputum it would be well to follow Gautier's advice, to commence by washing with water acidulated with acetic acid.

Properties of mucin. Mucin when freshly precipitated is a glutinous substance, which forms with water an opaque liquid in which it is held in suspension without being dissolved. It is soluble in weak solutions of the alkalies and alkaline earths, from which it is precipitated by dilute acids, acetic acid being usually employed for this purpose.

It is insoluble in dilute hydrochloric acid, containing from 0·1 to 1 p.c. of real acid; but it is soluble in hydrochloric acid of 5 p.c.

Mucin which has been precipitated by acids is insoluble in solutions of common salt.

Mucin is not digested by artificial gastric juice; it is dissolved by alkaline solutions of *trypsin*.

Mucin is precipitated by acetate of lead from neutral or weakly alkaline solutions, but by no other metallic salts. It is not precipitated by acetic acid and potassium ferrocyanide: it is also unaffected by tannic acid.

When treated with copper sulphate and caustic potash it prevents the precipitation of cupric hydrate; the solution is not reduced on boiling.

When boiled with Millon's reagent, mucin gives a rose colouration.

Elementary composition of Mucin. Mucin contains the elements C, H, N, O, but no sulphur. The following are the results of elementary analyses by various investigators.

	I. (Scherer). Mucin from mucous contents of a cyst.	II. (Obolensky). Mucin from submaxillary gland.	III. (Eichwald). Mucin from *Helix pomatia*.
Carbon	52·17	52·31	48·94
Hydrogen	7·01	7·22	6·81
Nitrogen	12·64	11·84	8·50
Oxygen	28·18	28·63	35·38

[1] Gautier, *Chimie appliquée à la Médecine*, Vol. II. p. 126.

It is impossible to study these analyses without concluding that, though agreeing in general chemical reactions, the mucin-like constituent of the tissues of invertebrates is a different substance from the mucin obtained from mucous membranes.

Products of decomposition of mucin. When boiled for twenty or twenty-five minutes with dilute sulphuric acid, mucin is decomposed with the formation of acid albumin and a body possessing the property of reducing salts of copper and bismuth similar to those of glucose. That this body is not a sugar is proved by the facts that it does not rotate polarized light, and that it is incapable of alcoholic fermentation; it appears to be a nitrogenous body[1].

When boiled with strong sulphuric acid for seven hours mucin yields *leucine* and *tyrosine* (Obolensky).

When boiled with caustic soda, on neutralizing and shaking with ether, the latter fluid dissolves a body which possesses the reaction of *pyrocatechin* ($C_6H_6O_2$), *i.e.* is coloured of an emerald green colour by solution of ferric chloride (Obolensky).

The products obtained when mucin is subjected to the action of pancreatic ferment, prolonged until putrefaction sets in, have been studied under Nencki's direction by Wälchli[2]; amongst them were found ammonia, indol, a large quantity of butyric acid, and a substance possessed of a sweet taste and reducing copper salts.

Relations of mucin. Mucin is unquestionably a product of the differentiation of the protoplasm of certain animal cells, and is obviously derived from the proteids. It is conceivable that it may result from a decomposition in which both collagen and mucin originate; what the nature of the decomposition may be is, however, quite unknown.

Sect. 2. Adipose Tissue.

Structural Elements of Adipose Tissue. Fat occurs in the animal body either in a state of solution or minute suspension in its juices, or deposited within the interior of cells. This is especially the case in cells which are developed in, and supported by, the connective-tissue of certain regions; these cells, which originally are identical with connective-tissue cells, undergo changes which ultimately result in the diminution of the cell protoplasm (at the expense of which, or through the agency of which, oily matter is deposited within the cell) and in the development of a well-marked cell-wall which serves to contain their oily contents. These oily contents undergo remarkable fluctuations according to the state of nutrition of the animal.

Fat cells are usually found in groups or clusters, supported by the fibrous elements of connective tissue, and surrounded by a network

[1] Obolensky, "Ueber Mucin aus der Submaxillardrüse." Pflüger's *Archiv*, Vol. IV. p. 336.

[2] Wälchli, *Ber. d. deutschen chem. Gesellsch.* XI. 1878, p. 509.

of capillaries. They develope with special frequency in areolar tissue, especially in the subcutaneous areolar tissue and in the connective tissue which lies around and between certain of the abdominal viscera. In certain situations (as *e.g.* in the orbit) the areolar tissue is never free from fat; in other situations, as in the subcutaneous connective tissue of the eyelids, of the penis, and scrotum, fat cells are never present.

The fully formed fat cell presents the appearance of a little bag distended with glistening oily contents, and with no appearance of a nucleus or of typical protoplasm. In reality, however, it can be shewn that even the fully formed fat cell contains a nucleus with remains of the original cell protoplasm around it, though these are so pressed upon and surrounded as to be invisible until some cause comes into operation to remove a part at least of the oily contents.

The contents of the fat cells are during life of fluid consistence and only solidify when the tissue containing them is cooled below 25° C. When solidifying, the oily matter often separates, at least in part, in the form of groups of needles; sometimes in small single needles.

When adipose tissue is treated with ether, this fluid extracts and dissolves more or less completely the fatty contents of the cells, in which the remains of the nucleus and protoplasm may be then detected.

The wall of the fat cell is not acted upon by acetic acid nor by dilute mineral acids; it is easily dissolved by natural or artificial gastric juice. When treated with a solution of perosmic acid, the fatty matter contained in fat cells is stained of an intense black colour; this reagent is admirably adapted for the microchemical detection of fatty bodies.

In this section a description will be given of the principal constituents of the adipose tissue of man and the higher animals, the discussion of the origin of fat in the economy being postponed to that division of this work in which certain general chemical processes of the body are treated of under the heading of Nutrition. A consideration of the fatty matters which occur in particular organs or fluids (as for instance in the nervous organs and in milk) is given in the chapters devoted to these subjects.

Mode of extracting the fats of adipose tissue.

The fatty matters which are contained in adipose tissue are best extracted by drying the tissue so as to expel the water which it contains, and then boiling the finely divided or comminuted tissue in ether, which dissolves all the fats. The etheral solution is then evaporated to dryness. The residue contains the fats, mixed with small quantities of other bodies soluble in ether, such as cholesterin and lecithin.

In some cases the fatty matters of adipose tissue can be separated in large quantities by the combined action of heat (which causes the fats to melt) and pressure; or by boiling the tissue with water, when the melted oil floats to the surface and can be skimmed off. These

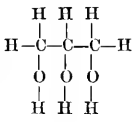
two methods are employed in the commercial separation of fats from certain animal products.

Chemical constitution of the neutral fats.

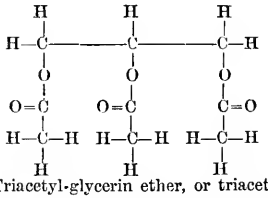
The fats which are contained within the fat cells of man and the higher animals are mixtures of the so-called neutral fats termed *stearin, palmitin,* and *olein,* of which the two former are solid bodies at ordinary temperatures, and are, at the temperature of the body, held in solution by the third.

The neutral fats are the most abundant of the non-nitrogenous organic proximate principles of the body and contain the elements carbon, hydrogen and oxygen. These fats consist of ethers derived from the triatomic alcohol glycerin $C_3H_5(OH)_3$.

We may form a true conception of the relations of a neutral fat to glycerin by examining the relations of an artificial neutral fat, or glycerin ether, *triacetin,* to *glycerin,* and these will be easily illustrated by the aid of the two graphic formulae here appended. The three carbon atoms of glycerin are seen to be linked to the O atoms of three OH groups; the H in any one or in all of these may be replaced by the oxidized radical of a fatty acid, *e.g.* by acetyl C_2H_3O; when all three of the hydrogens are thus replaced the neutral fat called triacetin is formed, thus :—



Glycerin.



Triacetyl-glycerin ether, or triacetin.

The neutral fats of adipose tissue are constituted on the same type as triacetin, except that, instead of acetyl, other acid radicals take the place of the H in the OH groups.

In the two more solid fats, *stearin* and *palmitin,* the oxidized radicals of *stearic* and *palmitic* acids occupy the position of the acetyl of triacetin; in the more liquid constituent of the fats, viz. olein, the oxygenized radicals of oleic acid occupy the same position.

Sometimes, instead of the terms *stearin, palmitin,* and *olein,* the more precise designations of tristearin, tripalmitin and triolein are employed.

The formulae of the three principal fats are appended and their relationship shewn to glycerin and the acids.

	Palmitin	$C_3H_5(OC_{16}H_{31}O)_3$	Palmitic acid	$C_{16}H_{31}O,OH$
Glycerin $C_3H_5(OH)_3$	Stearin	$C_3H_5(OC_{18}H_{35}O)_3$	Stearic acid	$C_{18}H_{35}O,OH$
	Olein	$C_3H_5(OC_{18}H_{33}O)_3$	Oleic acid	$C_{18}H_{33}O,OH$

General properties of the neutral fats.

The neutral fats are all solid at a certain temperature, above which they are fluid; this temperature is called their *melting-point.* They are all soluble in boiling alcohol, in ether, benzol, carbon disulphide

and chloroform. When fluid they render paper which is coated with them transparent (*grease spots*). When mixed with colloid substances and water, they admit of being broken up into fine drops, so that the fluid becomes white and opaque (an emulsion). Under the influence of certain ferments (*e.g.* one of the ferments contained in the pancreatic secretion) they combine with the elements of water, splitting up into glycerin and a fatty acid; thus:—

$$\underset{\text{Tripalmitin.}}{C_3H_5(OC_{16}H_{31}O)_3} + \underset{\text{Water.}}{3H_2O} = \underset{\text{Glycerin.}}{C_3H_5(OH)_3} + 3\,\underset{\text{Palmitic acid.}}{(C_{16}H_{31}O, OH)}.$$

The rancid smell of decomposing fats is due to volatile acids which are set free.

When the neutral fats are boiled with solutions of the alkaline hydrates or carbonates they undergo the process of *saponification, i.e.* they combine with the elements of water, and decompose into glycerin and fatty acids, the latter constituents combining at once with the alkaline metal to form a soluble salt, termed a *soap*. Thus when stearin, palmitin or olein is boiled with potash hydrate or with sodium hydrate, the results of the operation are, in the first case, potassium stearate, palmitate, or oleate, respectively and glycerin; in the second case sodium stearate, palmitate, or oleate, and glycerin.

When boiled with litharge the neutral fats yield an insoluble lead soap and glycerin.

The term *soap* is applied to the metallic salts of the fatty acids, and hence the term *saponification* is employed to designate the process which results in the formation of these compounds.

Stearin or Tristearin. $C_3H_5(O.C_{18}H_{35}O)_3$.

Stearin is the chief constituent of the more solid fats. Its melting-point is higher than that of any other neutral fat, but varies, according to the treatment to which it has been subjected, between 53° and 66°. It is nearly insoluble in cold alcohol and ether, though soluble in both these fluids when these are boiled. The insolubility in cold ether is taken advantage of in the preparation of pure stearin.

Stearin may be obtained from the suet of the sheep by extracting it repeatedly with cold ether, and dissolving the residue in boiling ether, which, on cooling, deposits crystals of stearin, in the form of little leaflets which shine like mother-of-pearl. From a boiling alcoholic solution stearin is deposited in brilliant scales, which are almost square rhombic crystals having angles of 90°·5.

Palmitin or Tripalmitin. $C_3H_5(O.C_{16}H_{31}O)_3$.

Is the more abundant of the two solid neutral fats in the adipose tissue of man. It is more soluble in cold and in hot alcohol and ether than stearin; it is deposited from saturated solutions in the form of fine needles, which radiate from a centre and appear as delicate filaments.

Its melting-point varies, like that of stearin, according to its treatment; the temperature at which it solidifies, after being melted, is said to be 45° C.

Margarin a mixture of stearin and palmitin.

From a mixture of stearin and palmitin, crystals often separate which consist of a mixture or perhaps of a combination of stearin and palmitin, but which were formerly supposed to be a special fat to which the name of Margarin was given and which was supposed to be a glycerin ether of margaric acid ($C_{17}H_{34}O_2$).

The crystals which form not unfrequently in fat cells were formerly supposed to consist of this compound.

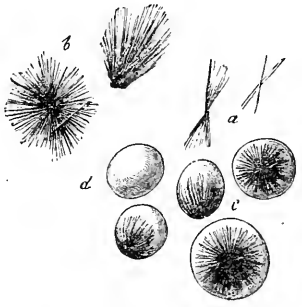


FIG. 51. CRYSTALS OF THE SO-CALLED MARGARIN.

a. single needles. *b*. larger groups. *c*. crystals within fat cells. *d*. a fat cell containing no crystals. (Funke.)

Olein or Triolein. $C_3H_5(O \cdot C_{18}H_{33}O)_3$.

This neutral fat is obtained in a state of comparative purity from the more liquid fats by exposing them to a temperature of 0° C. and then subjecting to pressure; the liquid portion expressed consists of olein.

When pure, olein is a colourless oil which is fluid at ordinary temperatures and which solidifies when the temperature falls below 0° C. When exposed to air olein absorbs oxygen, and in doing so it acquires a faint yellow colour.

It dissolves all the solid fats, especially at a temperature of 30° C. Olein is easily soluble in cold absolute alcohol or in ether.

Glycerin. $C_3H_5(OH)_3$.

Mode of preparation from the neutral fats.

As has been already stated, when the neutral fats are saponified, glycerin is set free. If the neutral fats be boiled with litharge and water, the fatty acids are all thrown down as insoluble lead soaps, and glycerin dissolves in the water. By passing a stream of sulphuretted hydrogen, it is freed from dissolved lead, and on filtering and evaporating the

solution a syrupy liquid—glycerin—is left. This instructive method of preparing glycerin is not at present employed in the arts, the substance being now obtained by decomposing and distilling the neutral fats by means of superheated steam.

Properties of Glycerin. Glycerin is a colourless, syrupy liquid of intensely sweet taste, having a specific gravity of 1·27, and soluble, in all proportions, in water. It becomes solid at -40° C. It boils at 280°. When heated with the fatty acids it combines with them, forming ethers which are constituted as the fats. Thus by the action of acetic acid on glycerin at 100° C. a body termed *monacetin* is obtained; by the action of acetic acid at a higher temperature *diacetin* is obtained, and again by reacting further with acetic acid on the latter body, *triacetin* is formed. The H of the three hydroxyls of glycerin is in this case successively replaced.

$C_3H_5\{OH\ OH\ OH$ Glycerin. $C_3H_5\{OH\ OH\ OC_2H_3O$ Monacetin. $C_3H_5\{OH\ OC_2H_3O\ OC_2H_3O$ Diacetin. $C_3H_5\{OC_2H_3O\ OC_2H_3O\ OC_2H_3O$ Triacetin.

When glycerin is subjected to the prolonged action of yeast, it yields propionic acid $\left.\begin{matrix}C_3H_5O\\H\end{matrix}\right\}O$.

When distilled with hydriodic acid glycerin yields isopropyl iodide:—

$$\underbrace{C_3H_8O_3}_{\text{Glycerin.}} + \underbrace{5HI}_{\text{Hydriodic acid.}} = \underbrace{C_3H_7I}_{\text{Isopropyl iodide.}} + \underbrace{3H_2O}_{\text{Water.}} + 2I_2.$$

When heated with phosphorus pentoxide, or acid potassium sulphate, or subjected to destructive distillation, glycerin yields acrolein (C_3H_4O), which is the aldehyde of allyl-alcohol (C_3H_5OH); this substance boils at 52·4°. Its vapour possesses an intensely irritating and characteristic odour.

Fatty matters found in the adipose tissue of certain of the lower animals.

Spermaceti. In addition to the three neutral fats which have been mentioned other fats occur in certain members of the animal kingdom. In spermaceti, which is a fatty substance contained in the cranial sinuses of whales, there are no glycerides, but the fats appear to be derivatives of cetyl-alcohol $\left.\begin{matrix}C_{16}H_{33}\\H\end{matrix}\right\}O$, a solid body melting at 50°, the chief compound being cetyl-palmitate; when saponified, spermaceti yields, in addition, stearic, myristic and lauric acid. It is worthy of notice that cetyl-alcohol can be artificially oxidized so as to yield palmitic acid.

Bees' wax. In Chinese wax which is produced by the *Coccus ceriferus*, and in bees' wax, the product of the common bee, the portion of the substance which is soluble in boiling alcohol contains ceryl-cerotate

$\left.\begin{matrix} C_{27}H_{55} \\ C_{27}H_{53} \end{matrix}\right\} O$, which when saponified by boiling with caustic potash yields ceryl-alcohol $\left.\begin{matrix} C_{27}H_{55} \\ H \end{matrix}\right\} O$, which is one of the series of primary alcohols, and cerotic acid, $C_{27}H_{54}O$, which is the normal fatty acid corresponding to the above alcohol. In addition to ceryl-cerotate, free cerotic acid is contained in bees' wax.

In the portion of bees' wax which is insoluble in alcohol there is contained myricyl palmitate, an ether derived from myricyl alcohol $\left.\begin{matrix} C_{30}H_{61} \\ H \end{matrix}\right\} O$.[1]

Analysis of the Fats.

Extraction and determination of the total amount of fat in a tissue.

A weighed quantity of the finely divided tissue in which the fats are to be separated and determined is evaporated to dryness in a water oven. The dry residue is then boiled with ether for a long time. The process may be carried on in a flask connected with an inverted condenser, the flask being heated on the water-bath. The apparatus shewn in Fig. 52, which was devised by Dr Drechsel[2], is perhaps superior to any other for the extraction of fats from animal matters. At *A* is a flask containing ether, into which is fitted a tightly-fitting cork or stopper,

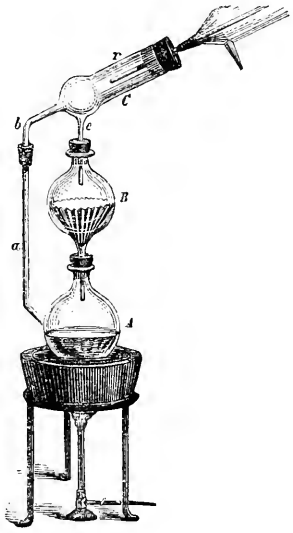


FIG. 52. DRECHSEL'S APPARATUS FOR THE EXTRACTION OF FATS.

perforated so as to allow the lower end of the bulb *B* to fit into it. *B* is closed by a stopper. Into *B* can be passed a plaited filter such as is shewn in the cut, and into this filter the solid is placed from which

[1] Consult Schorlemmer's *Organic Chemistry*, p. 174.
[2] Drechsel, *Journ. f. prakt. Chemie*, Vol. XV. (1877), p. 350 and Plate II.

the fats are to be extracted. *PCbc* is a glass connecter which communicates with an inverted Liebig's condenser, with a stopper which fits into the upper part of *B*, and with the tube *a* which is joined to the side of *A*. When the flask *A* containing ether is placed upon the water-bath, that liquid boils and the vapour passes through *a* and *b* to *bPC*; it ascends into the Liebig's condenser where it is condensed, and it then flows back into *B* over the matter placed upon the plaited filter, and thence into *A*. A continual circulation of ether is thus kept up, and the dissolved fats accumulate in *A*.

The ethereal solution is then evaporated to dryness in a weighed capsule, and the weight ascertained.

Separation of the fatty acids contained in the neutral fats.

The mixture of neutral fats is dissolved in boiling alcohol and the solution is poured into a silver basin, and then treated with an alcoholic solution of caustic potash. The fluid is boiled for some time over a water-bath and then evaporated to dryness. Water is then added to the residue so as to dissolve the soaps which have been formed, the solution is now acidified by the addition of hydrochloric acid, then boiled and allowed to cool. The fatty acids, which have been liberated from the neutral fats, set in the form of an insoluble mass, which is collected on a filter. This is then dissolved in hot alcohol and treated with an alcoholic solution of lead acetate which precipitates all the fatty acids in the form of insoluble lead salts. The precipitate is collected on a filter and dried, and subjected to the action of boiling ether, which dissolves only lead oleate, leaving lead stearate and palmitate. The ethereal solution of lead oleate may be agitated with dilute hydrochloric acid, which will decompose the salt, and the ether will then hold oleic acid in solution which will remain on evaporation; the treatment with HCl should be carried out in an atmosphere of CO_2. The mixture of lead stearate and palmitate is heated with hydrochloric acid and thereafter shaken with ether; the ethereal liquid is freed from acid by shaking with water and the ether is then distilled off, when a mixture of palmitic and stearic acids is obtained.

The melting-point of the mixture is then taken. In order to do this a very thin glass tube is made and a small quantity of the mixed acids is dropped in; the tube is then drawn out slightly. The glass tube is now attached by means of a little india-rubber band (which may be made by cutting a thin circular slice from the end of a narrow india-rubber tube) to a finely graduated thermometer, in such a manner that the part of the tube in which the fat lies is on the same level as the bulb of the thermometer. The latter is then plunged into a beaker containing water, which is immersed into a larger beaker also containing water. (See the arrangement employed for determining the temperature at which solutions of the proteids coagulate at p. 15). The latter is then heated so that the temperature of the water in the inner beaker rises very gradually. The observer watches very carefully the temperature at which the fat melts; he then withdraws the heat from the outer beaker and, as the temperature of the water surrounding the thermometer bulb falls, he notices the temperature of solidification of the previously melted fats.

With the aid of the appended table based upon the observations of Heintz[1] an approximate estimation of the composition of a mixture of stearic and palmitic acids can be made.

A mixture of			
Stearic acid	Palmitic acid	Melts at	Solidifies at
90 parts	10 parts	67°·2	62°·5
80 ,,	20 ,,	65°·3	60°·3
70 ,,	30 ,,	62°·9	59°·3
60 ,,	40 ,,	60°·3	56°·5
50 ,,	50 ,,	56°·6	55°·0
40 ,,	60 ,,	56°·3	54°·5
30 ,,	70 ,,	55°·1	54°·0
20 ,,	80 ,,	57°·5	53°·8
10 ,,	90 ,,	60°·1	54°·5

In order to demonstrate the separate presence of stearic and palmitic acid it is, however, essential to proceed further. The mixture of the acids is dissolved in boiling alcohol and treated with sodium carbonate, then evaporated to dryness on the water-bath; the residue is further heated in the air-bath to 130°. The residue is pulverized and boiled with absolute alcohol; the solution is filtered hot.

Fractional precipitation of a mixture of fatty acids.

This solution is now subjected to *fractional precipitation*, by adding either solution of chloride or acetate of barium. When one of these salts is added, little by little, to a solution containing both stearic and palmitic acids, the precipitate which first falls is composed entirely of barium stearate; the further gradual addition of the barium salt leads to the precipitation of a mixture of barium stearate and palmitate, and if the addition of barium salt be continued after this pure barium palmitate falls. Relying upon these facts the experimenter adds to the alcoholic solution of the mixed fatty acids one or two drops of a solution of barium chloride or acetate, filters, heats the filtrate to boiling, then adds to it one or two drops more of the barium solution, collects the new precipitate on a separate filter, heats the filtrate, and repeats these operations until the addition of barium salt occasions no new precipitate. Each precipitate is collected on a separate small filter, is washed with warm alcohol and dried at 120°C. The barium in each precipitate is then determined by igniting in a porcelain capsule, and adding first hydrochloric and then sulphuric acid to the ash, again igniting, and weighing the barium sulphate. If stearic acid is present the first precipitates should contain the amount of barium corresponding to barium stearate; if palmitic acid, the last precipitates should agree in composition with barium palmitate.

Composition of the barium salts of stearic and palmitic acids.

100 parts of barium stearate contain 19·49 parts of barium.
,, ,, ,, palmitate ,, 21·17 ,, ,,

[1] Poggendorff's *Annalen*, Vol. xcii. p. 588.

Sect. 3. Cartilage.

Structural elements of Cartilage.

Cartilage is a tissue composed of certain cells, termed cartilage cells, imbedded in a *ground substance* or matrix. According to the *predominating* character of this matrix the cartilage may be classified as (1) cellular cartilage, (2) hyaline cartilage, (3) white fibro-cartilage, (4) elastic or spongy cartilage. In the first of these varieties the matrix consists merely of a transparent and very thin envelope termed the *capsule*, surrounding each cartilage cell; these capsules possess the same chemical properties as the matrix of hyaline cartilage; in the second variety the matrix is composed of a translucent homogeneous substance, which occasionally presents an appearance resembling that of ground glass, and sometimes exhibits fibrillation; in the third, the cartilage cells are surrounded by capsules which lie imbedded in a preponderating mass of fibres identical with the white fibres of connective tissue; in the fourth the cartilage cells with their capsules are imbedded in a meshwork of elastic fibres.

Cartilage cells.

The cartilage cell is a mass of protoplasm with one or two nuclei, contained in a cavity which it completely fills and which is bounded by the so-called *capsule* of the cartilage cell, this being intercellular substance which is produced by the differentiation of the cell protoplasm.

It is the capability of producing this intercellular substance which is the very characteristic of the cartilage cell. In cellular cartilage we find an aggregation of cartilage cells, each of which is surrounded by its own capsule: in hyaline cartilage the homogeneous matrix has been produced by the fusion of concentric, and successively developed, cartilage capsules, as can be shewn by subjecting the fully formed and homogeneous tissue to the action of certain reagents, such as a mixture of hydrochloric acid and potassium chlorate, when the appearance of concentric stratification of the matrix around the cartilage cells, is revealed.

In young cartilage cells the protoplasm often contains *glycogen* ($C_6H_{10}O_5$); in the cells of the fully developed tissue fat is often seen.

General Composition of Cartilage.

Cartilage contains more than half its weight of water, though the proportion varies remarkably. Its solid constituents consist mainly of organic matter with a small proportion of salts, in which sulphates and phosphates preponderate.

The following analyses exhibit the relative proportions of water, organic matters and mineral matters in the cartilage of a young and healthy man (Hoppe-Seyler[1]):—

[1] Hoppe-Seyler, quoted by Kühne, *Lehrbuch*, p. 387.

	Costal Cartilages.	Articular Cartilages from knee joint.
Water in 100 parts	67·67	73·59
Organic matters	30·13	24·87
Mineral „	2·20	1·54

The following are the results of Hoppe's analysis of the ashes of human costal cartilage.

Potassium sulphate in 100 parts	26·66
Sodium „	44·81
„ chloride	6·11
„ phosphate	8·42
Calcium „	7·88
Magnesium „	4·55

Chondrigen.

The substance of which the matrix of hyaline cartilage and the capsules of the cartilage cells in the other forms of cartilage is composed, resembles in many particulars collagen, but differs from it in the product which it yields by the prolonged action of boiling water. As it is generally believed that by this action a body to which the term Chondrin has been given is formed, the mother substance has received the name of Chondrigen.

Chondrigen is unacted upon by cold water, and swells very slightly in acetic acid. It is dissolved by concentrated mineral acids and caustic alkalies. When heated (in sealed glass tubes or in a Papin's digester) in water at a temperature of 120° C. for three or four hours chondrigen dissolves; the solution contains chondrin.

Chondrin.

Preparation. Costal cartilage is boiled for a few minutes, and is then scraped so as to remove the perichondrium. It is then finely divided and boiled for twenty-four hours with water; or placed in a Papin's digester, and heated in water at 120° C., for three or four hours. The solution thus obtained is filtered so as to separate it from insoluble matters, such as elastic tissue, cellular elements, &c., and it is then precipitated with acetic acid. The precipitate is then extracted with alcohol or ether. It may be again dissolved in hot water and the solution poured into a large excess of absolute alcohol, when the chondrin which precipitates is separated and dried. When dry it presents the appearance of a hard, transparent mass, devoid of smell and taste [1].

General reactions. Chondrin is insoluble in cold water, in alcohol, ether, or chloroform. It is soluble in hot water, and aqueous solutions of chondrin gelatinize exactly like

[1] The description of the preparation of Chondrin is mainly borrowed from Gautier, *Chimie Appliquée, &c.*, Vol. I., p. 346.

solutions of gelatin. They are precipitated by the following reagents which have no such action when added to solutions of gelatin:—acetic acid, the precipitate being insoluble in excess of the precipitant, but soluble if some alkaline salt be added; solutions of alum, the precipitate being soluble in excess of the reagent; solutions of silver nitrate and copper sulphate, the precipitates being soluble in excess of the reagents; solution of lead acetate, the precipitate not soluble in excess. Solutions of chondrin have been said to be rendered only slightly turbid by mercuric chloride and by tannic acid. The Author is inclined to rely on the very positive statement of J. Müller that these reagents exert the same action on chondrin as on gelatin.

Elementary composition of Chondrin.

Very great discrepancies exist between the results of various analyses of this assumed chemical individual, as will be observed by a study of the following Table.

COMPOSITION OF CHONDRIN.

	Mülder.	Fischer und Boedeker.	Schützenberger und Bourgeois.	v. Mehring.
Carbon	49·3	50·0	50·16	47·74
Hydrogen	6·6	6·6	6·58	6·76
Nitrogen	14·4	14·4	14·18	13·87
Sulphur	0·4	0·4		0·60
Oxygen	29·3	28·6	29·08	31·04

The following table exhibits the relative composition of *gelatin, chondrin* and *mucin,* according to the analyses which appear to be most trustworthy.

	Gelatin (Hofmeister).	Chondrin (Schützenberger)	Mucin (Obolensky).
Carbon	50·75	50·16	52·31
Hydrogen	6·47	6·58	7·22
Nitrogen	17·86	14·18	11·84
Oxygen	24·92	29·08	28·63

Products of decomposition of Chondrin.

When finely divided cartilage is boiled with dilute hydrochloric or sulphuric acids there is formed a body resembling acid albumin, and a substance which possesses a sweet taste and reducing properties analogous to those of true sugars[1]. This body has been termed *Chondri-glucose.*

[1] Fischer und Boedeker, "Künstliche Bildung von Zucker aus Knorpel (Chondrogen), &c." *Annalen der Chemie und Pharm.*, Vol. CXVII. (1861), p. 111.

According to Fischer and Boedeker, this body is laevo-gyrous and is capable of undergoing the alcoholic fermentation. According to Hoppe-Seyler[1], the body which reduces cupric salts is a nitrogenous body and is identical with the body obtained by boiling mucin with dilute acids.

When chondrin is subjected to prolonged boiling with dilute sulphuric acid, it yields leucine, but no tyrosine or glycocine. (Hoppe[1]).

When chondrin is heated with barium hydrate, Schützenberger and Bourgeois[2] have found that the products of decomposition are somewhat different from those yielded by gelatin under the same circumstances. In both cases carbon dioxide, oxalic and acetic acid, and ammonia are obtained, in addition to a mixture of amido-acids. The quantity of acetic acid yielded by chondrin is, however, three times as great as that yielded by gelatin. In the mixture of amido-acids no glycocine is present.

Doubts as to the existence of Chondrin.

Amongst the tissues which are supposed to be composed mainly of chondrigen is the substance of the cornea. In an investigation on the chemical composition of this structure, Morochowitz[3] has arrived at the conclusion that the primitive fibrillae of the ground substance of the cornea consist of collagen, and that the supposed chondrin is a mixture of gelatin and mucin. After extracting the tissue with lime- or baryta-water or with 10 p.c. solution of NaCl, it yields on being treated with boiling water pure gelatin. From the alkaline solutions mucin can be thrown down by the addition of an acid. Morochowitz has further investigated cartilage from various sources, and has found that after treatment with reagents which dissolve mucin, as lime- or baryta-water, 10 p.c. solution of NaCl, or $\frac{1}{2}$ p.c. solution of caustic soda, whilst mucin is removed, the substance which is left undissolved is on boiling readily converted into perfectly normal gelatin. According to this author chondrin is to be looked upon as no pure substance, but as a mixture of gelatin, mucin and salts.

If these views be, as the Author believes, correct, all the tissues belonging to the connective tissue group, possess common chemical character in that their ground substance is in all cases a body transformed into gelatin by the prolonged action of boiling water; this being mixed in greater or less proportion with mucin which, as we have shewn, undoubtedly plays the part in many forms of connective tissue of a connecting or cementing substance.

[1] Hoppe, "Ueber das Chondrin und einige seiner Zersetzungsproducte." *Journ. f. prakt. Chemie*, Vol. LVI. (1852), p. 129.

[2] Schützenberger et Bourgeois, "Recherches sur la constitution des matières collagènes." *Comptes Rendus*, LXXXII. 262.

[3] Morochowitz, "Zur Histochemie des Bindegewebes." *Verhandl. d. naturhist. med. Vereins zu Heidelberg*, Vol. I. part V.

Sect. 4. Osseous Tissue or Bone.

Structural Elements of Bone.

The hard tissue which forms the scaffolding and support of the soft parts of our bodies, although on superficial examination, appearing so different from the other members of the group of connective tissues, possesses the closest affinity to them, as is evident not merely from developmental considerations but from a study of its chemical composition.

All the bones of the skeleton are invested by a fibro-vascular membrane, the *periosteum*, which conveys to them the great majority of the blood-vessels which supply them, and which contains on its inner layer certain cells—*osteoblasts*—which are the active agents in the growth of bone, and in virtue of which the periosteum possesses the power of forming new bone.

Those bones which articulate with others have no periosteal covering over their articular ends, which are tipped with cartilage.

The external part of all bones has a very dense structure; the interior is either hollowed out into a cavity termed the *medullary cavity*, or is occupied by a trellice-work of bony plates which constitute the *cancellated* tissue. Those bones which possess a medullary cavity present near their articular ends much cancellated tissue.

The medullary cavity lodges the *medulla* or yellow marrow, which is composed of fat cells supported by a frame work of connective tissue, and abundantly supplied with blood-vessels; the *cancelli* or spaces of the cancellated tissue afford support to the so-called *red marrow*, which is a tissue in which a large number of cells identical with the colourless cells of the blood are found, besides certain cells which resemble the nucleated coloured corpuscles of embryonic blood.

It also contains large giant cells with many nuclei to which the name of *myeloplaxes* is applied, and which are identical in appearance and probably in functions with those cells which under the name of osteoclasts are supposed to be the active agents in the formation of the *medullary cavities* of growing bone.

Though the chief blood-supply of bone is drawn from the periosteum, both arteries and veins of considerable size enter by so-called 'nutritious foramina' and are distributed to the marrow and so-called *endosteum*, as the connective tissue lining the medullary cavity is called; some of the branches of the nutrient vessels anastomose with the blood-vessels of the hard tissue.

The blood-vessels contained in the hard substance of bone lie in canals—the *Haversian canals*. Around these canals the bony substance is arranged in concentric *lamellae*. In these lamellae are cavities, also arranged concentrically, called *lacunae*, and from these proceed minute canals, the *canaliculi*, which establish a communication between adjacent lacunae, and between the lacunae which are in

the circle nearest the Haversian canal and the canal itself. The lacunae lodge nucleated masses of protoplasm—the *bone corpuscles* or *bone cells*—which do not send processes into the canaliculi.

The walls of the calcified lacunae and canaliculi, as well as of the Haversian canals, appear to be composed of a tissue resembling elastic tissue[1] and are left surrounding the bone cells when softened bone is boiled for many hours in water or when it is subjected to digestion with trypsin[2].

Besides the lamellae or sheets of bony substance which are arranged in concentric layers around the Haversian canals, and which may be termed the *lamellae of the Haversian systems,* other lamellae are arranged concentrically around the medullary canal and immediately beneath the periosteum; these may be termed *fundamental lamellae.*

The minute structure and arrangement of the soft parts of bone can only be studied by making preparations of decalcified or softened bones. In these preparations it may be shewn that the ultimate lamellae of bone are transparent sheets which exhibit intercrossing fibres, which possess the characters of the white fibres of connective tissue. It may further be shewn that the fundamental lamellae are perforated by fibres—the so-called *perforating* fibres of Sharpey—which dip into the bone from the periosteum, and which appear to have mainly the chemical characters of yellow elastic tissue.

The Water found in Bone.

All bones contain, when fresh, a considerable quantity of water. The estimates of various observers differ remarkably in reference to this matter. Volkmann estimates the mean percentage of water at 48·6 p.c. of the fresh bone. According to Aeby's determinations (which are certainly too low) bones just removed from the dead body contain between 11 and 12 p.c. of water. According to this author the water exists in a state of chemical combination, probably analogous to that of water of crystallization. This view is based partly on the constancy of the amount of water, but partly on the fact that heat is evolved when dried bone is placed in water[3].

The Animal or Organic basis of Bone.

Methods of preparing decalcified or softened bone.

When a bone is placed in a dilute mineral acid for some days, it gradually loses its rigidity, and although retaining its form and general appearance, it becomes comparatively soft and pliable, so that a long and comparatively thin bone, such as the clavicle or the radius, may be tied into a knot.

[1] Hoppe, Virchow's *Archiv*, Vol. v. (1853) p. 170.

[2] De Burgh Birch, "Erscheinungen bei Trypsinverdauung an Knochen." *Centralblatt f. d. med. Wissenschaft.* 1879, p. 945.

[3] Aeby, "Der Grund der Unveränderlichkeit der organischen Knochensubstanz." *Centralblatt f. d. med. Wissenschaft.* 1871, No. 14.

The following solutions may be employed for softening bones.

(1) A mixture of one part of hydrochloric acid and five of water.

(2) A mixture of nitric and chromic acids of the following composition: chromic acid 5 grms., nitric acid 10 cub. c., water 1000 c. c.[1]

(3) A solution containing from 2 to 5 parts of chromic acid in 1000 parts of water (Ranvier).

(4) A saturated aqueous solution of picric acid.

In the case of solutions 3 and 4 it is important that the fragments of bone to be softened shall be very small.

Characters of the organic basis of bone.

The organic basis of softened bone is insoluble in cold water, but is for the most part soluble on prolonged boiling in water. The solution contains gelatin, which is identical in its reactions with that body as it is obtained from white fibrous tissue. The structures which are undissolved by boiling water are the perforating fibres, and apparently the decalcified walls of the lacunae, canaliculi, and of the Haversian canals, which appear to be formed of a substance resembling *elastin*.

The organic basis of bone (which has by some writers been termed *ossein*) then consists mainly of a body identical in chemical reactions with *collagen*, mixed with a certain amount of elastin and with the proteid matter of the bone cells. It is to be noticed that the animal matter of cartilage before ossification does not consist of normal collagen but of chondrogen (or, if we adopt Morochowitz's theory, of a mixture of collagen and mucin). In the process of ossification, which consists essentially in an intrusion of periosteal elements into cartilage, which is *pari passu* removed by absorption, the animal matter assumes all the characters of connective tissue proper.

There appears to be always some fat in bone, but its relations to the organic basis are not known.

All organic matters are destroyed when bone is incinerated. The following are the results of some analyses shewing the relative proportion of organic and mineral matters in bone (Zalesky).

		Organic matters.	Mineral matters.
Bone of man	(mean of 4 analyses)	34·56	65·44
„ „ ox	(mean of 6 analyses)	32·02	67·98
„ „ guinea pig	(mean of 2 analyses)	34·70	65·30

The Mineral Matters of Bone.

The mineral matters of bone are deposited in the organic basis in such a manner as to be invisible on microscopic examination. They may for the most part be dissolved by employing the processes already described as producing the decalcification of bone. They may be obtained in a solid form by igniting or incinerating bone, and they then retain the form of the original bones (*calcined bone*).

[1] Rutherford, *Outlines of Practical Histology*, pp. 3 and 82.

The following analyses[1] illustrate the composition of the mineral matters of bone.

I. RESULTS OF THE ANALYSES BY HEINTZ[2].

	Ox.	Sheep.	Man. (1)	Man. (2)
Ca	38·52	38·52	38·59	38·56
PO_4	52·98	53·29	53·75	53·87
CO_3	6·04	5·65	5·44	5·51
Fl	1·89	1·96	1·74	1·58
Mg	0·57	0·58	0·48	0·48

II. ANALYSES OF THE BONES OF CHILDREN. (RECKLINGHAUSEN.)

	Bones of skull of child 3 days old.	Bones of child 14 days old. Skull.	Bones of child 14 days old. Femur.	Bones of child of 6 years. Femur. Cortical layers (diaphysis).	Bones of child of 6 years. Femur. Epiphyses.
Ca	38·41	36·43	37·66	37·98	37·97
PO_4	56·20	56·96	54·81	54·86	56·73
CO_3	4·85	6·02	7·06	6·88	4·97
Mg	0·54	0·59	0·47	0·28	0·33

III. ANALYSES OF THE BONES OF MAN AND THE OX. (ZALESKY.[3])

	Man.	Ox.
Ca	40·13	40·69
PO_4	52·16	53·50
CO_3	7·81	8·45
Cl	0·18	0·20
Fl	0·23	0·30
Mg	0·29	0·28

From all the analyses which have been made we may legitimately conclude that the proportion of mineral to organic matters in bone, and even the relative proportion of the different elements, vary remarkably little in animals of different species, and of different ages.

The chief salts present in bone are five in number, of which four are compounds of calcium, and one a compound of magnesium. They are calcium phosphate, $Ca_3 2(PO_4)$; calcium carbonate, $CaCO_3$; calcium chloride, $CaCl_2$; calcium fluoride, $CaFl_2$; magnesium phosphate, $Mg_3 2(PO_4)$. In addition to these, very small quantities of sulphates and chlorides are always present.

[1] Extracted *verbatim* from Hoppe Seyler, *Physiologische Chemie*, p. 105.

[2] Heintz, "Ueber die chemische Zusammensetzung der Knochen." Poggendorff's *Annalen*, Vol. LXXVII. (1849) p. 267.

[3] Zalesky, "Zusammensetzung der Knochen von Menschen und Thieren." *Med. chem. Untersuchungen* von Hoppe-Seyler. Part 1. p. 19 et seq.

The following exhibits the probable composition of the mineral matters of bone calculated from the analyses of Zalesky.

Calcium phosphate ($Ca_3 2PO_4$)	83·889
Calcium carbonate ($CaCO_3$)	13·032
Calcium in combination with fluorine, chlorine and organic acids	0·350
Fluorine	0·229
Chlorine	0·183
	98·722

The occurrence of considerable quantities of a fluoride in bone has, since it was first discovered, attracted the attention of many investigators (Chevenix, Morichini[1], Gay Lussac, Berzelius[2], G. Wilson and others). The presence of this element is readily proved by heating powdered bone with strong sulphuric acid in a leaden or platinum capsule, when hydrofluoric acid is given off, as can be proved by its etching glass.

Constitution of the mineral matters of bone.

It has been surmised that a combination of calcium phosphate and calcium fluoride having the same *constitution* as the mineral *Apatite* exists in bone; the composition of this mineral is shewn by the formula $Ca_{10}Fl_2, 6(PO_4)$.

In bone, however, the fluorine is present in very minute quantities, the main compound having probably the composition $Ca_{10}CO_3, 6(PO_4)$. This matter is discussed again in connection with dentine and enamel (see p. 291).

Zalesky has shewn that chlorides exist in bone in two conditions, a portion being soluble in water, and another portion being only dissolved by acids.

Influence of food on mineral matters of bone.

The influence of food, rich or poor in earthy salts, upon the composition of bone has been studied by various writers with entirely different results. Thus Forster[3] observed a diminution in the proportion of calcium in the bones of dogs fed upon a diet in which calcium salts were deficient. In similar experiments performed on dogs, Zalesky[4] obtained altogether negative results. Weiske[5] came to similar con-

[1] An account of Morichini's discovery of fluorine in fossil teeth was given in a letter addressed by Gay Lussac to Berthelot in the *Annales de Chimie* of 30 Fructidor, an 13 (1805).

[2] Berzelius, "Extrait d'une lettre à M. Vauquelin sur le fluate calcaire contenu dans les os et dans l'urine." *Ann. de Chim.*, Vol. LXI. (1807) p. 256.

[3] J. Forster, "Ueber die Verarmung des Körpers speciell der Knochen an Kalk bei ungenügender Kalkzufuhr." *Zeitschrift f. Biolog.*, Vol. XII. p. 464.

[4] Zalesky, *Op. cit.*, p. 44 et seq.

[5] Weiske, "Einfluss verschiedener der Nahrung beigemengter Erdphosphate auf die Zusammensetzung der Knochen." *Zeitschr. f. Biolog.*, Vol. VIII. p. 239. "Ueber Knochenzusammensetzung bei verschiedenartiger Ernährung." *Zeitschr. f. Biol.*, Vol. X. p. 410.

clusions. This matter will be referred to again (p. 282) in considering the etiology of Rickets.

It was asserted by Papillon[1] that when animals are supplied with food specially rich in magnesium, aluminium, and strontium salts, these elements enter into the composition of the mineral matter of the bones. J. König[2] contradicts the researches of Papillon in so far as compounds of magnesium and aluminium are concerned, but confirms them in respect to strontium. In the bones of rabbits fed with strontium phosphate, he found as much as 5·37 p.c. of strontium. According to Weiske[3] both Papillon and König have fallen into error. In the bones of rabbits fed with strontium phosphate, Weiske found only minute traces of strontium.

The Composition of the Marrow of Bone.

As has been already said, it is customary to distinguish between the yellow marrow, which is contained in the medullary cavity of the long bones, and the red marrow which is lodged in the cancellated tissue of spongy bone.

The former on microscopical examination has all the characters of adipose tissue, being composed of fat cells supported by connective tissue fibres and blood-vessels; the latter contains cells which resemble the white cells of the blood, and certain cells which resemble the nucleated coloured corpuscles of the blood of the embryo.

The dried yellow marrow consists chiefly of fat which appears to have the normal composition of the fatty matter of adipose tissue. The red marrow is said to contain albumin and a free organic acid, supposed by Berzelius[4] to be lactic acid.

Heymann[5] has detected hypoxanthin in marrow of healthy bones, and Nasse[6] has found in the red marrow of the ribs of old horses, microscopic agglomerations of granules, having a diameter of from 0·007—0·015 mm., which contain oxide of iron (probably also ferric phosphate) and organic matters and are coloured intensely blue by ferrocyanide of potassium; these are identical with similar bodies found in the spleen of man and the horse.

These chemical facts, taken in connection with the observations of cases of myelogenic leukaemia, give great countenance to the view

[1] Papillon, "Recherches expérimentales sur les modifications de la composition immédiate des os." *Comptes Rendus*, Vol. LXXVI. (1873) p. 352.

[2] König, "Substitution des Kalkes in den Knochen und Einfluss kalkarmer Nahrung auf die Zusammensetzung der Knochen." *Zeitschrift f. Biolog.*, Vol. X. p. 69.

[3] Weiske, *Zeitschr. f. Biol.*, Vol. X. p. 410.

[4] Berzelius, quoted by Gorup-Besanez, *Phys. Chem.*, p. 631.

[5] Heymann, "Ueber das Vorkommen von Hypoxanthin im normalen Knochenmarke." Pflüger's *Archiv*, Vol. VI. p. 184.

[6] Nasse, "Ueber das Vorkommen eisenhaltiger Körner im Knochenmarke." Abstracted in Maly's *Jahresbericht*, Vol. VII. (1878) p. 300. "Ueber den Eisengehalt der Milz." Maly's *Jahresbericht*, Vol. IV. (1874) p. 91.

entertained by many histologists that the red marrow is an organ concerned in the transformation of the coloured cells of the blood.

In the marrow of the bones of rabbits, Rustitzky[1] found mucin; he was unable to discover this substance in the fat marrow of ox bones.

RESULTS OF COMPARATIVE ANALYSES OF BONES BELONGING TO DIFFERENT MEMBERS OF THE ANIMAL KINGDOM. (FRÉMY.[2])

Name of Bone.	Ash per cent.	Calcium Phosphate.	Magnesium Phosphate.	Calcium Carbonate.
Male foetus, 4 months; femur	61·7	60·2		
„ „ 6 months „	62·8	60·2		
Female foetus „	63·0			
„ „ 7 months; humerus	62·8			
Girl, born at term; femur	64·8	60·8		
Boy, 18 months „	64·6	61·5		
Woman, 22 years; scapula	63·3	60·0		
„ „ cranium	64·1	57·8		
„ „ femur	64·6			
„ „ humerus	64·1			
Man; spongy part of femur	61·0			
„ dense „ „	65·0			
Man, 40 years; femur	64·2	56·9	1·3	10·2
Woman, 80 „ „	64·6	60·9	1·2	7·5
„ 81 „ „	64·5	58·1	1·2	10·0
„ 88 „ „	64·3	57·4	1·3	9·3
„ 88 „ spongy part of femur	59·7	54·0	1·2	7·0
„ 97 „ femur	64·9	57·0	1·2	9·3
Egyptian mummy, female; femur	65·0	58·7	1·7	5·9
Saky; femur	64·0			
Kinkajou; femur	62·0			
Genet	70·2			
Bitch; femur	62·1	59·0	1·2	6·1
Young lioness; femur	64·7	60·0	1·5	6·3
Panther; femur	65·6			
Walrus	63·1	53·9	1·5	9·3
Rabbit; femur	66·3	58·7	1·1	6·3
Guinea pig	71·8			
Indian elephant	66·8	62·2	1·2	5·6

[1] Rustitzky, *Centralblatt f. d. med. Wissenschaft.* 1872, p. 562.

[2] E. Frémy, "Recherches chimiques sur les os." *Annales de Chimie et de Physique*, ser. 3, Vol. XLIII. pp. 47—107.

Name of Bone.	Ash per cent.	Calcium Phosphate.	Magnesium Phosphate.	Calcium Carbonate.
Java rhinoceros	65·3	60·0	2·3	5·2
Horse; femur	70·4			
Calf, still-born; spongy part of femur	61·5	60·5	1·2	
„ „ dense „ „	64·4	59·4	1·7	5·2
„ 5 months; femur	69·1	61·2	1·2	8·4
Cow, full grown „	70·7			
„ old „	71·1			
„ „ „	71·3	62·5	2·7	7·9
Ox; humerus	70·4	61·4	1·7	8·6
„ „	70·2	62·4	1·7	7·9
Bull; femur	69·3	59·8	1·5	8·4
Lamb „	67·7	60·7	1·5	8·1
Sheep „	70·0	62·9	1·3	7·7
Kid „	68·0	58·3	1 2	8·4
Cachalot „	62·9	51·9	0·5	10·6
Whale; spongy part of femur	57·5			
Eagle	70·5	60·6	1·7	8·4
Vulture	66·2			
Owl	71·3	61·6	1·5	8·8
Ostrich; dense part of femur	70·0			
„ spongy part „	67·0			
Bustard	71·1			
Chicken	68·2	64·4	1·1	5·6
Turkey	67·7	63·8	1·2	5·6
Partridge	70·7	65·4		
Heron	70·6	62·5	1·5	10·2
Thrush	66·6	63·0		
Humming bird; bones of head	55·0			
„ „ „ limbs	59·0			
Teal	73·5	68·4	1·3	5·6
Turtle; carapace	64·3	58·0	1·2	
Land tortoise; carapace	64·0	56·0	1·2	10·7
Crocodile; cutaneous bone	64·6	58·3	trace	9·7
Crocodile	64·0	58·3	0·5	7·7
Serpent	67·5			
Cod	61·3	55·1	1·3	7·0
Barbel	60·2			
Sole	54·0			
Shad	50·0			
Carp	61·4	58·1	1·1	4·7
Pike	66·9	64·2	1·2	4·7
Eel	57·0	56·1	traces	2·2
Dogfish	62·6			
Ray; cartilage	30·0	27·7	trace	4·3

Composition of fossil bones.

Fossil bones contain a smaller quantity of organic matter than recent bones. This appears, however, to yield normal gelatin on boiling. They contain the same mineral matters as recent bones.

ANALYSES OF VARIOUS FOSSIL BONES. (FRÉMY.[1])

Name of Bone.	Ash per cent.	Calcium Phosphate.	Magnesium Phosphate.	Calcium Carbonate.	Calcium Fluoride and Silica.	Organic matter.
Ox, from the caves of Oreston; metatarsal bone, external portion having the aspect of wood	80·74	71·1	1·5	11·8		10·3
— internal portion of same (very friable).	80·6	71·5	1·7	11·3		11·0
— spongy portion of same	84·2	63·3	1·2	5·2	17·2	8·0
Rhinoceros from Sansan (Gers)						
— vertebrae	83·4	59·0		41·3	2·6	trace
— ribs	83·1	66·8		27·5	1·4	trace
Hyena, from the caves of Kirkdale; long bone	75·5	72·0	1·3	4·7		20·0
Rhinoceros; dorsal vertebrae	69·5	25·7	0·4	57·5	8·5	
Rhinoceros; humerus	73·0	32·4	0·4	64·0	6·2	
Bear; dense part of bones	83·9	59·7	0·4	23·6	9·8	
„ spongy part	76·7	23·1	1·2	67·5	14·0	
Anoplotherium; caudal vertebra	84·0	53·1	0·4	20·4	19·4	
Tortoise; vertebrae	87·0	61·1	0·7	10·6	18·6	

THE CHANGES WHICH BONE UNDERGOES IN DISEASE.

Osteomalacia.

Changes of the bone in Osteomalacia or Malacosteon.

By the name of *Mollities Ossium, Osteomalacia* or *Malacosteon,* a disease is designated in which the bones become deprived of a large part of their mineral matter and liable to bend or to break. Not only are the mineral matters removed, but the organic basis undergoes marked structural alterations; the medullary cavity of long bones is enlarged and is often filled with hyperaemic red marrow; in some cases a yellow, in others a mucoid, marrow is found.

In some cases of osteomalacia the bones do not yield gelatin when boiled; in other cases they do.

[1] Frémy, *Op. cit.* page 88.

The fatty matter of bone seems to be very greatly increased in this disease.

Some observers have noticed that the bone possesses an acid reaction and that this is due to the presence of lactic acid[1]. It has indeed been surmised that the development of lactic acid is the primary cause of the morbid change in the bones[2]. This view has been supported by a narrative of experiments in which animals were subjected to large and long-continued doses of lactic acid with the result that they became affected with rickets which afterwards passed into osteomalacia (Heitzmann[2]).

It has been justly remarked that the experience of physicians who have experimented with Cantani's method of treating *diabetes mellitus*, which consists in giving large doses of lactic acid, does not support Heitzmann's statements, as no one has observed osteomalacia to result[3].

ANALYSES OF THE BONES IN OSTEOMALACIA[4].

In 100 parts.	I. Femur of man aet. 40 (Lehmann).	II. Rib from same case as I. (Lehmann).	III. Femur of man aet. 60 (von Bibra).	IV. Vertebrae of child (Marchand).
Organic basis	48·83	50·48	32·54	75·22
Fats	29·18	23·13	4·15	6·12
Soluble salts	0·37	0·63	1·35	1·98
Calcium phosphate	17·56	21·02	53·25	12·56
„ carbonate	3·04	3·27	7·49	3·20
Magnesium phosphate	0·23	0·44	1·22	0·92

Rachitis.

Changes in bone in Rachitis or Rickets.

Rickets is a general disorder of nutrition, accompanied by changes especially affecting the epiphyses of bones. An abnormal proliferation of cartilage cells occurs, leading to an enlargement of the epiphyses, whilst the growing bones being deficient in earthy salts become distorted. When calcification occurs the deformities which have been produced are often rendered permanent. It is a disease which affects bones in the process of development or rather cartilage which is being converted into bone—and it therefore differs fundamentally from osteomalacia, in which a morbid process causes the absorption

[1] C. Schmidt, "Knochenerweichung durch Milchsäurebildung." *Annalen d. Chemie und Pharm.*, Vol. LXI. (1847) p. 142.

[2] Heitzmann, "Ueber die Wirkung der Milchsäurefütterung auf Thiere." *Anzeiger der kais. Akad. d. Wissensch. Wien*, 1873, No. 17. Abstracted in Maly's *Jahresbericht*, Vol. III. (1874) p. 229.

[3] The reader may consult a paper by Dr Ernst Heiss entitled "Kann man durch Einführung von Milchsäure in den Darm eines Thieres den Knochen anorganische Bestandtheile entziehen?" *Zeitschr. f. Biologie*, Vol. XII. p. 151. Heiss found that the results were negative, even though lactic acid was administered to animals fed upon a diet deficient in lime salts.

[4] Gautier, *Chimie appliquée à la Physiologie, à la Pathologie, &c.*, Tome II. p. 541.

of the salts of fully formed bone and further serious changes in the decalcified framework.

In rickets the bones become specifically lighter than in health; the unossified cartilage contains an increased proportion of water; the long bones contain an increased quantity of fatty matter. The amount of fat is, however, much less in the bones of rachitis than in those affected with osteomalacia. It has been found by Lehmann and Marchand that occasionally in rickets the bones do not yield a normal gelatin when boiled.

COMPOSITION OF BONE IN RACHITIS[1].

In 100 parts.	Femur (Marchand).	Tibia (Lehmann).	Humerus (Ragsky).
Inorganic matters	20·60	33·64	18·88
Organic matters	79·40	66·36	81·12
Calcium phosphate	14·78	26·94	15·60
Magnesium phosphate	0·80	0·81	
Calcium carbonate	3·00	4·88	2·66
Soluble salts	1·02	1·08	0·62
Fats	7·20	6·22	81·12
Collagen	72·20	60·14	
Calcium fluoride and loss	1·00	0·99	

Etiology and Pathology of Rickets.

Very different views have been advanced on the etiology and pathology of rickets. Petit[2] first suggested that the disease is caused by the too early weaning of infants; since his time others, with no less reason, have maintained that too prolonged lactation often acts as a predisposing cause, the impoverished milk being incapable of supplying the growing infant with all the materials which its organism requires.

Whilst some have considered that rickets is induced by an improperly adjusted diet, in which the different groups of food constituents are not in their proper proportions, a majority of writers have advocated the view that the disease specially depends upon a deficiency in the lime salts of the food, or in a deficient absorption of lime salts. All these views have been supported by experimental researches which have led to diametrically opposite conclusions; certain experimenters having, for instance, succeeded in inducing rickets by feeding young growing animals upon meat instead of milk[3],

[1] Extracted from v. Gorup-Besanez, *Lehrbuch d. phys. Chemie*, p. 635.

[2] Petit, *Traité des maladies des os*, 1741.

[3] T. Guérin, *Thèse de Paris*, 1859, p. 24. Quoted, at secondhand, by Léon Tripier, *Dictionnaire encyclopédique des sciences médicales*, Troisième série (Paris, 1874). Article "Rachitisme."

others by cutting off the salts of lime more or less completely (Lètellier, von Bibra, Chossat, Milne Edwards[1]), whilst not a few have found that although the animals subjected to these conditions suffered in health and even died, they shewed no symptoms of rickets (Léon Tripier[2], Weiske[3]). We think, from a careful perusal of the experimental evidence bearing on this question, that we may draw from it the following conclusion. *When young animals are subjected to an insufficient diet or one in which certain of the alimentary constituents are deficient, there is engendered a predisposition to rickets, although there is no evidence to shew that such insufficient or improper diet can, acting alone, induce the disease.*

Amongst the views which have been promulgated and adopted by eminent writers on this subject is that which ascribes the chief part in the production of the disease to the formation of lactic acid in the alimentary canal; the acid thus formed is supposed to be absorbed into the blood and to act 'as an irritant on the osteoplastic tissues' and 'as a solvent on the calcareous salts deposited in the bones, promoting their elimination[4].'

This theory rests upon the most unsatisfactory evidence, as, that the amount of lime excreted in rickets is increased (a fact which has not been established by one single properly conducted observation): that rachitic bones have been found to contain lactic acid after death: and that the urine of rachitic children contains lactates.

Even assuming that large quantities of lactic acid were generated in the alimentary canal these would necessarily be converted into lactates in the blood. No one has been bold enough to assume that in rickets, or any other disease, the blood loses its alkaline reaction, for no one could conceive of an acid reaction of the blood being compatible with a prolonged continuance of its functions; and yet in order that lactic acid could exert any solvent action, it would be necessary that it should exist in a free condition in the blood or that, by an unknown chemical decomposition, alkaline lactates should be decomposed in the bones. This theory like all crude

[1] Amongst more recent researches which confirm the older writers on the possibility of inducing rachitis by a diet poor in lime salts are those of T. Lehmann, "Ueber den Einfluss der Nahrung auf die Knochenbildung." Abstracted in Maly's *Jahresbericht*, Vol. VIII. (1879) p. 272.

[2] Léon Tripier. See the admirable article referred to in note 3, p. 282.

[3] Weiske, "Einfluss Kalk- oder Phosphorsäure armer Nahrung auf die Zusammensetzung der Knochen." *Zeitschrift f. Biologie*, Vol. VII. pp. 179—183 and pp. 333—337.

[4] This view is adopted by Senator in his article on Rickets in Ziemssen's *Cyclopaedia of the Practice of Medicine*, English edition. Vol. XVI. p. 178. We quote his very words, "To sum up: the morbid process which underlies the development of rickets may, in accordance with the results of experiments and the clinical observations we possess, be explained in the following manner. Owing to digestive disturbance, either preexistent or brought on by improper feeding, lactic acid is generated in the system; this operates, on the one hand, as an irritant on the osteoplastic tissues; on the other, as a solvent on the calcareous salts deposited in the bones, promoting their elimination. At the same time the supply of earthy matter is reduced, either directly (as in cases of protracted lactation) or indirectly (as when diarrhoea carries off the lime-salts from the intestines before they are absorbed)."

chemical theories of disease does not stand the test of even a superficial criticism.

We shall probably form a nearly correct idea of the essential nature of rachitis if we look upon it as a morbid process having its seat in the ossifying epiphyses, and in newly-formed bone—a morbid process which is the local expression of a general disorder of nutrition. As a result of the latter, the cartilage cells undergo an abnormal proliferation and the newly-formed bone cells are more or less unfit to separate from the blood the lime salts which are needed for the hardening of the newly-formed ground substance in which they lie. As a result of the excessive proliferation of cartilage cells, the bones enlarge, especially at their epiphyses, and, because as they grow they do not concomitantly harden, they yield to external pressure and become deformed.

Caries.

Changes in the bone in Caries.

The following is a tabular view of the composition of the bone in caries, according to the analyses of Becquerel and Rodier[1].

	Metacarpal bone.	Met. bone, articular end.	Phalanx of finger.	Femur affected with caries.	Lumbar vertebra of a woman aet. 40.
Calcium phosphate } „ fluoride }	49·77	31·36	49·36	51·53	44·05
Calcium carbonate	7·24	4·07	8·08	5·44	3·45
Magnesium phosphate	1·11	0·83	0·98	3·43	1·02
Other salts	0·30	0·30	0·40	0·91	1·70
Collagen	37·97	59·36	37·47	35·69	41·42
Fats	3·61	4·08	3·00	3·00	8·36

Necrosis.

Changes in the bone in Necrosis.

In necrosis the organic matter of bone is gradually removed.

The following is an analysis[2] of necrosed bone by von Bibra.

Collagen	in 100 parts	19·58
Fats	„ „	1·22
Calcium phosphate with a little calcium fluoride	„ „	72·63
Calcium carbonate	„ „	4·03
Magnesium phosphate	„ „	1·93
Soluble salts	„ „	0·61

[1] Becquerel et Rodier, *Traité de Chemie pathologique*, p. 546.
[2] Quoted by Gautier, *Op. cit.* Vol. II. p. 543.

METHODS FOLLOWED IN THE QUANTITATIVE ANALYSIS OF BONE.

Preliminary preparation of bones to be subjected to analysis.

The bones to be analyzed are carefully denuded of their periosteum. They are then divided with a saw. The cancellated tissue is carefully removed, by means of a chisel, from the compact bone, and the latter is then divided into somewhat small pieces. Each of these is then wrapped in paper and being placed on an anvil is struck with a hammer so as to crush it into minute fragments. These are then powdered in a steel mortar and the powder passed through a very fine sieve.

Some writers recommend that the crushed bones should, before pulverization, be tied in a small muslin bag which is suspended in distilled water, which is to be renewed several times, with the object of separating from the bone soluble constituents which do not properly belong to it, but which are of the nature of accidental contaminations. If this process be followed, the fragments of bone after extraction with cold water are dried in an oven and thereafter pulverized.

Determination of the quantity of Fat in Bone.

A weighed quantity of powdered bone which has been dried at 130° C. is extracted with ether as in the apparatus of Drechsel (see p. 265). The ethereal solution is evaporated to dryness and weighed.

Determination of the total quantity of Ash in Bone.

A quantity of the fat-free powder which has been dried at 130° C. is weighed in a platinum crucible and ignited until the ash is perfectly white. The residue is moistened with solution of ammonium carbonate, and then heated gently. The object of this operation is to restore the carbon dioxide which may have been expelled from the bases by the strong heat to which they have been subjected.

Determination of the quantity of Chlorine in the Ash.

The ash resulting from the preceding operation is finely powdered and dissolved, with the aid of heat, in dilute nitric acid. The solution is concentrated and then treated with silver nitrate, which precipitates all the chlorine as silver chloride. This is washed by decantation, ignited and weighed according to the ordinary rules of analysis. 1 part of AgCl corresponds to 0·24724 parts of chlorine.

Determination of the amount of Calcium in Bone.

The filtrate from the last operation is treated with solution of NH_4Cl, so as to precipitate completely, as AgCl, the silver which it contains; the filtrate is saturated with ammonia, and then acetic acid is added so as to cause complete solution of the precipitate. In the case of old bones which have been macerated or buried, a certain quantity of phosphate of iron is present in the bone ash, and it forms that part of the ammonia precipitate, just mentioned, which is not dissolved by an excess of acetic acid. It may be collected on a filter, washed, dried, ignited and weighed as $Fe_2(PO_4)_2$.

From the acetic acid solution of the ammonia precipitate, the calcium is thrown down by adding solution of ammonium oxalate. The fluid, with the precipitate, is heated on a water-bath, set aside in a warm place for 24 hours, and then thrown on a filter of which the amount of ash is known; the filtrate is collected and kept. The precipitate is washed with water holding a little ammonia in solution. It is then dried, and the precipitate and filter-paper with adhering precipitate are separately ignited, as directed in works on quantitative analysis. 100 parts of the resulting $CaCO_3$ correspond to 40·00 of Ca.

Determination of the Magnesium.

The filtrate from which calcium has been precipitated by means of ammonium oxalate is evaporated to a small bulk. It contains all the magnesium of bone in the form of phosphate, which is precipitated as ammoniaco-magnesian phosphate ($MgNH_4PO_4 + 6H_2O$) on saturating with ammonia. With this object an excess of ammonia is added and the fluid is set aside for 24 hours in a warm place; it is then filtered through a small filter, the precipitate is washed with ammoniacal water, dried and ignited.

100 parts of $Mg_2P_2O_7$ correspond to 21·622 of Mg.

Determination of Phosphoric acid.

The filtrate from the precipitate of ammoniaco-magnesian phosphate in the last operation is now treated with *magnesia mixture*[1] and set aside for 24 hours. Again a precipitate of ammoniaco-magnesian phosphate forms, which corresponds to all the phosphoric acid not combined with magnesium. The precipitate is treated as in the last operation.

100 parts of $Mg_2P_2O_7$ correspond to 78·37872 of $2(PO_4)$.

[1] "*Magnesia mixture* is made by dissolving one part of recrystallized magnesium sulphate and one part of pure ammonium chloride in eight parts of water, and adding to the mixture four parts of moderately strong ammoniac solution. The liquid is allowed to stand for a few days in a corked flask; it is then filtered and preserved in a well-stoppered bottle." Thorpe's *Quantitative Chemical Analysis*, p. 111.

Determination of Carbonic acid.

About 5 grammes of the bone dried at 130° C. are employed for this determination, which may be conveniently effected with the aid of Geissler's apparatus as figured below. (Fig 53.)

The weighed quantity of bone is introduced through the tubulature *a* into the bulb *A*, and then three or four cubic centimetres of distilled water are added.

The stopper *a* is then inserted and the stopcock *b* leading from *B* is turned so as to shut off the latter from *A*. The stopper which fits into the upper part of *B* having been removed, pretty strong, but yet non-fuming, hydrochloric acid is poured into *B*. The stopper is then replaced. The small perforated stopper *e* at the upper part of *C* is

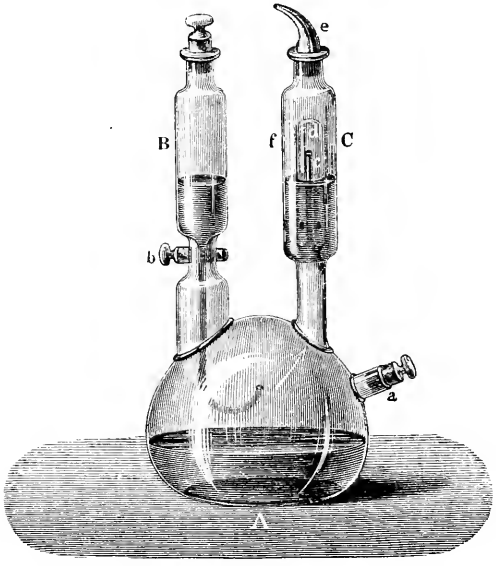


Fig. 53. Geissler's apparatus for the analysis of carbonates.

now removed, and with the aid of a small funnel, concentrated sulphuric acid is poured into *C* to about the level shewn in the drawing. The perforated stopper is then replaced. The whole apparatus is then carefully dried with a clean cloth, placed in the balance case for half an hour and then very carefully weighed. After being taken from the balance the stopcock *b* is momentarily opened, so as to allow a small portion of the contents of *B* to flow into *A*. Carbonic acid is disengaged, and this passes through the narrow tube *c* into the wider tube *d*, and thence, through two small holes situated near its base, it bubbles through the sulphuric acid contained in *c*. The effect of this passage of the moist carbon

dioxide through the concentrated sulphuric acid is to dry the gas and to retain the moisture in the apparatus. When the evolution of CO_2 has ceased, stopcock *b* is again opened for an instant so as to allow a fresh quantity of hydrochloric acid to act upon the bone. When the evolution of CO_2 has ceased and does not recommence on the addition of a few drops more of the acid, the whole apparatus is placed on a water bath so as to heat it gently for a few minutes. The stopcock *b* is then opened, the stopper at the upper part of *B* is temporarily removed and a piece of narrow india-rubber tube is slipped over the upper narrow portion of the perforated stopper *e*. The experimenter then placing the free end of the india-rubber tube in his mouth, draws air through the whole apparatus for about a minute, or rather until the gas which is aspirated has lost the peculiar taste of CO_2. The india-rubber tube is then taken away, the stopper of *B* is replaced, the whole apparatus once more wiped with a clean and dry cloth, placed in the balance case for half an hour, and then again weighed. On subtracting the weight after, from the weight before decomposition, the weight of dry CO_2 evolved is readily ascertained.

Determination of Fluorine.

Most chemists who have published analyses of bone have estimated the amount of calcium fluoride indirectly, as follows. The whole of the CO_2 of the bone is supposed to exist as calcium carbonate, ($CaCO_3$), then the whole of the phosphoric acid which does not exist as magnesium phosphate is calculated in combination with calcium. In a properly conducted analysis it will be found that on adding together the calcium combined with carbonic and phosphoric acid and subtracting the amount from the total weight of calcium found, there is a small excess of lime left, which obviously must have existed in some other form of combination. This is calculated as existing in combination with fluorine.

Zalesky[1] determined the quantity of Fl *directly*, by a modification of the method first suggested by Kobell. This consists in gently heating for a long period of time a weighed quantity of bone with strong sulphuric acid and a weighed quantity of glass, the amount of silica in which has been previously determined. In presence of the silica and sulphuric acid all the fluorine contained in the bone unites to form fluosilicic acid, $SiFl_4$. The amount of fluorine present in the bone is ascertained by determining the loss of weight which the glass undergoes[2].

Calculation of the results of the Analysis of the ash of bones.

The whole of the magnesium found is calculated as magnesium phosphate ($Mg_2P_2O_8$). The amount of phosphoric acid in this com-

[1] Zalesky, *Op. cit.* p. 36.

[2] Kobell's original paper was published in the *Journ. f. prakt. Chemie*, Vol. 162, p. 385. The reader who desires to know the improvements introduced by Zalesky is referred to the previously quoted memoir by this author.

pound is then deducted from the total weight of phosphoric acid, the difference being calculated as calcium phosphate. The amount of calcium in this compound is calculated and deducted from the total amount of calcium found. Thus is found the calcium which exists in other states of combination than as phosphate, *viz.* as carbonate, chloride and fluoride. The whole of the carbon dioxide found is assumed to have been derived from the decomposition of calcium carbonate, so that the amount of the latter is easily calculated. The chlorine found is calculated as present in calcium chloride. By deducting then the calcium in combination with phosphoric and carbonic acids and with chlorine from the total quantity of calcium found, the amount of calcium present as $CaFl_2$ is obtained.

Sect. 5. Tooth.

A tooth is a composite organ presenting for examination several tissues; of these, three constitute the hard portion of the tooth, viz. *enamel, dentine,* and *crusta petrosa* or *cementum.* In the interior of the tooth is the so-called *pulp cavity,* which lodges the *pulp,* which consists of a framework of connective tissue, to which are distributed blood-vessels and nerves, and whence proceed processes which are prolonged into the *dentinal tubules.*

Although two only of the hard tissues of tooth—viz. *dentine* and *crusta petrosa*—belong to the group of connective tissues, the *enamel,* which is a modified epithelial structure, will also, for reasons of expediency, be considered in this place.

Dentine.

Dentine, or ivory, constitutes the chief part of the teeth. On making a longitudinal section through a tooth it will be found that the pulp cavity is bounded on all sides, except where the cavities of the fangs open into it, by dentine; the same tissue forms the body of the crown (which is only covered by an external layer of the harder enamel) and nearly the whole thickness of the fang or fangs.

Dentine mesoblastic in its origin.

Dentine is distinctly mesoblastic in its origin, being formed through the agency of certain cells, termed '*odontoblasts,*' which are modified connective-tissue corpuscles arranged circumferentially over the surface of the papillary protrusions which rise from the connective tissue of the buccal mesoblast so as to meet and indent the downward dipping epiblastic cells which give rise to the *enamel organ.*

Microscopic structure and micro-chemical reactions of dentine.

On examining very thin sections of dentine it is found to consist of very fine tubes—the *dentinal tubules,* which are surrounded by a homogeneous ground substance; these tubules open internally in the pulp cavity, from which they pass outwards, dividing and inter-communicating. Sections made at right angles to their long axes exhibit the tubes as minute round holes scattered through a translucent homogeneous matrix.

When teeth are placed in the acid solutions which have been recommended for decalcifying bone (see p. 274), the mineral matters which give intense hardness to the hard tissues are dissolved, and it then appears that around the *lumen* of the dentinal tubule there is a structure which may be called the *dentinal sheath,* which, as it resists the action of acids, obviously differs from the matrix more external to it; the dentinal sheath possesses apparently the characters of yellow elastic tissue. Under similar circumstances, the dentinal sheath may occasionally be seen to contain a fine fibre, the *dentinal fibre,* which is a process from the pulp, probably a process from the *odontoblasts* of the pulp.

Relation of dentine to bone.

If we except the substance which constitutes the dentinal sheaths and which is not affected by prolonged boiling, nor by the action of acids or alkalies, dentine has a composition which very closely resembles, or rather which is almost identical with, that of bone; it consists, namely, of a collagenous organic basis in which are deposited mineral matters identical with those of bone.

The collagenous organic basis impregnated with salts is the result of the activity of those connective-tissue cells which we term *odontoblasts,* just as the matrix of bone proper was originally formed through the activity of those connective-tissue cells which we designated *osteoblasts.* Though differing somewhat in arrangement and in texture, the two tissues, dentine and bone, are, on developmental as well as on chemical grounds, seen to be identical.

As Hoppe-Seyler has well shewn, the *dentinal sheaths* correspond to the more internal portion of the ground substance of bone which may be separated as a distinct investment bordering the *lacunae, canaliculi* and *Haversian canals* (see p. 273).

Water and organic matter of dentine.

Fresh dentine, when dried, loses about 10 per cent. of water; the quantity of organic matter contained in it varies between 26 and 28 per cent., on an average being about 28 per cent.

Constitution of the mineral matters of dentine.

Although a large number of analyses of tooth have been made, we possess fewer absolutely reliable analyses of dentine than of enamel. Dentine, like bone, contains, as its chief mineral ingredients, calcium and phosphoric acid; the proportion of carbonic acid found in its ash by most analysts is smaller than in bone. Hoppe-Seyler

is of opinion that in dentine, as in bone and enamel, the chief mineral ingredient is a definite compound ($Ca_{10}CO_3$, $6(PO_4)$) of calcium phosphate and carbonate, constituted like apatite ($Ca_{10}Fl_2$, $6(PO_4)$).

Recalculating the results of an analysis of dentine of the ox made by Aeby[1], Hoppe-Seyler states its composition as follows :

$Ca_{10}CO_3$, $6PO_4$	in 100 parts	72·06
$MgHPO_4$	,, ,,	0·75
Organic matter	,, ,,	27·70
		100·51

Numerous analyses of dentine by various chemists will be given in the table exhibiting the general results of quantitative analyses of tooth.

Enamel.

This tissue, the hardest in the body, as well as the richest in mineral constituents, covers the crown or exposed surface of the tooth.

In its adult condition enamel is composed of polygonal (usually hexagonal) prismatic columns which rest upon the dentine and radiate out from it.

As has been already said, enamel is epiblastic in its origin, being developed through the agency of the columnar epithelial cells of the enamel organ, a structure produced by the proliferation and growing downwards of the deeper epithelial cells of the oral mucous membrane.

When enamel is digested in acids, only a small quantity (2—6 per cent.) of organic matter is left, which does *not* yield gelatin on boiling. Enamel is thus seen, on chemical as well as on developmental grounds, to differ from the connective tissues.

The mineral matters of enamel are essentially the same as those of bone and dentine, and, according to Hoppe-Seyler, there are good grounds to believe that they consist mainly of the same compound of calcium phosphate and carbonate. Adult enamel contains a small quantity of a fluoride, but Hoppe-Seyler failed to detect fluorine in the growing enamel of the pig. It might be surmised that the enamel consists of a mixture of apatite and bone earth, but there are good reasons for believing that this is not the case.

The following formulae exhibit the relations between apatite and the peculiar salt which Hoppe-Seyler believes to be the characteristic mineral ingredient of bone, dentine and enamel.

Crystallized apatite	$Ca_{10}Fl_2$, $6(PO_4)$
,, ,, (another variety)	$Ca_{10}Cl_2$, $6(PO_4)$
The bone earth salt	$Ca_{10}CO_3$, $6(PO_4)$

In the annexed tables are given, firstly the results obtained by Hoppe-Seyler from his analyses of enamel, and secondly the probable amounts of the mineral compounds which he assumes to have been present.

[1] Aeby, *Centralblatt f. d. med. Wissenschaft.* 1873. No. 7.

I. TABLE EXHIBITING THE RESULTS OF HOPPE-SEYLER'S ANALYSES OF ENAMEL OF RECENT AND FOSSIL TEETH[1].

Constituent.	Newborn child.			Pig.		Dog.	Horse.	Fossil Elephant.	Mastodon.	Rhinoceros.	Palaeotherium.
	I.	II.	III.	Rudimentary enamel.	Developed enamel.						
PO_4	40·85	47·75	48·99	52·26	54.31	58·38	53·82	51·98	53·01	54·28	53·79
Cl	—	0·15	—	0·30	0·40	0·51	0·43	0·28	0·38	0·42	0·37
Ca	29·59	32·08	32·16	34·76	36·84	36·76	36·50	35·51	37·76	36·59	37·42
Mg	0·43	0·47	0·30	0·44	0·55	1·36	0·34	0·55	0·18	0·45	0·35
Fe	—	0·24	—	0·34	0·33	—	—	0·20	0·12	0·91	0·28
Salts soluble in water and	22·29	0·35	15·43	0·24	0·15	—	4·74	—	—	0·01	0·21
organic matters		15·43		9·71	2·06	?		4·54	1·24	3·16	2·31

II. TABLE SHEWING THE PROBABLE MINERAL CONSTITUENTS DEDUCED FROM THE ABOVE ANALYTICAL RESULTS[1].

Constituent.	Newborn child.			Pig.		Dog.	Horse.	Fossil Elephant.	Mastodon.	Rhinoceros.	Palaeotherium.
	I.	II.	III.	Rudimentary enamel.	Developed enamel.						
$Ca_{10}CO_3 6PO_4$	75·94	82·40	82·81	89·09	94·30	93·91	93·40	91·03	96·69	93·63	95·84
$CaCl_2$	—	0·23	—	0·46	0·62	0·80	0·66	0·44	0·59	0·65	0·57
$MgHPO_4$	2·16	2·37	1·50	2·22	2·73	—	1·68	2·75	0·90	2·25	1·77
Soluble salts and		0·35		0·24	0·15	6·81		—	—	0·01	0·21
organic matters	22·29	15·59	15·40	9·71	2·06		4·74	4·54	1·24	3·16	2·32

[1] Hoppe-Seyler, Virchow's *Archiv*, Vol. xxiv. p. 13, and *Physiologische Chemie*, p. 182 and 183.

Crusta Petrosa or Cement.

The cementum, or crusta petrosa, is found as a thin covering over the dentine of the fangs and is developed from the periosteum

COMPARATIVE ANALYSIS OF TEETH AND PARTS OF TEETH OF VARIOUS ANIMALS (VON BIBRA [1]).

Nature of body analysed.	Calcium phosphate with a little Calcium fluoride.	Calcium carbonate.	Magnesium phosphate.	Soluble salts.	Total Inorganic matter.	Organic matter.
Enamel:						
Woman, molar	81·63	8·88	2·55	0·97	94·03	5 97
Man „	89·82	4·37	1·34	0·88	96·41	3·59
Wolf „	87·82	1·21	1·10	0·83	90·96	9·04
Fox „	88·24	1·72	1·20	0·75	91·91	8·09
Lion, canine	83·33	2·94	3·70	0·64	90·01	9·39
Bear „	84·38	2·20	6·01	0·77	93·36	6·04
Seal „	85·60	1·94	1·00	0·63	89·17	10·83
Horse, molar	89·01	1·19	1·95	0·60	92·75	7·25
Ox, incisor	83·77	7·00	1·32	0·61	92·70	7·30
Dentine:						
Woman, molar	67·54	7·97	2·49	1·00	79·00	21·00
Man „	66·72	3·30	1·08	0·83	71·99	28·00
Wolf „	68·81	1·04	0 97	0·80	71·62	28·38
Fox „	71·84	0·90	0·99	0·78	74·51	25·49
Lion „	60·03	3·00	4·21	0·77	68·01	31·99
Bear „	64·88	1·34	6·40	0·80	73·42	26·58
Seal „	68·46	1·09	0·97	0·78	71·30	28·70
Elephant (Indian), tusk	38·48	5·63	12·01	0·70	56·82	43·18
„ „	46·48	3·86	7·84	0·77	58·95	41·05
Dolphin	66·37	1·84	1·36	0·99	70·56	20·44
Boar, tusk	60·00	2·51	6·43	0·43	69·37	30·63
Horse, molar	61·28	6·08	1·75	0·74	69·85	30·15
Ox, incisor	58·33	7·39	0·97	0 75	67·44	32·56
Goat, incisor	63 04	2·83	1·70	0·93	68·50	31·50
Stag, molar	63·51	3·99	3·72	0 58	71·80	28·20
Crocodile	53·47	6·33	10·75	1·36	71·91	28·09
Crusta Petrosa:						
Dolphin	69·42	1·79	1·47	0 93	73·61	26·39
Ox, incisor	58·00	7·22	0 99	0·73	66·94	33·06
Crocodile	53·39	6·29	9·99	1·42	71·09	28·91
Whole Tooth:						
Saw-fish	61·99	3·64	1·70	1·81	69·14	30·86
Pike	63·98	2·54	0·73	0·97	68·22	31·78
Black-fish	59·94	9·01	2·00	1·77	72·72	27·28
Plaice	57·20	1·34	0·88	1·82	61·24	38·76

[1] Reprinted from Watts' *Dictionary*, Vol. v., p. 706.

which covers them; histologically it is found to be composed of true osseous tissue, presenting lamellae, lacunae and canaliculi, perforating fibres, and occasionally Haversian canals; chemical analysis reveals no difference between it and bone proper.

COMPOSITION OF VARIOUS FOSSIL TEETH (VON BIBRA[1]).

	Calcium phosphate.	Calcium fluoride.	Calcium carbonate.	Calcium sulphate.	Magnesium phosphate.	Silica, iron, alumine, calcium chloride.	Organic matter.
Rhinoceros Tichorhinus							
Upper molar, Enamel	83·11	4·14	7·66	0.95	0·73	0·24	3·17
„ Dentine	54·65	3·09	12·80		0·80	5·63	23·03
Elephas primigenius, molar	62·83	4·15	14·90		2·11	0·32	15·60
„ „ „	68·43	3·72	15·40		1·34	1·91	9·14
Cave Bear, molar	64·03	2·51	1·46		8·25	0·30	23·45
Fish (*Acrodus*)		90·01	1·80		0·52	5·50	2·17
Fish from chalk		85·01	9·11		0·45	14·89	0·54

Analysis of Dental Tissues.

This is conducted according to the methods which have been described for the analysis of bone.

[1] Reprinted from Watts' *Dictionary*, Vol. v., p. 706.

CHAPTER VIII.

EPITHELIAL TISSUES OR EPITHELIUM. KERATIN. CHITIN. PIGMENTS DEPOSITED IN THE EPITHELIAL STRUCTURES. CERTAIN OTHER ANIMAL PIGMENTS.

By the term *epithelium* is designated a tissue, composed entirely of cells, which covers the whole external surface of the body, and lines the cavities which open externally. The term is generally held to include also the tissue composed of a single layer of tesselated or tile-like cells which lines the arteries, capillaries and veins, the serous sacs and the lymphatics. This tissue to which the terms *endothelium* or *epithelioid tissue* are more properly applied, as indicating that it differs from epithelium in its development, in its characters, and in the uses which it subserves, will be considered in this work in discussing the chemistry of the so-called ductless glands and the lymphatics.

Confining our attention to epithelium proper we might classify it in various ways: firstly, according to the form and arrangement of the cells of which it is composed: secondly, according to the regions in which it occurs: thirdly, according to the mode in which it is developed: fourthly, according to the chemical characters which it possesses; we shall not, however, strictly follow any of these modes of classification. There is no other tissue of which the individual anatomical elements exhibit such marked differences in the chemical operations of which they are the seat.

Speaking broadly we may, however, say that the epithelium covering the external surface of the body is composed of cells which are, even in their most active stages, the seat of but slow and unimportant chemical changes, whilst a large number of them cease to be the seat of any material exchanges whatever, or to manifest any phenomena which characterize them as living, long before they cease to form part of the living body.

The function of such epithelium—and we are referring to that which composes the cuticle and its appendages—is in the strictest sense *tegumentary*. This epithelium possesses two characters which may be taken together. Firstly, it is entirely derived from the external layer of the blastoderm or epiblast. Secondly, however

different the arrangement of the cells and the physical characters of the tissue which they compose, the main product which characterizes them is an undefined *horny* substance to which the term *Keratin* has been applied.

The epithelium, on the other hand, which covers the mucous membranes and lines the interior of secreting glands, is composed of cells, whose protoplasm is the seat of the most active and remarkable chemical operations, tending to separate, from the blood, constituents of which it has no longer need, or to build up, at the expense of certain of those constituents, new bodies which are to serve important functions in the organism.

This epithelium is mainly derived from the hypoblast, though in some cases it takes its origin in the epiblast (epithelium of mouth and salivary glands), in others from the mesoblast (certain portions of the epithelium of genito-urinary tract). In short, the epithelium of the mucous membranes is possessed of diverse chemical attributes and is developed in several ways; it does not therefore possess any common characters which permit of a general description.

We shall, therefore, in this chapter, confine ourselves, in the first place, to a consideration of the chemistry of the keratin-forming epiblastic tissues, postponing the exposition of the chemistry of other epithelial tissues to future sections of this work, where they will be treated of in relation to the organs in which they occur, and the functions which they specially subserve.

SECT. 1. EPIBLASTIC KERATIN-PRODUCING EPITHELIAL TISSUES. THE HORNY SUBSTANCE OF CUTICLE, NAILS, HORN, HAIR, AND FEATHERS.

Structure of Epidermis.

The cuticle or epidermis is composed of many layers of epithelial cells which overlie the derma or true skin. The cells of the most superficial layers present the appearance of distorted, shapeless, hardened scales; those of the deeper layer are more or less spheroidal, soft, and present a well-marked nucleus.

The most superficial cells, which are horny throughout, are unacted upon by acetic acid; this reagent renders the deeper cells more transparent and their nucleus more evident.

The cells are connected together by a cementing substance which, like the cementing substance of the connective tissue, is dissolved by alkalies. In order to dissolve this connecting substance and effect the dissociation of the epidermal cells, maceration in cold, or even warm, solutions of caustic potash or soda should be had recourse to. These reagents not only dissociate the cells but, in the case of the more superficial cells, render their structure more evident.

The caustic alkali, at first, leaves the nucleus and the cell substance intact, merely causing the latter to swell and become more

transparent than previously; subsequently, the nucleus may disappear, leaving the cell body. In some cases, the separate anatomical elements are best seen if after the action of alkali the tissue is placed in water. Cold concentrated sulphuric acid also brings out the cells of the epidermis, effecting to a certain extent their dissociation. When heated, it dissolves the deepest cells (those of the *rete mucosum*) but leaves undissolved the more superficial, in which the cell protoplasm has undergone conversion into horny substance.

Structure of Nails. The Nails possess essentially the same structure as the epidermis, and their cells may, like those of the latter structure, be divided into an upper horny layer and a lower softer stratum. The action of caustic potash or soda on the cells of nail is similar to that exerted by these reagents on the cells of the cuticle.

Structure of Horn. Horn is constituted exactly as nail; in the deeper layers of cells pigment is sometimes present, as it is in the *rete Malpighii* of the epidermis.

Structure of Hoof. Hoof is composed of compressed epithelial cells arranged concentrically around canals which run from above downwards. The individual cells are made apparent by treatment with solutions of caustic alkalies.

Structure of Hair. Hairs have a more complex structure than the epidermal tissues yet enumerated, and for a description we must refer to treatises on Histology. It may be mentioned however that the stem of the hair is seen to be covered by layers of imbricated cells which are evidently modified epidermic epithelial cells; within these is the so-called *fibrous substance* which makes up the greater part of the stem and which may be resolved into elongated scales; and in the very centre of the hair is the *medulla* or *pith* in which sometimes air spaces are seen, sometimes cells which are filled with fatty and pigmentary particles. The lower part of the stem or shaft of the hair dips into the so-called *hair-follicle*, where it is attached to, or rather grows upon, the *papilla;* the imbricated scales which cover the root of the hair are continuous with the innermost layer of cells of the epidermic lining of the hair-follicle.

Horny Substance or Keratin.

By the term *Keratin* is understood the organic substance, or perhaps the mixture of organic substances, left as an insoluble residue when cuticle, horn, nails, hairs, feathers, &c., are successively boiled in ether, alcohol, water, and dilute acids. This insoluble residue retains the form of the tissues from which it is prepared; it is little affected by boiling with water at ordinary pressure, but is dissolved when subjected to the prolonged action of water under pressure (as

in sealed glass tubes heated to 150°—200° C.), yielding a turbid solution which furnishes on evaporation a dry mixture insoluble in water. Keratin swells, and subsequently is dissolved by boiling in alkalies, and on the addition of acids to the alkaline solutions sulphuretted hydrogen is given off.

Horny substances swell when immersed in dilute acetic acid, and are in great part dissolved by boiling glacial acetic acid.

When boiled with dilute sulphuric acid, Keratin yields aspartic acid, volatile fatty acids, leucine, and tyrosine. Nitric acid dissolves it, and oxalic acid is formed as an ultimate product.

When strongly heated, horny substances burn, evolving the characteristic smell of burned feathers.

Results of ultimate analyses of Horny Tissues.

Though we cannot obtain by any known process a definite substance Keratin, which can be considered as pure, yet the horny tissues present a very close resemblance in the proportion in which their elements are contained, as will be seen by perusing the analyses here appended[1].

	Hair, (v. Laer).	Nails, (Mulder).	Cow's horn, (Tilanus).	Horse's hoof, (Mulder).
C	50·60	51·00	51·03	51·41
H	6·36	6·94	6·80	6·96
N	17·14	17·51	16·24	17·46
O	20·85	21·75	22·51	19·49
S	5·00	2·80	3·42	4·23

The sulphur which is contained in these horny substances is very loosely combined; it varies also remarkably in quantity in various samples of the same tissue, as for example in human hair between 3 and 8·23 per cent. When heated with barium hydrate and water in sealed glass tubes, nearly the whole of the sulphur is obtained in the form of $Ba(SH)_2$ (Hoppe-Seyler[2]).

Inorganic Matters contained in the Horny Tissues.

All these tissues contain inorganic matters. In the nails the ash is said to be specially rich in calcium phosphate. Hair contains from 0·5 to 7 per cent. of mineral constituents, and the latter contain alkaline sulphates, iron and silica (40 per cent. of the ash). The proportion of iron in the hair varies, and it has been stated that it is larger in dark than fair hair; but this statement must be received with some doubt.

The composition of the mineral matters of feathers varies, according to von Bibra, with the nature of the food upon which birds feed; thus, the silica may vary between 27 and 40 per cent. of the total mineral matters.

[1] Hoppe-Seyler, *Physiologische Chemie*, 1 Theil, p. 90.
[2] Hoppe-Seyler, *Op. cit.*, p. 91.

Sect. 2. Tissues which yield Chitin, Spongin, Tunicin, and Hyalin.

The tissues of many groups of invertebrate animals contain certain of the proximate principles which have been enumerated and described as obtained from the tissues of man and the higher vertebrates. Thus mucin is present in organisms low in the scale; as we ascend, we find chondrin-yielding tissues, and in the Cephalopoda tissues which yield gelatin[1] when boiled.

In certain invertebrates we find, however, that the tissues contain substances which do not occur in vertebrates. Amongst these are the substances now to be considered, viz. Chitin, Conchiolin, Spongin, Tunicin, and Hyalin.

Chitin.

Distribution of Chitin in the Animal Kingdom.

Chitin usually occurs throughout Invertebrates in the form of an investment to the outermost cellular layer or ectoderm. The exceptions real and apparent to this statement are noted in the following table of its distribution, which however must be accepted as only approximately accurate, in the absence of full chemical investigation, in any case except that of the Arthropoda[2].

Protozoa. Membrane of all "loricate" forms, cuticle of Infusoria, &c. Oesophageal lining of toothed Ciliata (*Nassula, Prorodon*). Central capsule of Radiolaria. Cyst wall of all encysted forms.

Coelenterata. Membrane of *fertilized* ovum. Mesodermal (?) skeleton of Hydro-medusae (*Velella*).

Vermes. Membrane of ovum. Cuticle in all cases, including the ectocyst of Polyzoa, and cuticular appendages, such as the setae of Annelida. Oesophageal armature of Rotifera and some Annelida. Mesodermal branchial skeleton of *Balanoglossus.*

Echinodermata. The presence of chitin is not indicated in this group.

Mollusca. Membrane of ovum. Setae of larval Brachiopoda. Byssus, shell-ligament and shell (in many cases, if not universally, the organic base of the shell is composed not of chitin but conchiolin, q. v.). Rings and hooks of suckers of Cephalopoda. Upper lip and jaws of Cephalopoda and Gasteropoda. Radula of odontophore. Mesodermal branchial skeleton of Lamellibranchiata.

[1] Hoppe-Seyler, "Ueber Unterschiede im chemischen Bau und der Verdauung höherer und niederer Thiere." Pflüger's *Archiv*, Vol. XIV. p. 395—400. "Ueber das Vorkommen von leimgebendem Gewebe bei Avertebraten." *Med. Chem. Untersuchung.* p. 580.

[2] It would seem that in many cases a chitinous composition has been ascribed to a structure solely on the ground of its insolubility in caustic alkalies and dilute acids, or even in only one of these two classes of reagents.

Arthropoda. Membrane of ovum. Cuticle with its appendages external and internal (setae, apodemata, large tracts of alimentary canal, gizzard when present, all excretory ducts, tracheae of Onychophora, Arachnida, Myriapoda and Insecta).

Chitin is frequently found impregnated with calcareous matter, as in Crustacea, or with silica, as in the radula of the higher Mollusca.

Preparation. The wing-cases of the cockchafer are boiled in dilute solution of caustic soda until they have become colourless; they are then washed with water, dilute acids, and lastly with boiling alcohol and ether (Hoppe-Seyler).

From the shell of the crab or lobster it is obtained by the same treatment, after previous digestion in hydrochloric acid, so as to dissolve the earthy matters deposited in the chitinous tissue. The chitin thus prepared may be dissolved in cold pure concentrated hydrochloric acid, and the solution precipitated by the addition of a large excess of water.

Properties. Chitin is a colourless, amorphous body, which retains, when prepared by the first of the above-mentioned methods, the form of the parts composed of it; when prepared by the second method it appears as an amorphous gelatinous body. It is insoluble in water, alcohol, ether, acetic acid, in dilute mineral acids, and in solutions of the alkalies. It is dissolved by concentrated mineral acids. Chitin resists in a very remarkable manner the action of alkalies, and can be boiled in their concentrated solutions for long periods of time without undergoing decomposition.

Elementary composition and formula. Chitin has been subjected to analysis by many observers. The following is the mean of twelve analyses made by Ledderhose[1], who has investigated the constitution of chitin under the direction of Professor Hoppe-Seyler.

Carbon	in 100 parts	45·69
Hydrogen	„ „	6·42
Nitrogen	„ „	7·00
Oxygen	„ „	40·89

Ledderhose[1] ascribes to Chitin the formula $C_{15}H_{26}N_2O_{10}$.

Products of decomposition. Berthelot pointed out[2] that when chitin is dissolved in concentrated sulphuric acid it yields a fermentable sugar; this statement has been disproved. The researches of Ledderhose carried out under the direction of Professors Hoppe-Seyler and Baumann have thrown great light

[1] Ledderhose, "Ueber Chitin und seine Spaltungsprodukte." *Zeitschrift für physiol. Chem.* Vol. II. (1878), p. 213. Ledderhose: "Ueber Glykosamin." *Ibid.* Vol. IV. (1880), p. 139.

[2] Berthelot, *Comptes Rendus*, XLVII. 227.

upon the decomposition, and have shewn that when heated with acids chitin combines with the elements of water and splits up into a nitrogenous body *glycosamine* and into acetic acid, thus:—

$$2\,(C_{15}H_{26}N_2O_{10}) + 6H_2O = 4(C_6H_{13}NO_5) + 3(C_2H_4O_2).$$

Glycosamine, $C_6H_{13}NO_5$.

Preparation. Chitin is soluble in cold concentrated HCl, and the solution remains colourless when exposed to air, the dissolved body not undergoing decomposition and being thrown down unchanged by the addition of water.

When the solution is boiled it becomes black, in consequence of a decomposition which is completed in about an hour. On evaporation, impure hydrochlorate of glycosamine is obtained, and is purified by recrystallizing repeatedly.

The amount of this compound formed amounts to 70 or 75 p.c. of the weight of the chitin dissolved.

Properties. The hydrochlorate of glycosamine is easily soluble in water, but soluble with difficulty in alcohol; its solution has a sweet taste and an acid reaction. It reduces alkaline solutions of cupric and silver salts, and its solutions react like solutions of glucose when boiled with caustic alkalies. It is dextro-rotatory $(\alpha)\,D = +\,70^{\circ}{\cdot}6$. The pure base (prepared by the action of barium hydrate on the sulphate of glycosamine) crystallizes from alcohol in the form of needles. It is not fermentable.

Probable constitution. Ledderhose considers glycosamine to be an amido-derivative of grape sugar (dextrose), thus:

$$\underbrace{COH\,.\,(CHOH)_4\,.\,CH_2OH}_{\text{Dextrose.}} \qquad \underbrace{COH\,.\,(CHOH)_4CH_2NH_2}_{\text{Glycosamine.}}$$

Conchiolin.

The organic matter of the shells of Mussels and Snails was formerly supposed to be identical with chitin; this is not, however, the case, and to the substance the name of Conchiolin has been applied.

Preparation. The shells of mussels are macerated in dilute hydrochloric acid; then boiled in aqueous solutions of the caustic alkalies.

Elementary composition. The differences in composition between conchiolin and chitin are shewn by the two analyses quoted below:

	Conchiolin.	Chitin.
Carbon	50·7	46·32
Hydrogen	6·5	6·40
Nitrogen	16·7	6·14
Oxygen	26·1	41·14

Reactions of Conchiolin. Conchiolin is insoluble in dilute acids and in alkaline leys. It is soluble in hot concentrated hydrochloric acid. When boiled with dilute sulphuric acid it furnishes leucine and no sugar-like body. By the two last characters it is as clearly distinguished from chitin, as by its much smaller amount of nitrogen.

Spongin.

When sponge is boiled with dilute hydrochloric acid, then with caustic soda, water, ether and alcohol, there is left a body to which the name of *Spongin* has been given.

This body (if a definite body it be), so far as it has been investigated, appears to have the following composition:

Carbon	47·44
Hydrogen	6·30
Nitrogen	16·15
Oxygen	30·11

When boiled with water it yields no gelatin.

When boiled with dilute sulphuric acid it yields leucine and glycocine, but no tyrosine.

Hyalin.

This term is applied to the principal constituent of the walls of *hydatid cysts.*

Preparation. Hydatid cysts, emptied of their contents, are boiled in water, then in alcohol and ether. The residual matter is soluble when heated in water (under pressure) at 150° C. The solution is precipitated by alcohol, neutral and basic lead acetate, and by mercuric nitrate.

Elementary composition. The composition is said to vary according as the substance is prepared from old or young cysts.

Composition of hyalin (Lücke[1]).

	(1) From young cysts.	(2) From old cysts.
C	44·1	45·3
H	6·7	6·5
N	4·5	5·2
O	44·7	43·0

Products of decomposition. When dissolved in strong sulphuric acid or boiled in dilute sulphuric acid, hyalin is said to yield 50 p.c. of its weight of a dextrogyrous sugar, susceptible of the alcoholic fermentation.

Tunicin or Animal Cellulose, $C_6H_{10}O_5$.

This body, closely resembling, if not identical with, the cellulose so widely distributed throughout the vegetable kingdom, occurs in the mantle of the *Tunicata.*

[1] Lücke, Virchow's *Archiv*, Vol. XIX. p. 189.

Preparation. The cartilaginous investment of Ascidians, *e.g.* the "mantle" of *Phallusia* and *Cynthia* and the external coat of *Salpa* consist mainly of Tunicin and may be employed in its preparation.

These structures are digested in hot water, then for a short time in dilute acids and alkalies, lastly in alcohol and ether; the residue, which preserves the original form of the structures, consists of tunicin.

Tunicin is by the action of acids converted entirely into a reducing sugar susceptible of the alcoholic fermentation (dextrose?). It is maintained by Berthelot[1] that tunicin presents certain differences from ordinary cellulose, as, for instance, that it is coloured yellow by iodine and is less affected by certain reagents.

Sect. 3. On Certain Colouring Matters of the Epithelial Tissues of Vertebrates.

Brown and black Pigments. Melanin.

The cells of the *rete Malpighii* of the human skin often contain granules of a black pigment; this is especially the case in the skin of the negro, which owes its colour to these pigment-bearing cells. A similar pigment is found in the hexagonal epithelial cells which constitutes the most external layer of the retina, and which used formerly to be considered as belonging to the choroid; also in the connective tissue cells of the outer layer of the choroid. In the bronchial lymphatic glands, of adults and aged persons, in the lung tissue and in melanotic tumours, similar brown or black pigments are discovered, which are all included under the name of Melanin, though it is certain that the substance obtained from these various sources does not present an uniform composition; in all probability, however, all these colouring matters derive from the decomposition of haemoglobin. The formation of such a black pigment has actually been traced in the interior of the red blood-corpuscles, in cases of pernicious intermittent fevers (see p. 163).

Characters and reaction of Melanin. Melanin occurs in the form of minute amorphous granules which when suspended in water exhibit *Brownian* movements. It is soluble in ether, alcohol, water and acids. When boiled with solution of caustic potash the black colouring matter is slowly and imperfectly dissolved, a brown liquid being formed, which is discolourized by chlorine.

In the lung tissue, particles of carbon sometimes occur; these are sometimes in a finely granular condition, though occasionally they present the appearance of minute fragments of coal. The latter are distinguished from melanin by their complete insolubility in boiling caustic potash, in boiling sulphuric acid, and when boiled in strong hydrochloric acid and potassium chlorate.

[1] Berthelot, *Ann. de Chim. et de Phys.*, Vol. LVI. p. 149.

Percentage composition of Melanin. The analyses which have been made of the various pigmentary matters included under the term Melanin have led to widely discordant results. The carbon in 100 parts has varied between 51·7 and 58·3; the H between 4·02 and 5·09; the N between 7·1 and 13·8; the O between 22·03 and 35·44[1].

Pigments of the Feathers of Birds.

The brilliant colours of the plumage of birds is due in part to the optical characters of the surface of the feathers (*interference-colours*): in part to the presence, within the feathers, of colouring matters, which may usually be extracted from them by alcohol, ether, or hot acetic acid, and which, as a rule, are very unstable, becoming decolourized by exposure to air.

These colouring matters have hitherto not been subjected to a thorough chemical investigation, with the exception of the one to be described in the ensuing paragraph.

Turacin.

In various species of birds belonging to the family Musophagidae and which, from the nature of their food, are designated Plaintain-eaters, the primary and secondary pinion-feathers, are more or less of a crimson colour. The colour is due to a pigment which has been separated and analysed by Professor Church, who has applied to it the name Turacin, from *Touracon*, the name by which the Plaintain-eater is designated by the natives on the shores of the Gambia.

Mode of separating Turacin. The barbs constituting the red part of the web are stripped from the shaft of the feathers, placed in a beaker, and washed with ether, then with alcohol. They are then dried, by pressure between folds of filtering paper, and placed in a very dilute cold solution of pure caustic soda, a solution containing one part of soda in a thousand of distilled water being quite strong enough. The crimson pigment is soon dissolved; its solution is then poured into dilute hydrochloric acid (1 of acid to 4 of water), when the red colouring matter is precipitated. It is then washed, first with water, until all acid reaction is removed, and then in alcohol and ether, and dried.

Properties of Turacin. Occurs in scales which have a deep violet-purple colour by reflected light, and a crimson tint when seen in small fragments by transmitted light.

It has not yet been obtained in a crystalline form. It is very slightly soluble in pure water, giving a pale rose-pink solution. It is not soluble in alcohol or ether. It is insoluble in acid, but soluble in alkaline liquids.

Spectrum of Turacin. Turacin and the feathers containing it possess a spectrum which is almost identical with that of oxy-

[1] See a paper by Hodgkinson and Sorby entitled "Pigmentum Nigrum, the black colouring matter contained in hair and feathers." *Journ. Chem. Soc.* 1877, p. 427.

haemoglobin; there is, namely, a shading of the blue end of the spectrum and two absorption bands between D and E; no change is, however, produced by the addition of reducing solutions.

The author has carefully measured the positions of the bands of Turacin (from *Turacus persa*) and he finds that the centre of the band corresponding to that designated α in the spectrum of oxy-haemoglobin has a wave-length of 578; the band in the green has a wave-length of 538—540.

Composition of Turacin.

The remarkable feature of this red-colouring matter is the constant presence of copper in it.

Church has made many analyses of several specimens of this body, and these have yielded concordant results. From these analyses Church has deduced the empirical formula $C_{50}H_{55}CuN_5O_{19}$, which demands the following percentages:—

	Theory.	Experiment (Mean).
C_{50} . .	54·87	54·63
H_{56} . .	5·12	5·22
Cu . .	5·81	5·90
N_5 . .	6·39	6·38
O_{19} . .	27·81	27·87
	100·00	100·00

The quantity of Turacin in a single bird does not exceed two or three grains[1].

Sect. 4. Certain other Colouring Matters occurring in the Animal Kingdom.

The study of animal colouring matters apart from those found in the blood has hitherto, with few exceptions, met with but little attention. While a number have been examined spectroscopically with the results given below, but few have been chemically analysed with anything like thoroughness[2].

[1] The above account is drawn from Professor Church's Memoir entitled "Researches on Turacin, an animal pigment containing copper." *Philosophical Transactions*, Vol. CLIX., Part ii. (1870), pp. 627—636.

[2] The chief papers on this subject are the following:—E. Ray Lankester: "Report on the Spectroscopic Examination of certain Animal Substances," *British Association Reports*, 1869. "Abstract of a Report on the Spectroscopic Examination of certain Animal Substances, presented to the Brit. Assoc. at Exeter, 1869," *Journ. of Anat. and Phys.*, Nov., 1869, p. 119. "On Blue Stentorin, the colouring matter of *Stentor coeruleus*," *Quart. Journ. of Micros. Sc.*, April, 1873. "Preliminary notice of some observations with the spectroscope on Animal Substances," *Journ. of Anat. and Phys.*, 1868, p. 114. H. C. Sorby: "On the colouring matters derived from the decomposition of some minute organisms," *Month. Micro. Journ.*, Vol. VI. (1871), p. 124. "On the colouring matter of some *Aphides*," *Quart. Journ. of Micr. Sc.*, 1871, p. 352. "On the colouring matter of *Spongilla fluviatilis*," *Quart. Journ. of Micros. Sc.*, 1871, p. 352. "On the colouring matter of *Bonellia viridis*," *ibid.*, p. 166. H. N. Moseley: "On Actiniochrome," *Quart. Journ. Micros. Sc.*, 1873, p. 143. "On colouring matters of various animals," *ibid.*, 1877, p. 1. This is a most important paper, giving the fullest account of the spectroscopic examination of a very large number of pigments.

The pigments, to be referred to in this section, occur either diffused through the tissues, as in many marine animals, or in the form of granules contained in certain cells or layer of cells, usually dermal or subdermal, sometimes deeper in the mesoderm, very rarely in the endoderm. Such granular mesodermal deposits are frequent in cephalopoda, fishes, amphibia and even lizards. The *Chlorophylls* and associated 'vegetable' pigments, when present in animals, are always in granules, whether in the striae of the myophane of *Infusoria*, the tissues generally of *Spongilla*, the sub-muscular mesoderm of *Convoluta*, or the endoderm of *Hydra viridis*.

The following is a list of such pigments as have hitherto been described, arranged in the order of the animals yielding them[1].

Sub-kingdom.	Colouring Matters.
Protozoa.	*Chlorophyll*. *Blue Stentorin*.
Porifera.	*Chlorophyll*. Various other pigments shewing no bands.
Coelenterata.	*Chlorophyll*, &c. in *Hydra viridis* and in *Anthea Cereus*, var. *smaragdina*.
	Actiniochrome in *Bunodes crassicornis*.
	Polyporythrin in many simple Anthozoa and some Hydroids.
	Two distinct pigments with characteristic absorption bands in *Adamsia* sp.
	A red pigment with one band in *Coenopsammia*.
	Other pigments yielding no bands.
Echinodermata.	*Purple Pentacrinin* in many species of *Pentacrinus*.
	Red Pentacrinin in a species from Meangis Is.
	Antedonin from an Antedon and a deep-sea Holothurian.
	Hoplacanthinin from *Hoplacanthus* sp.
	These four pigments all have absorption spectra with definite bands, the other pigments obtained from animals belonging to this class yield no bands.
Vermes.	*Chlorophyll* (?) in *Convoluta*[2].
	Bonellein in *Bonellia viridis*.
	Other pigments yielding no bands, including a blue one, reddened by acids, in a *Rhyncodemus* sp.
Crustacea.	*Chlorophyll* in *Telotea viridis*.
	Crustaceorubrin in many deep-sea Decapods ; in a *Pandarus* infesting *Carcharius brachyurus ;* in surface Entomostraca.
	Other pigments yielding no definite absorption spectra.
Insecta.	*Cochineal* from *Kermes cacti*.
	Aphidein from an *Aphis* on the apple.
	Lac-dye from *Coccus Laccae*.
	Other pigments not yet examined, or yielding no definite spectra.

[1] This list is compiled chiefly from Moseley's previously-quoted paper (*Quart. Journ. of Micr. Sc.*, 1877, p. 1).

[2] Geddes, "Physiology and Histology of Convoluta Schultzii." *Proceedings of the Royal Society*, Vol. XXVIII. p. 449.

Mollusca.	*Aplysio-purpurin* from *Aplysia*[1] and *Doris*.
	Janthinin in *Janthina*.
	Tyrian purple[2] in several species of *Murex* and *Purpura*.
	Other pigments yielding no bands.
Vertebrata.	In addition to colouring matters referred to in other parts of this book—a bluish-green pigment with a single band, extending from B to beyond C, destroyed by heat, acids and alkalies; found in *Odax*, 3 spp. and in *Labrichthys Richardsonii*[3].

A short account of the chief characters of certain of the above-named colouring matters will now be given.

Blue Stentorin[4].

This blue colouring matter obtained from *Stentor coeruleus* is characterized by a spectrum with two absorption bands; of these the darker is on the red side of C; a second lighter band, between D and E, occupies approximately the space intervening between the middle of the α band of oxy-haemoglobin and the β band of the same body. The colour is unaffected by acetic, hydrochloric, and sulphuric acids; caustic potash causes the colour to become darker, the band between D and E disappears, and that between B and C becomes darker and is shifted somewhat nearer towards B.

Actiniochrome[5].

This is a red colouring matter obtained by Moseley from *some* specimens of *Bunodes crassicornis*. It possesses an absorption band having approximately the position of the band α of oxy-haemoglobin.

Bonellein[6].

This is a colouring matter obtained by Sorby from *Bonellia viridis*. According to Sorby it resembles blue chlorophyll in many respects, but differs in only being temporarily altered by acids, the original colour returning on neutralization. It occurs in fine granules in the epidermal protoplasm, and is insoluble in water, soluble in alcohol, ether and carbon disulphide. The following are the wave-lengths of the centres of the absorption bands of Bonellein (expressed in millionths of a millimetre):

1. Alcohol solutions, alkaline or neutral
   662, 636, 611, 587, 520, 490.

[1] An Italian chemist has asserted that an aniline base is present in Aplysia, (Moseley, *op. cit.*, p. 13).

[2] Lacaze-Duthiers, "Mémoire sur la Pourpre," *Annales des Sciences Naturelles, Zool.* Sér. IV., Vol. XII. pp. 5—84.

[3] George Francis, *Nature*, Vol. XII. p. 167.

[4] Lankester, *Op. cit.*, (see foot-note to p. 305).

[5] Moseley, *Op. cit.*, (see foot-note to p. 305).

[6] Sorby, *Op. cit.*

2. Alcohol solutions, slightly acidulated
636, 611, 588, 565, 543, 522, 492.
3. „ strongly acid
617, 590, 565, 552, 517.

Solutions of Bonellein are fluorescent.

Carminic acid.

The female Cochineal insect (*Coccus cacti*) contains from 26—50 p. c. of a splendid red colouring matter, to which the name of Carminic acid is given, and from which commercial carmine is prepared. This colouring matter is found in other species of *Coccus*, and occurs in the vegetable kingdom, being found in the blossoms of *Monarda didyma*.

Mode of preparation. One part of powdered cochineal is boiled in 40 parts of water for half an hour; the solution is then decanted, precipitated with lead acetate, care being taken to avoid an excess of the precipitant; the precipitate is washed with boiling water so long as the washings give a precipitate with solutions of mercuric chloride; it is then decomposed by sulphuretted hydrogen; the filtrate from the precipitate of lead sulphide is evaporated to dryness at a very low temperature, and the residue is extracted with alcohol, which dissolves the carminic acid.

Composition and properties. Carminic acid, $C_{17}H_{18}O_{10}$, is an amorphous red powder easily soluble in water, and alcohol, and in hydrochloric and sulphuric acids. It forms no salts of constant composition. The ammonium salt exhibits two absorption bands between D and E nearer E than those of oxy-haemoglobin; these bands are more closely approximated than those of oxy-haemoglobin and have less distinct edges. Aqueous and alcoholic solutions of cochineal on the other hand absorb all but the red rays.

The author has determined the position of the centres of the two bands of carminate of ammonia. The centre of the band which corresponds to that designated as a in the spectrum of oxy-haemoglobin is approximately 530, the centre of the band corresponding to the band β of oxy-haemoglobin is approximately 570. An ammoniacal solution of carmine also exhibits two absorption bands, of which the centres are respectively 570 and 528.

A solution of picro-carmine exhibits a spectrum which at first sight very closely resembles that of oxy-haemoglobin. It will be observed, however, that the band near the red (a) is less dark than the one in the green, the centres being respectively approximately 565 and 520, and there is a third band in the blue, very close to its junction with the green.

Carminic acid a Glucoside. When boiled with dilute acids carminic acid combines with the elements of water to form an unfermentable sugar, which is optically inactive, and a new pigment carmine-red ($C_{11}H_{12}O_7$):

$$C_{17}H_{18}O_{10} + 2H_2O = C_6H_{10}O_5 + C_{11}H_{12}O_7.$$[3]

Tyrian Purple[1].

This colouring matter, which was employed in remote antiquity to dye the robes of royalty, and which even in the luxurious days of Imperial Rome retained its position as the dye of greatest beauty and value, is derived from the secretion of a glandular organ which is situated at the lower part of the mantle, between the gill and the rectum, of various species of *Murex* and *Purpura*. The secretion when first poured out is colourless or yellowish, but when exposed to the light, especially if it be first diluted with water, it assumes first a bluish-green, then a red and lastly a purple-violet colour, at the same time emitting a strong alliaceous smell. This change occurs spontaneously in the case of *Murex trunculus* even though the juice be kept in the dark, in sunlight it occurs in a few minutes. In *Murex brandaris* the colour is produced only in the light and more slowly. The dried juice, when powdered, appears red; it is insoluble in water, alcohol and ether, in dilute acids and cold alkaline leys.

Punicin the colouring matter obtained by the action of light from the Chromogen of Purpura lapillus.

Schunck[2], to whose investigations we owe so much of our knowledge of certain animal colouring matters, has examined the bright purple colouring matter obtained by exposing to light the secretion of the *purpurogenous* gland of Purpura lapillus. This colouring matter is insoluble in water, alcohol, and ether; it is slightly soluble in boiling benzol, and in boiling glacial acetic acid. It dissolves entirely and with comparative ease in boiling aniline. The solution is at first green, but as it approaches saturation, it becomes purplish-blue; on cooling, it again becomes green, depositing at the same time small granular masses of colouring matter, and retains at last only a faint greenish tinge. The solution at its darkest stage, while still warm, shews a broad but well-defined band, beginning near the line *C* of the spectrum, and extending beyond *D*, but as the solution cools, depositing the substance contained in it, the band becomes gradually narrower, until it occupies the space midway between *C* and *D*, and it then disappears. The masses of colouring matter deposited from the solution in aniline, are seen under the microscope to consist of star-shaped groups of irregular crystalline needles. Punicin, when cautiously heated, furnishes a crystalline sublimate.

Chlorophylloid Colouring Matters.

The consideration of these will be postponed to Book III. (Respiration).

[1] The reader interested in the subject of this paragraph is referred to the fine memoir of Lacaze-Duthiers (see foot-note 2, p. 307), and to an interesting article by Dr Schunck, entitled "Note on the Purple of the Ancients." *Journal of the Chemical Society*, 1879, p. 589.

[2] Edward Schunck, "Note on the Purple of the Ancients." *Journal of the Chemical Society*, No. 202 (1879), p. 589.

CHAPTER IX.

THE CONTRACTILE TISSUES.

Sect. 1. Introductory.

The Structure of the Contractile Tissues.

The properties of Protoplasm.

While Schwann and other observers, about the year 1835, were tracing the resemblances of vegetable and animal tissues, Dujardin[1], a French naturalist, was investigating in some of the lower animals a remarkable substance to which he gave the name of *sarcode*. This substance is amorphous, gelatinous of various degrees of consistence, and elastic; and it always contains granules of greater or less fineness. It occurs in fragments whose shape is indefinite and indeed variable. It is capable of developing within its mass *vacuoles* or cavities filled with a pellucid fluid, which afterwards close so perfectly that no crack or scar betrays their former presence. But it is chiefly remarkable for its power of extending portions of its surface, at will, into processes which may or may not inosculate, and which again, at will, are withdrawn into the general mass. To this property the name *contractility* is given. In what manner the protrusion is effected it is impossible to decide; but it is easy to imagine that the normal form of the contractile mass of sarcode is spherical and that contraction may be exerted in any *chord:* in which case the corresponding *segment* would be pressed out as a process (Hermann). The projection of columns or processes is not the only movement exhibited by sarcode. The granules imbedded in its mass may undergo gliding and dancing movements resembling the mechanical *Brownian movements* which are seen when very minute particles are suspended in a liquid. In each case the granules are passive. In the case of the gliding movements, which are well seen along the extended processes of *foraminifera,* the agent is the contractile sarcode; but in the case of the dancing movements the cause may be the same as that of the Brownian movements referred to. It is true that they may be seen in contractile tissues which are unquestionably alive; but

[1] Dujardin, "Recherches sur les organismes inférieurs." *Ann. des Sciences naturelles,* 2nd Ser. (1835), Vol. iv. p. 343.

it is also true that they are exhibited in dead tissue, and that they often seem to depend upon a diluted state of the sarcode[1].

It soon became apparent that the most remarkable properties of sarcode, or, as it is now termed, *protoplasm*, were not peculiar to it. Siebold[2] discovered contractile powers in the yolk-spheres of Planarian ova, and Wharton Jones[3] in the white corpuscles of vertebrates, while Kühne[4] contrasted muscular tissue, Amoebae and Vorticellae in respect of their excitability and death-changes. Thus the way was prepared for the doctrine of the analogy of sarcode to the body or contents of the animal cell, and the doctrine of the cellular nature of infusorians[5]; from which we derive the unity of the contractile power in such creatures as the Amoeba and in the specialized muscular tissues of man.

Limited powers of contraction are enjoyed by very many cells of the bodies of higher animals. The connective-tissue corpuscles of the cornea[6], the cells of hyaline cartilage[7], and the walls of capillaries[8], seem capable of contracting, at least when stimulated by electrical currents. The gliding motion of granules in the pigment cells of the frog's skin may be readily demonstrated. White blood corpuscles and lymph cells exhibit movements in no respect different from those of primitive sarcode; while ciliated epithelia and spermatozoa offer the simplest examples of movement *as a specialized function*. But it is in muscles that contraction becomes prominently the function of the tissue, and where its laws have been most fully examined.

Classification of muscles according to their structure.

Of muscles there are, from the histological point of view, three sorts: (1) the smooth involuntary muscular tissue of intestines, uterus, arterial walls, &c.; (2) the striated muscles of the general voluntary system; and (3) the striated involuntary muscle of the heart.

Structure of unstriped involuntary muscle.

This variety of muscular tissue consists of innumerable small fibre-cells (0·045 to 0·230 × 0·004 to 0·01 of a mm.) extended in

[1] Recklinghausen, "Ueber Eiter- und Bindegewebs-Körperchen." Virchow's *Archiv f. path. Anat. u. Physiol.*, Vol. XXVIII. p. 166, 1863.

[2] Siebold, *Froriep. Notizen*, No. 380, p. 85. Quoted by Stricker, "Ueber die Zelle." *Handbuch der Lehre von den Geweben*, chap. I. p. 2.

[3] Wharton Jones, "The blood corpuscle considered in its different phases of development in the animal series." *Phil. Trans. Roy. Soc. Lond.* 1846, pp. 63—106.

[4] Kühne, "Untersuchungen ü. Bewegungen u. Veränderungen der contractilen Substanzen." *Archiv für Anat. Physiol. u. wiss. Med.* (Reichert u. du Bois-Reymond), 1859, p. 816.

[5] M. Schultze, "Ueber Muskelkörperchen und das, was man eine Zelle zu nennen habe." *Archiv f. Anat. Physiol. u. wiss. Med.* (Reichert u. du Bois-Reymond), 1861, p. 17.

[6] Kühne, *Protoplasma*, &c., p. 125. Rollett, Stricker's *Handbuch*, p. 1103.

[7] Heidenhain, "Zur Kenntniss des hyalinen Knorpels." *Studien des physiol. Inst. zu Breslau*, Part 2 (1863), p. 1.

[8] Stricker, "Untersuchungen ü. die Contractilität der Capillaren." *Wiener Sitzungsber. d. math.-naturwiss. Classe*, LXXIV. p. 313, 1877.

the axis of contraction[1], overlapping their neighbours, to which they are united by means of an intervening substance well seen in hardened transverse sections of the tissue. The importance of this interposed substance has been called in question by Engelmann[2]. In perfectly fresh specimens, not only of the ureter but also of other smooth muscular tissues, it is impossible to detect any demarcation of cell from cell; the tissue forms, to all appearance, a homogeneous mass, interrupted only by the nuclei; it is an optical *continuum*. This homogeneity persists, under favourable circumstances, for a short time; but frequently, after a few minutes have elapsed, fine lines begin to appear, which speedily cut up the field into elongated elliptical areas, enclosing the nuclei, and clearly foreshadowing the cells. Thus the homogeneity claimed by Engelmann for involuntary muscular tissue is the homogeneity of an absolutely fresh cornea. It is merely optical and does not imply a perfect structural continuity in the sense sometimes ascribed to Engelmann's words[3].

The cells are commonly spindleshaped, but sometimes forked and flattened. They were formerly considered to possess no membrane; but lately a sheath has been described, with annular swellings which produce an appearance of transverse striae[4]. Their substance is granular, and speckled with a varying number of refractile particles soluble in alcohol; and they contain an elongated oval or rod-shaped nucleus. Inside the nucleus one or more distinct nucleoli are found; and beyond each pole of the nucleus, in the substance of the fibre-cell, is a short row of larger granules, which diminish in size as they approach the end of the fibre. The fibres frequently display a longitudinal striation, especially when treated with reagents[5]; and, although they are properly described as non-striated in a transverse direction[6], yet it is no uncommon thing, when they have been macerated in certain hardening fluids, to find them snapped sharply across so as to leave a truncated, praemorse surface. When examined with polarized light, fibre-cells, like the transversely striated muscle

[1] The power of contraction along *two* axes at right angles to one another has been suggested by Mr Gaskell in the case of the muscles of arterial walls. (*Studies from the Physiol. Lab. of the University of Cambridge*, Part III. p. 164. Also *Journ. Anat. and Physiol.*, Vol. XI.)

[2] Engelmann, "Zur Physiologie des Ureter." Pflüger's *Archiv*, Vol. II., 1869, pp. 247, 274. "Beiträge zur allgemeinen Muskel- u. Nervenphysiologie." Vol. III., 1870, p. 248.

[3] See the discussion in the *Archiv f. mikrosk. Anat.* by Dogiel, Foster and Dew-Smith, &c. Hermann (*Physiology*, 2nd ed. by A. Gamgee, p. 300) so understands Engelmann: but Engelmann always refers to a *physiological continuity* merely, although he speaks of the ureter as a 'colossal fibre.'

[4] E. Klein, "Observations on the Structure of Cells and Nuclei." *Quarterly Journal of Microscop. Science*, New Series, July 1878, p. 331.

[5] Flemming, "Ueber die Beschaffenheit des Zellkernes." *Arch. f. mik. Anat.*, Vol. XIII. p. 693. Klein, *Op. cit.*

[6] See however Meissner ("Ueber das Verhalten der muskulösen Faserzellen im contrahirten Zustande." *Zeitschr. f. rat. Med.*, 2nd Ser., Vol. II., 1858, p. 316) who saw transverse markings on contracted fibres; also Klein, *Op. cit.*

about to be described, are found to contain doubly refracting, positive, uniaxal particles scattered through their substance.

Structure of voluntary muscle.

The second kind of muscular tissue is commonly known as *voluntary*, and *transversely striated*. It consists of *elements* or *fibres*, which are exceedingly large when compared with fibre-cells, being about $\frac{1}{500}$th of an inch in diameter (10 to 80 μ[1]), and as much as from 1 to $1\frac{1}{2}$ inches long[2]. Each fibre is enclosed in a structureless elastic sheath or *sarcolemma*, rounded at its extremities, which either become attached to tendons or aponeuroses, or lie overlapped by neighbouring fibres. The contents of the sarcolemma when examined in a perfectly fresh condition, as they may be in the case of cold-blooded animals, are of a pale grey translucent appearance. They exhibit a very regular series of transverse markings, but hardly a trace of longitudinal striation, if care have been exercised in the preparation. The transverse striation is due to an alternation of dim and bright lines which commonly run continuously across the long axis of the fibre, but which are sometimes interrupted by 'faults,' (to use a geological term,) as if one portion of the fibre had slipped to a lower level than the rest. The striae in the frog's muscle are exceedingly fine and somewhat confusing. If we examine in the normal condition the muscles of animals lower in the scale, we find the corresponding elements both larger and more complex. This examination may be made with very little preparation in the case of the limb-muscles of *Hydrophilus*, fragments of which may be snipped out and mounted without any addition, after the chitinous covering of the thigh has been split, while in the case of *Cyclops* no preparation whatever is needed other than fixing the specimen beneath a covering glass[3]. A muscle of small diameter and at rest should be selected for observation. In such a specimen the most striking feature will still be the alternation of darker and lighter bands. But the dark, or, more strictly speaking, the *dim* band will be found more or less marked by longitudinal lines, and to be traversed by a zone or region less cloudy than the rest, to which the name of *Hensen's disc* is given. The lighter stripe, in its turn, is still more clearly divided by a thin dark line called *Krause's membrane*, which under a sufficiently high power in hardened specimens appears as a series—often as a double series—of dots[4]. If such a muscular fibre were seen in cross section, and in a perfectly normal state, it would present the appearance of a homogeneous clear substance, thickly and evenly

[1] Ranvier, *Traité technique d'Histologie*, p. 468.

[2] Quain's *Anatomy*, eighth ed., Vol. II. p. 115.

[3] As was demonstrated to the author by Mr Marcus Hartog, in Cyclops the structure of striated muscle, and the end-organs of the nerves in muscle, may be perfectly studied in the living, uninjured animal.

[4] This line is said to have been first seen by Dobie (*Ann. of Nat. Hist.*, 2nd Ser., 1849, Vol. III. p. 109).

studded with fine dots; but if the muscle were first frozen before the section was made, it would be seen to be divided by fine lines into a number of angular areas, known as *Cohnheim's areas;* as if the whole

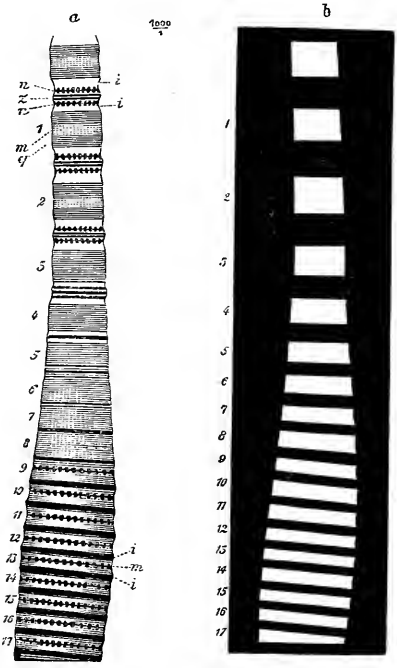


FIG. 54. DIAGRAM ILLUSTRATING THE STRUCTURE OF A STRIPED MUSCULAR FIBRE. (After Engelmann.)

The specimen was taken from the abdominal muscles of *Telephorus melanurus*, and made rigid by being plunged into 50 p.c. alcohol: *a* represents the fibre in various stages of contraction, when viewed in common light; *b* is a schema of the same fibre in polarized light.

1, 2, represent the broad dim bands in the fully relaxed condition; *m*, Hensen's disc (Mittelscheibe); *q*, darker portion of broad dim band (Querscheibe); *z*, Krause's membrane, appearing double (Zwischenscheibe); *n*, accessory band (Nebenscheibe); *i*, *i*, intermediate substance, forming the broad bright stripe or band.

Of the various segments, 1 and 2 are fully relaxed; 3, 4 and 5 are in the beginning of contraction; 6, 7 and 8 constitute the homogeneous stage of contraction; and from 9 onwards the segments are in the stage of transposed bands, the original bright stripe being now dimmer than the original dark stripe.

b shews that there is no transposition of doubly refracting and singly refracting substance on contraction.

For the sake of simplifying the diagram the double refraction of Krause's membrane and the accessory bands is not indicated.

fibre consisted of a number of compressed columns or prisms surrounded by the sheath of the sarcolemma. Were we to irrigate these fresh specimens of muscular tissue with dilute acetic acid, the muscle would swell up, and the transverse striation would become faint, while here and there a third element of the tissue, viz. *the nuclei,*

would become prominent. These in the fresh state are oval, flattened, structures containing *nucleoli* and usually surrounded by, or associated with, a small fragment of unstriated granular protoplasm; but under the influence of the acid they frequently become shrivelled and linear. In the frog and in the water-beetle they may be found at any depth in the mass of the fibre; while in mammalian muscles they are situated immediately beneath the sarcolemma.

If the leg of a water-beetle be torn from the body of a recently killed specimen, and its chitinous covering split; and if it be then plunged into absolute alcohol; portions of fibres may be found in all stages of contraction. Most frequently it happens that in the contracted portion the sarcolemma is raised up from the contractile substance opposite the level of each dim band in the form of a regular fold encircling the whole fibre. In consequence of this, the sarcolemma at the edge of a longitudinal section (or *optical* longitudinal section) of a fibre appears very regularly festooned, the festoons being opposite the ends of the dim bands, and the fixed points opposite the ends of the so-called membranes of Krause. Upon this very remarkable appearance, taken in conjunction with the appearance known as the areas of Cohnheim, Krause has founded his theory that a muscular fibre is partitioned off into superposed prismatic cavities or cells, by horizontal diaphragms which are Krause's membranes, and by vertical walls which are the boundaries of Cohnheim's areas.

Older views. Bowman's Sarcous elements.

The older views of the muscular fibre were chiefly based upon the effects of certain reagents on the tissue. If a muscular fibre is steeped and hardened in a solution of chromic acid or in alcohol—and this applies to mammalian as to other fibres—the sarcolemma becomes brittle, and the whole contents resolve, at the slightest touch, into innumerable fine *fibrillae*, each of which exhibits an alternation of light and dark parts corresponding with the light and dark bands of fresh fibres. If, again, a fibre has been macerated in hydrochloric acid, the tendency which it exhibits is, not to split longitudinally but rather transversely, through the centre of the principal bright band, thus breaking up into a number of superposed discs called the *discs of Bowman*. It is clear that, if we imagine cleavage to occur at the same time in both the longitudinal and the horizontal plane, the muscular fibre will become broken up into a number of short prisms, rods, or particles, which may be regarded as the structural units of the *dead* fibres. To these Bowman[1] gave the name of *sarcous elements*, several of which are included in each area of Cohnheim. The researches of Bowman remodelled the old representations of the striated muscular fibre, and gave to them a form which they have more or less preserved for forty years.

[1] Bowman, "On the minute structure and movements of voluntary muscle." *Phil. Trans. Roy. Soc. Lond.* 1840, p. 457.

The behaviour of muscle to polarized light.

The property of double refraction in muscular tissues has already been mentioned. Its discovery was made[1] in striated muscle; where also its conditions have been more fully observed than in the smooth variety. A convenient apparatus for demonstrating double refraction in microscopic objects consists of two Nicol's prisms, one—the polarizer—fixed between the illuminating mirror of the microscope and the object stage, and the other—called the analyser and capable of rotating about the optical axis of the instrument—interposed between the ocular and the observer's eye. When the planes of polarization of the two Nicols are at right angles the prisms are said to be crossed, and the field of view is darkened; when they coincide the field is brightest. If, when the Nicols are crossed, a doubly refracting body is interposed between them; if for example a plate of doubly refracting crystal, cut parallel to its axis, is laid upon the stage of the microscope; the analyser no longer blocks the rays, and the field again becomes bright. The degree of brightness varies according to the direction of the axis of the doubly refracting plate: it is greatest when this axis makes an inclination of 45° with each Nicol's plane; and it is *nil* when it coincides with either of these. If muscle, or any part of muscle, behaved like such a plate of crystal, we should ascribe to it similar double-refracting properties. A more beautiful way of demonstrating the optical properties of muscular tissue is to interpose a very thin plate of doubly refracting selenite or mica between the crossed Nicols. In this case, as in the above experiment, light is transmitted or not through the analyser according to the inclination of the axis of the plate; but the light is not white, it is *coloured*. The particular colour depends upon the thickness of the plate; and the most useful thickness is that which gives a purple tint to the field with the proper inclination of the axis. Supposing this to be attained, and supposing also that we have that relation of the plate to the prisms which secures the highest intensity or fulness of colour, we shall find that, as we rotate the analysing Nicol, the intensity of the tint will diminish to its vanishing point, at 45°, beyond which the *complementary* tint will appear, and increase to its maximum fulness at 90°; and so alternately through every quadrant. If now we place upon the mica plate a doubly refracting body, its colour will be found to differ from that of the field according to its doubly refracting character, its thickness and the inclination of its axis to the crossed Nicol planes. The advantage of the arrangement is that we may discriminate between isotropous and doubly refracting bodies, not merely by different intensity of light, but by more easily detected differences of colour.

By the aid of such appliances Brücke[2] was able to determine

[1] C. Boeck (in 1839); reported in *Arch. f. Anat. Physiol. u. wiss. Med.* (J. Müller), 1844, p. 1.

[2] Brücke, "Muskelfasern im polarisirten Lichte." Stricker's *Handbuch*, Chap. vi. p. 170.

that muscular fibres consist of isotropous and anisotropous or doubly refracting substance; that the latter is found in the broad dim band which is made up of a series of sarcous elements; and that these optically resemble uniaxal crystals the axes of which coincide with the length of the fibre. By comparing the optical phenomena of muscles and rock-crystal he assigned to muscle a place among positive double-refracting bodies; and on purely physical grounds he assumed the double-refracting powers to be due to the presence in the sarcous elements of innumerable doubly-refracting particles, to which hypothetical particles he ascribed the name of disdiaclasts (δίς, twice, and διακλάω, I break in twain).

The rest of the muscular fibre is isotropous in all meridians and all positions; except Krause's membrane, which, like the sarcous elements, is doubly refracting[1].

Engelmann's views. Of late years the scheme of striated muscle in Arthropoda, and especially in Insecta, has grown to be still more complicated than in this description. With suitable powers, that which has been called Krause's membrane becomes resolved into three narrow bands, an *intermediate* and two *accessory*[2]. The intermediate band is continuous, when in the fresh state, and sometimes double; but broken into granules when hardened. The accessory bands are usually more or less granular. The intermediate band is double-refracting, as may best be seen in hardened specimens of broad-banded muscles; whereas double refraction in the accessory bands is faint and uncertain. The diagram on page 314 should be consulted; it will be noticed that the double refraction of Krause's membrane is omitted for the sake of simplicity.

Subdivision of voluntary muscles into pale and red. While the above general description includes all conditions of voluntary striated muscular tissues, varieties are distinguished in the muscles of some animals: these are the *pale* and the *red*, of which the unlikeness of colour persists after bleeding. In the former, transverse striation is extremely regular and longitudinal striation merely indicated: the *recti*, the *vasti* and the *adductor magnus* of the rabbit's hind limb are instances. In the latter, or red variety, of which the *adductor brevis* and the *soleus* are types, the transverse bands are broken up by a well-marked longitudinal striation[3]. Still more interesting physiological differences will be spoken of hereafter.

[1] See following note.

[2] Engelmann, *Proces verbaal d. k. Akad. van wetenschappen. Afdeel. Natuurk.* No. 6, Dec. 1871; and No. 7, Jan. 1872. Referred to in a paper by the same author ("Ueber die quergestreifte Muskelsubstanz") in Pflüger's *Archiv f. d. ges. Physiol.*, Vol. VII., 1873, pp. 36, 42, 50. Unhappily the term 'strie intermédiaire' has been applied by Ranvier (*Traité Technique d'Histologie*, p. 481) to Hensen's disc.

[3] Ranvier, "De quelques faits relatifs à l'Histologie et à la Physiologie des Muscles striés." *Arch. d. Physiol. norm. et pathol.*, 2nd Ser., Vol. I. p. 5, 1874. E. Meyer, "Ueber rothe und blasse quergestreifte Muskeln." *Arch. f. Anat. Physiol. u. wiss. Med.* (Reichert und du Bois-Reymond), 1875, p. 217. W. Krause seems to have been the first to notice the distinction of colour in red and pale muscles (*Anatomie des Kaninchens*, 1863).

The blood-vessels of muscular tissue.

The blood-vessels of striated muscular tissue are very abundant, and the capillaries are small. The latter are distributed upon the fibres, in elongated meshes; but they nowhere pierce the sarcolemma.

The capillaries of the red variety of voluntary muscles are wider than those of the pale. They are disposed in shorter meshes, and are marked by peculiar aneurismal dilatations[1].

The structure of the Muscular Substance of the Heart.

The third kind of muscular tissue consists of quadrate cells with a faint longitudinal striation and a rough transverse striation. In the centre of each cell is an oval nucleus, usually associated with a small amount of granular protoplasm; and not unfrequently the cell-substance contains a few scattered fat particles. The cells are joined end to end, or side to side, and often by means of stout truncated processes. They are apparently destitute of sarcolemma.

The heart-muscle of amphibia differs somewhat from the above description. The cells are not quadrate, but spindle-like, overlapping the neighbouring cells. They are transversely striated; and both Krause's membrane and Hensen's disc are said to have been seen[2]. The dark striae are doubly refracting.

In mammals the size of the cells is from 50—70 μ long by 15—23 μ broad[3].

Terminations of Nerves in Muscle.

The mode of union of muscles and motor nerves cannot as yet be said to have acquired more than anatomical significance. The cells of smooth muscle are entangled in a net of nervous fibres, from which fine offsets seem to end abruptly on the surface or in the substance of the cells, or even to pierce the nuclei[4].

A similar disposition of nerves has been claimed for the heart-muscle[5].

In the case of striated voluntary muscle, the medullated nerve fibres reach the sarcolemma, and pierce it. The sheath of Schwann or neurilemma becomes continuous with the sarcolemma. The

[1] Ranvier, "Note sur les vaisseaux sanguins et la circulation dans les muscles rouges." *Op. cit.*, p. 446. E. Meyer, *loc. cit.*

[2] Langerhans, "Zur Histologie des Herzens," Virchow's *Archiv*, Vol. LVIII. p. 56 Gerlach, L., "Ueber die Nervenendigung in der Musculatur des Froschherzens." Virchow's *Archiv*, Vol. LXVI. p. 187.

[3] Schweigger-Seidel, "Das Herz," Stricker's *Handbuch*, chap. vii. p. 179.

[4] Consult Arnold, "Gewebe der organischen Muskeln," Stricker's *Handbuch*, chap. iv. p. 144; and, of the more recent authorities, Löwit, *Wien. Acad. Sitzungsb.* III. Abth., Vol. LXXI. 1875, p. 355. Gscheidlen, R., *Arch. f. mikr. Anat.*, Vol. XIV. p. 321. Ranvier, *Comptes Rendus*, Vol. LXXXVI. p. 1142.

[5] Consult Schweigger-Seidel, Stricker's *Handbuch*, chap. vii.; and also P. Langerhans, Virchow's *Archiv*, Vol. LVIII. p. 65. L. Gerlach, Virchow's *Archiv*, Vol. LXVI. p. 187. E. Fischer, *Arch. f. mikr. Anat.*, Vol. XIII. p. 365.

axis-cylinder divides in Mammals, Birds and Reptiles, into a short dendriform structure called an *End-plate*, which rests upon a granular nucleated mass called a *Protoplasmic foot*. In Amphibia, there is no protoplasmic foot, and the divisions of the axis-cylinder are nucleated and long, extending mainly in the long axis of the fibre, immediately beneath the sarcolemma. In all classes the white substance of Schwann terminates somewhat abruptly at the entrance to the sarcolemma or at a little distance within it[1]. In muscular fibres destitute of sarcolemma the nerve ends in a granular *Eminence of Doyère*[2], on the side of the fibres which usually bears a nucleus.

Chemical constitution of normal living Muscle, so far as it can be known or inferred.

On the distribution of liquid and solid parts in a voluntary muscular fibre.

In the section which has preceded, an account has been given of the appearances presented by muscular fibres when subjected to a high magnifying power; and we have shewn that, according to all observers, there is contained within the elastic sarcolemma a substance in which doubly refracting and isotropous structures alternate. In the sequel it will be shewn that in the process of contraction the fibre becomes shorter and thicker, and that at the same time the anisotropous elements become broader and shorter, the intermediate isotropous substance also exhibiting some diminution in height and perhaps (though this admits of doubt) diminishing in amount. Whilst these changes in the form are proceeding, differences in light-transmitting powers are perceived, though the behaviour to polarized light remains as before.

We have referred to minor points in which different observers disagree, but without laying very much stress upon them. We have now, however, to discuss a question which is of paramount importance, in reference to the physical constitution of voluntary muscle.

When we examine a dead muscular fibre, especially one which has been acted upon by various hardening reagents, the contents of the sarcolemma all unquestionably possess a solid consistence. Can we, however, infer from such observations that they possess the same characters during life? Certainly not. It was shewn by Kühne[3] that

[1] Kühne, Stricker's *Handbuch*, chap. v.

[2] Doyère, "Mémoire sur les Tardigrades." *Ann. des Sci. Nat.*, Sér. II. Vol. XIV., 1840, p. 269.

[3] Kühne, *Archiv f. Anat. u. Physiol.*, 1859, p. 748. *Untersuchungen über das Protoplasma und über die Contractilität.* Leipzig, 1864. (Consult section entitled "Methoden zur Gewinnung des Muskelinhalts," p. 2.) *Lehrbuch der physiologischen Chemie*, Leipzig, 1866, p. 272.

by subjecting yet living and rapidly frozen muscular fibres to pressure we can express *from the interior of the fibre* a somewhat viscous but yet perfectly liquid substance, to which he gave the name of the *muscle-plasma*, which shortly afterwards, if the temperature be favourable, sets as a soft jelly: doubtless in consequence of the coagulation of a proteid body of which the precursor or precursors existed in solution. Kühne had the good fortune to observe on one occasion living muscular fibres, within the sarcolemma of which a living nematode (subsequently again seen by Eberth[1] and indentified by him as the *Myoryctes Weismanni*) freely moved about. This worm was able to make its way from one end to the other of the muscular fibre, displacing in its course (but only temporarily) the sarcous elements, and in a way which left no room for doubt that the creature was moving in a fluid medium in which were suspended the anisotropous constituents of the fibre. Kühne was thus led to the conception that in the voluntary muscular fibre the contents consist of anisotropous solid bodies—the sarcous elements—which are suspended in a viscous liquid, contraction consisting essentially of a change in the form of the suspended bodies.

Objections have been raised to this view of Kühne's, some of which are based upon microscopic observations, others upon the difficulty which their advocates have experienced in accounting satisfactorily for the orderly arrangement of the anisotropous elements, on the hypothesis that these are simply suspended in a viscous liquid.

Krause, as we have already pointed out, believes that the structure, which since his description of it has gone by the misleading term of 'Krause's membrane' (viz. the anisotropous structure in the light band of resting muscle), is attached to the sarcolemma, so that according to him a muscular fibre would be divided into a series of transverse compartments. But excellent observers who have followed him (Engelmann), deny the existence of a membrane, the existence of which is absolutely disproved by the fortunate observation of Kühne.

Such a view as Krause's might have been held before the time of Kühne's famous observation, but the latter, it appears to us, supplies a certain criterion for rejecting the former. On physical grounds it has been shewn by Brücke, and is maintained in his most recently published writings by Hermann, that the existence of a system of transverse partitions in the muscular fibre would oppose a great (and useless) resistance to the forces which bring about the changes in its form.

The difficulties which some have experienced in explaining the orderly arrangement of the anisotropous elements are, as Kühne points out, dispelled if we surmise that this orderly arrangement is dependent (1) on the pressure exerted upon the contents of the fibre by the sarcolemma, and (2) on the mutual attraction which leads solid bodies, floating in a liquid, to adhere one to another.

[1] Eberth, *Zeitschrift f. wissensch. Zoologie*, Vol. XII. (1863), p. 530.

The facts then that (1) a large portion of the contents of the sarcolemma can be expelled from it in the condition of a liquid, and (2) that living bodies move in the interior of the living fibre, as in a liquid holding solid bodies in suspension, appear to us to settle definitely the great problem of the physical condition of the doubly refracting and isotropous elements of muscular fibre. The most weighty consequences follow the conclusion to which we are led. We cannot, for instance, for one moment suppose that a liquid can change its form in consequence of internal forces acting within it, unless these lead to its becoming solid; we are therefore led on theoretical grounds to the conclusion that the sarcous elements must be the structures which are directly concerned in the change of shape of the fibre. Engelmann's observations, which in all respects are the most consistent and satisfactory which have been advanced, since the earlier classical investigations of Bowmann and Brücke were published, seem to shew that in contraction the sarcous elements undergo a change in form *and in volume*, increasing in bulk at the expense of the isotropous substance, so that the combined volume of the contents remains sensibly the same during contraction and during rest.

Were it possible, we should wish, in the first place, to study the chemical history of the various structural elements which make up the muscular fibre; but this ideal aim can but most imperfectly be realized; so far as possible, we feel however bound to attempt the task.

Chemical characters of the Sarcolemma.

The delicate transparent sheath which in voluntary muscle encloses, as in a sac, the contractile matter which forms the chief substance of the muscular fibre, was formerly supposed to be of the same nature as elastic tissue; like the latter, it is unacted upon by acetic acid, and resists long boiling with water, though it is ultimately dissolved. It differs, also, from elastin in being slowly dissolved when heated in dilute solutions of acids and alkalies. The fact that it is dissolved gradually at the temperature of the body by the ferments of the stomach and pancreas has also been adduced as proving that the sarcolemma is not identical with elastic tissue; in point of fact even elastic tissue is slowly dissolved by these ferments, and particularly by pepsin.

Chemical nature of the doubly-refracting elements of voluntary muscle.

The doubly refracting (anisotropous) matter of voluntary muscular fibre is, during life, as after death, of solid consistence. It loses its peculiar optical properties when the fibre containing it is subjected to the action of either acids or alkalies, or when it is heated to boiling. For these reasons it has been surmised that this matter is proteid in nature. It has, however, been remarked that neither alcohol

nor salicylic acid—reagents which coagulate the proteids—affect the doubly refracting sarcous elements, so that one would be inclined to believe that they consist rather of some derivative of the proteid bodies than of proteid bodies pure and simple.

It is stated that 'Krause's membrane,' though sharing the optical properties of the sarcous elements, has a different deportment towards dilute acids; thus a three-per-cent. solution of acetic and a one-per-cent. solution of hydrochloric acid are said to annul the anisotropous character of the sarcous elements, but not of Krause's membrane, which is, however, affected by caustic alkalies in the same manner as the sarcous elements.

The Muscle Plasma[1].

Kühne's[2] method of obtaining Muscle Plasma.

The liquid to which the name of *muscle plasma* is given, and which constitutes, as has been shewn, the isotropous material of the voluntary muscular fibre, can only be obtained from muscle which has not passed into the state of *rigor mortis*, for when this change occurs, a solidification of a proteid matter previously in solution occurs, and muscle plasma, properly so called, ceases to exist. Cold delays the coagulation of the plasma, as it does that of the liquor sanguinis, and it is by its aid that the plasma can be obtained.

The muscles of cold-blooded animals alone preserve their vitality sufficiently long to permit of the plasma being removed before rigor has had time to occur; practically those of the frog are always employed, and the process is the following:

The frog is bled, and salt solution ($\frac{1}{2}$ p.c.) is injected into the aorta, so as to wash the whole of the blood out of the muscles. The muscles are then cut up into small pieces, and washed in, or kneaded with, more of the same salt solution cooled to 0° C., with the object of getting rid of lymph. The fragments are then collected together, enclosed in fine linen, and tied up so as to constitute a compact ball, which is exposed to a temperature of about − 7° C. until it is in such a condition that it can, by means of cooled knives, be conveniently cut into very fine slices; this operation can only be carried out in very cold weather. The frozen slices are then pounded in cooled mortars, the pounded muscle tied up in strong linen, and expressed in a strong press at the temperature of the room. The muscle thaws at 0°, so that the liquid which flows from the press has this temperature; it is then filtered through small paper filters moistened with ice-cold salt solution; as the filters speedily clog, the fluid must frequently be transferred to fresh filters.

[1] This account of the Muscle Plasma and Muscle Serum, is taken *almost verbatim* from Kühne's *Lehrbuch der physiologischen Chemie*. It would have been vain to attempt to give a more succinct or a more satisfactory account than that of the eminent physiologist to whom we owe almost every fact known in relation to the proteids of muscle.

[2] Kühne, *Untersuchungen über das Protoplasma*, p. 2. *Lehrbuch*, p. 272.

The filtrate obtained by the above operation is a faintly yellow opalescent liquid. It is muscle plasma.

Properties of the muscle plasma.

Muscle plasma is of syrupy consistence; it flows, however, forms drops, and possesses all the characters of a liquid. It has a faint alkaline reaction.

At ordinary temperatures muscle plasma coagulates exactly like blood plasma. Coagulation is accelerated by contact with foreign matter and commences at the points of contact; it is also accelerated by stirring with a glass rod.

Myosin.

The solid body which separates from muscle plasma when this liquid coagulates has received the name of Myosin. This body differs from fibrin in being a gelatinous mass when first formed, and though it subsequently contracts, it never becomes fibrous, nor has the opacity of blood fibrin.

Reactions of muscle plasma depending upon Myosin.

The separation of myosin is hindered by cold. At temperatures about 0° C. it occurs very slowly, whilst at 40° C. almost instantaneously.

When muscle plasma is diluted with cold water, myosin is instantly precipitated, so that a drop of muscle plasma allowed to fall into water sets instantly in the form of a solid elastic ball. Dilute acids, and solutions of NaCl containing from 10 to 20 per cent. of the salt, cause instantaneous coagulation.

Muscle plasma may be mixed with ice-cold salt solutions containing from 5—7 p.c. of NaCl without myosin separating.

When plasma is allowed to flow *guttatim* into dilute hydrochloric acid (containing 0·1 per cent.), the little balls which are at first formed dissolve as they sink through the column of liquid, and give rise to an opalescent solution.

Preparation of myosin.

Pure myosin is obtained by dropping muscle plasma into distilled water, whereby a precipitate consisting of little balls is obtained, which is easily washed with water.

Myosin which has been thoroughly washed with water has a neutral reaction, is quite insoluble in pure water, but readily soluble in solutions of common salt containing between 5 and 10 per cent. of NaCl.

Another method of preparing myosin is based upon the solubility of coagulated myosin in weak solutions of common salt. Muscle is thoroughly washed with water, is finely divided, and rubbed up to the consistence of a fine paste with powdered common salt, the amount of salt which has been added being determined. Water is then added in such quantities as to form with the salt of the muscle a solution containing 10 per cent. of NaCl. The mixture of finely divided muscle, salt, and water, which should have the consistence of a thin magma, is set aside for 24 hours, then pressed in linen, and filtered through paper. The yellowish, syrupy, solution when poured

into water furnishes at once pure myosin, and resembles muscle plasma in all respects, except in not coagulating spontaneously.

Coagulation of myosin. When gradually heated a solution of myosin begins to be turbid at 55° C., and deposits flakes of proteid matter at 60° C., which consists of a coagulated product which resembles other proteids coagulated by heat.

Powdered common salt, added in excess, precipitates myosin from its solutions in common salt.

Myosin, like fibrin, decomposes peroxide of hydrogen.

Myosin-Syntonin. Liebig[1] shewed that when muscle is placed in dilute hydrochloric acid containing 1 part of acid in 1000, the proteid matter is in great part dissolved, to be precipitated when the solution is neutralized. Liebig believed that the body dissolved in the acid was a special body, *muscle-fibrin;* it is now known, however, that the solution merely contains acid-albumin or syntonin, differing in no respect from acid-albumin obtained from other proteids. It has been suggested that the ease with which it is converted into syntonin, under the influence of dilute hydrochloric acid, specially distinguishes myosin; it is probable that the rapid formation of acid-albumin is due to the fact that muscle always contains a trace of pepsin.

Characters of a solution of Syntonin. The facility with which a solution of acid-albumin can be obtained from muscle, causes us to examine in this place the reactions of such a solution in greater detail than was thought advisable in Chapter I.

To prepare acid-albumin from muscle this tissue is finely divided and then placed in a large quantity of dilute hydrochloric acid (1 part of HCl, 1000 parts of water). The solution is after some hours filtered. On neutralizing, gelatinous flakes are obtained, which are collected on a filter and washed. These contain in 100 parts:—C, 54·06; H, 7·28; N, 16·05; S, 1·11; O, 21·50.

Acid solutions of syntonin are not coagulated by heat; they are precipitated by sodium chloride, ammonium chloride, calcium chloride, sodium sulphate and magnesium sulphate.

Syntonin is soluble in a solution of sodium carbonate of 1 p. c., and the solution is not coagulated by heat. It is soluble in cold solution of lime water, and the solution does not coagulate when boiled; it froths when shaken.

Muscle Serum.

Following the analogy of the blood, we may designate, by the name of *muscle serum*, the liquid which remains after the separation of the spontaneously coagulating substance from the muscle plasma. The muscle serum from which myosin has separated at a low temperature has a neutral or faintly alkaline reaction. Kept at the ordinary temperature of our dwelling-rooms it acquires an acid reaction, in consequence of the development within it of sarcolactic acid.

Proteids of the muscle serum. Muscle serum contains three proteids in solution:—

1. A proteid body which coagulates when the muscle serum is cautiously heated (if needs be after

[1] Liebig, *Ann. d. Chem. u. Pharm.* Vol. LXXIII. pp. 125—129.

careful neutralization) to 45° C. This body is not myosin, being distinguished from it by its insolubility in weak solutions of NaCl[1].

2. An alkaline (potassium) albuminate, which is only precipitated when the reaction is made strongly acid.

3. Albumin apparently identical with serum-albumin, and coagulating, like it, at a temperature between 70° and 75° C. and not coagulated by the addition of ether. This proteid is much more abundant than either the first or second mentioned.

The great majority of the constituents to be discussed in the sequel are contained in the muscle serum; they will, however, for convenience, be considered under separate headings.

The Haemoglobin of Muscles.

We have already stated that certain of the voluntary muscles are distinguished by their red colour, due to the presence of haemoglobin which colours the contents of the sarcolemma. In warm-blooded animals, indeed, the majority of muscles are red, whilst in cold-blooded animals frequently the heart is the only red muscle. In certain gasteropod molluscs (Limnaeus and Paludina) Lankester made the remarkable observation that whilst haemoglobin is not present in the blood, it colours the muscular fibres which occur in the walls of the pharynx, these muscles being among the most active in the body.

Wherever haemoglobin occurs in the substance of muscle it colours the plasma and not the anisotropous sarcous elements; when the plasma coagulates, a portion of the colouring matter adheres to the myosin, whilst a portion remains in solution in the muscle serum. To demonstrate the presence of haemoglobin in muscle, the blood-vessels are washed out with salt solution, and thereafter the blood-free muscle is held between a light and the slit of the spectroscope. The muscular portion of the diaphragm of the rabbit lends itself particularly well to this observation. Crystals of haemin may by suitable treatment be obtained from the red muscles, or from the plasma which they yield (Kühne).

Nitrogenous (non-Proteid) organic Constituents of Muscle.

Extract of meat.

When finely divided dead muscle is repeatedly treated with cold water, this liquid dissolves the whole of the constituents of the muscle serum and, in addition, perhaps, soluble matters derived from the insoluble anisotropous sarcous elements. The solution thus obtained has a red colour due to the haemoglobin extracted from the muscular fibres and (unless the blood-vessels have been thoroughly washed out with salt solution) derived from the blood contained in the vessels of the tissue.

[1] The reader is referred for some recent observations on the proteids of Muscle to a paper by Demant entitled "Beitrag zur Chemie der Muskeln." *Zeitschrift f. physiol. Chemie*, 1879, p. 241.

When the solution is boiled, the haemoglobin and the soluble proteids which it contains are coagulated and, on filtering, a clear liquid is obtained containing the salts of muscle, certain non-nitrogenous organic bodies, such as glycogen, inosit, lactic acid, &c., and a mixture of nitrogenous organic bodies, mostly basic in their character; these are creatine, creatinine, carnine, xanthine, hypoxanthine, and perhaps urea. To the residue obtained by evaporating an aqueous infusion of muscle, the name of *extract of meat* is given; several extracts of meat exist in commerce, which are substantially obtained in the way we have mentioned and which may be employed in the laboratory for the preparation of the various nitrogenous organic bodies to be now described. Beef-tea is an aqueous extract of meat, and contains the same substances as are present in the solid extract of meat.

Creatine. $C_4H_9N_3O_2 + H_2O$.

Preparation.

This body occurs in only two of the elementary tissues of the body, viz. in muscular and nervous tissue. It has never been found in any glandular organ. In small quantities it occurs in the blood, but it is present in muscle in largest amount.

I. (Liebig's method[1]). Muscle is reduced to a fine state of division, as for example by the use of a sausage machine, and then mixed with one half its weight, or its own weight, of cold water, and set aside for some hours. The insoluble matter is separated on a linen filter from the liquid, and the former is subjected to strong pressure. It is then treated with a quantity of water equal to that first used, and, after some hours, the process of filtration and pressing repeated as before; the water used for the second extraction may be employed afterwards to extract a fresh quantity of meat. The liquid thus obtained is then boiled, by which means the albumin which it contains is coagulated; after removing the albumin by filtration, baryta water is added to the filtrate, so as to precipitate the whole of the phosphates present. The excess of baryta present in the solution is removed by passing through it a current of CO_2, and, after filtering, the filtrate is concentrated by evaporation on the water-bath, until it has a syrupy consistence; it is then set aside for some days. Creatine separates out in the form of crystalline crusts adhering to the bottom of the vessel; the mother liquor is poured off and the crystals washed with cold alcohol; these are then dissolved in boiling water, and the solution decolorized by means of animal charcoal. On evaporation, crystals of creatine separate which are purified by recrystallization.

II. (Neubauer's method[2]). The watery extract of muscle is precipitated by solution of lead acetate, the solution is treated with sulphuretted hydrogen to remove the excess of lead, and is cautiously

[1] Liebig, *Ann. d. Chem. u. Pharm.* Vol. 62, p. 257.
[2] Neubauer, *Ann. d. Chem. u. Pharm.* Vol. 119, p. 27.

evaporated until, on cooling, crystals commence to separate. It is then set aside for some days to crystallize. The liquid from which the crystals have separated is then treated with twice or three times its own volume of 88 per cent. alcohol, and the crystals which readily fall from this mixture of mother liquor and spirit are collected on a filter and, if necessary, weighed. They are at first yellow, but are obtained perfectly colourless by recrystallizing.

III. (Städeler's process[1]). Finely divided meat is digested on the water-bath with twice its volume of alcohol. The insoluble matter is pressed and the filtrate is heated on the water-bath so as to drive off a great part of the alcohol. Solution of basic lead acetate is then added and the process continued as in II.

Properties. Creatine crystallizes in the form of transparent, colourless, shining, oblique, rhombic columns, which when heated to 100° C. lose their water of crystallization (12·17 p. cent.) and become opaque.

The crystals belong to the monoclinic system. Inclination of the clinodiagonal to the principal axis = 70° 20′. Inclination of the faces ∞ P : ∞ P in the plane of the orthodiagonal and principal axis = 132° 2′ (nearly). Specific gravity of the crystals 1·35 to 1·34.

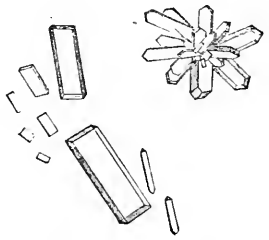


FIG. 55. CREATINE.

Creatine is soluble in 74 parts of cold water at 18° C.: freely soluble in hot water: slightly soluble in spirits of wine: but almost insoluble in absolute alcohol and ether. One part of creatine requires 9400 parts of absolute alcohol at ordinary temperatures to dissolve it.

Compounds of Creatine. Though solutions of creatine have a neutral reaction, the body is a weak base which, when dissolved in hydrochloric, sulphuric, and nitric acids, forms compounds which crystallize well. The following are the formulae of these compounds:

Hydrochlorate of Creatine	$C_4H_9N_3O_2 . HCl$
Sulphate „ „	$C_4H_9N_3O_2 . H_2SO_4$
Nitrate „ „	$C_4H_9N_3O_2 . HNO_3$.

A compound of mercury and creatine is formed in which two atoms of hydrogen are replaced by a single atom of mercury ($C_4H_7HgN_3O_2$).

[1] Städeler, *Journ. f. pract. Chem.* Vol. 72, p. 256.

Derivatives.

I. When creatine is dissolved, in the cold, in the strong mineral acids, or heated for some time in dilute mineral acids, or heated for a much longer time (several days) with water, a molecule of water is removed and *Creatinine* is formed, thus:

$$\underbrace{C_4H_9N_3O_2}_{\text{Creatine.}} = \underbrace{C_4H_7N_3O}_{\text{Creatinine.}} + \underbrace{H_2O}_{\text{Water.}}$$

II. When boiled with baryta water, creatine yields urea, sarcosin, methylhydantoin, methylparabanic acid and ammonia; these bodies are products of several reactions which go on side by side, as shewn by the following equations:

$$(a)\quad \underbrace{C_4H_9N_3O_2}_{\text{Creatine.}} + \underbrace{H_2O}_{\text{Water.}} = \underbrace{CH_4N_2O}_{\text{Urea.}} + \underbrace{C_3H_7NO_2}_{\text{Sarcosin.}}$$

$$(b)\quad \underbrace{C_4H_9N_3O_2}_{\text{Creatine.}} = \underbrace{C_4H_6N_2O_2}_{\text{Methylhydantoin.}} + \underbrace{NH_3}_{\text{Ammonia.}}$$

$$(c)\quad \underbrace{C_4H_9N_3O_2}_{\text{Creatine.}} + O_2 = \underbrace{C_4H_4N_2O_3}_{\text{Methylparabanic acid.}} + \underbrace{NH_3}_{\text{Ammonia.}} + \underbrace{H_2O}_{\text{Water.}}$$

III. When boiled with mercuric oxide, an aqueous solution of creatine yields oxalate of methyluramine and water, thus:

$$\underbrace{C_4H_9N_3O_2}_{\text{Creatine.}} + \underbrace{2HgO}_{\text{Mercuric oxide.}} = \underbrace{C_2H_7N_3 . C_2H_2O_4}_{\text{Oxalate of Methyluramine.}} + \underbrace{2Hg}_{\text{Mercury.}}$$

Synthesis and constitution of creatine.

When the alcoholic solution of 2 parts of sarcosin (methylglycosine) is heated for some hours at 100° C. with 1 part of freshly prepared cyanamide, creatine is formed; thus:

$$\underbrace{C_2H_3O_2N\begin{cases}CH_3\\H\end{cases}}_{\text{Methylglycosine, or Sarcosin.}} + \underbrace{NC.NH_2}_{\text{Cyanamide.}} = \underbrace{C_2H_3O_2N\begin{cases}CH_3\\C(NH)NH_2\end{cases}}_{\text{Methyl-guanidine acetic acid, or Creatine.}}$$

Quantity of creatine present in muscle.

The quantity of creatine in the muscles of different animals and in different muscles of the same animal does not vary greatly. The following are the results of Voit's[1] determinations.

100 parts of Muscle of	Frog	yield	0·21—0·35	pts. of	Creatine.
,, ,, ,,	Fox	,,	0·206—0·237	,, ,,	,,
,, ,, ,,	Ox	,,	0·219—0·276	,, ,,	,,
,, ,, ,,	Dog	,,	0·223—0·248	,, ,,	,,
,, ,, ,,	Rabbit	,,	0·269—0·336	,, ,,	,,
,, ,, ,,	Man	,,	0·282—0·301	,, ,,	,,

According to Voit the muscular substance of the heart contains less creatine than the voluntary muscles, and not, as Liebig stated, a larger quantity. According to Demant the amount of creatine[2] increases remarkably in starvation.

[1] Voit, *Zeitschr. f. Biolog.* Vol. IV. (1868) p. 77.

[2] Demant, "Zur Kenntniss der Extractivstoffe der Muskeln." *Zeitschrift f. phys. Chemie*, Vol. III. (1879) p. 387.

Creatinine. $C_4H_7N_3O$.

This body, which is a strong base, and which, as has already been stated, can be obtained from creatine by the prolonged action of dilute acids or water, is according to Neubauer[1] and Nawrocki[2] not present in muscle. On the other hand, C. Voit believes it to be occasionally present in that tissue.

Creatinine forms with zinc chloride a sparingly soluble compound having the composition represented by the formula $(C_4H_7N_3O)_2\ ZnCl_2$, and it is by conversion into this compound that it is always estimated. Creatinine will be treated of more fully in the Chapter on Urine.

Hypoxanthine or Sarcine. $C_5H_4N_4O$.

This body was first discovered by Scherer in the splenic pulp, but was shewn by Strecker to be constantly present in muscle. Unlike creatine, hypoxanthine is pretty widely distributed, being found in the blood, in many glands, in the marrow of bones, &c.

Preparation. The mother liquor from which creatine has separated is diluted considerably with water, and ammonia is added until the reaction is alkaline; it is then treated with an ammoniacal solution of silver nitrate, which throws down a flocculent, gelatinous precipitate ($C_5H_2Ag_2N_4O$), which is allowed to subside, and first washed *by decantation* with weak solution of ammonia, and then collected on a filter. The precipitate is then boiled in nitric acid of specific gravity 1·1, which dissolves the hypoxanthine compound, leaving undissolved any silver chloride which may be mixed with it. The latter is separated from the solution by decantation. On cooling, the nitric acid deposits a white crystalline compound of hypoxanthine and silver nitrate, having the composition $C_5H_4N_4O\,.\,AgNO_3$; these crystals often present the form of isolated spindles or of rosettes formed of spindles radiating from a centre.

The silver compound is suspended in water, and treated with sulphuretted hydrogen, then heated; the clear fluid filtered from the precipitate of silver sulphide deposits, on concentration, crystals of nitrate of hypoxanthine; when this compound is dissolved in hot water and treated with ammonia, it deposits crystalline nodules (never needles) of hypoxanthine[3].

Properties. As stated in the foregoing paragraph, hypoxanthine crystallizes in the form of nodules which never (Kühne) exhibit any needles. It is scantily soluble in alcohol; it is soluble in

[1] Neubauer, *Zeitschr. f. anal. Chemie*, Vol. II. (1863) p. 22.

[2] Nawrocki, *Centralbatt f. d. med. Wissensch.* 1865, p. 417. *Zeitschr. f. anal. Chemie*, 1865, p. 336.

[3] Very admirable woodcuts exhibiting the crystalline forms of the compound of hypoxanthine with silver nitrate and of nitrate of hypoxanthine, are to be found in Kühne's *Lehrbuch, etc.* p. 295 and 296.

78 parts of boiling and 300 parts of cold water; it is soluble in dilute acids and alkalies. It forms compounds with acids, bases, and metallic salts. Certain of the latter have already been referred to; a compound with platinum, having the composition of $C_5H_4N_4O \cdot HCl \cdot PtCl_4$, may be mentioned, as well as one with copper which is formed when a solution of hypoxanthine is boiled with solution of cupric acetate; this compound is a brownish flocculent body insoluble in water, which does not admit of purification; it yields however impure hypoxanthine when it is decomposed by H_2S.

Relations of Hypoxanthine to other bodies.

Hypoxanthine is very closely related to xanthine and to uric acid, as would appear probable from an examination of their formulae.

Uric acid . .	$C_5H_4N_4O_3$.
Xanthine . .	$C_5H_4N_4O_2$.
Hypoxanthine . .	$C_5H_4N_4O$.

From the two first of these bodies, hypoxanthine can be obtained by the action of sodium amalgam; when oxidized with nitric acid it yields xanthine.

Proportion of hypoxanthine found in muscle.

According to Neubauer[1] the flesh of the ox contains 0·022 per cent. and that of the rabbit 0·026 per cent. of hypoxanthine.

Xanthine. $C_5H_4N_4O_2$.

Xanthine a rare constituent of urinary calculi, of guano and of urine.

This constituent of muscle was first discovered by Marcet[2], as a constituent of a urinary calculus, and by him called xanthic oxide. It was afterwards analyzed by Liebig and Wöhler[3] and Unger[4]. It has been discovered in guano[5], and in some cases in the urine of man[6] and the lower animals[7].

Preparation from muscle, Neubauer's method.

In preparing hypoxanthine that body was directed to be precipitated with ammoniacal solution of nitrate of silver, and the precipitate dissolved in nitric acid of sp. gr. 1·1. It was stated that when the acid cooled the compound of hypoxanthine and silver nitrate separated. Now the first precipitate (viz. that thrown down by ammoniacal silver nitrate) contains, in addition to hypoxanthine, a silver compound of xanthine; the latter compound being more soluble in nitric acid of

[1] Neubauer, *Zeitschrift f. anal. Chem.* VI. 33.

[2] Marcet, *Essay on the Chemical History and Chemical Treatment of Calculous Disorders.* London, 1819.

[3] Liebig und Wöhler, Poggendorff's *Ann.* Vol. XLI. p. 393.

[4] Unger, *Ann. d. Chem. u. Pharm.* Vol. LVIII. p. 18.

[5] Unger and Phipson, *Chem. News*, Vol. VI. 1862, p. 16.

[6] Bence Jones, *Quart. Journ. of Chem. Soc.* Vol. XV. p. 78.

[7] Weiske, "Xanthin und Harnsäure im Hain eines kranken Schafbockes." *Zeitschr. f. Biol.* XI. p. 254.

sp. gr. 1·1 than the former, remains in solution after the hypoxanthine compound has crystallized out. On supersaturating with ammonia, a gelatinous compound of xanthine and silver ($C_5H_2Ag_2N_4O_2 + H_2O$) separates. By dissolving in warm nitric acid, the compound $C_5H_4N_4O_2 . AgNO_3$ is again formed, and from the latter xanthine can be prepared by following a process analogous to that which has been described in the case of hypoxanthine.

Properties of Xanthine. Xanthine when freshly separated from its solutions presents the appearance of white amorphous granules.

Xanthine is almost completely insoluble in cold water, requiring about 14000 parts of water at 16° C. to dissolve it, and 1400 parts of boiling water. It is easily soluble in solution of ammonia, which deposits it, on evaporation, in the form of indistinctly crystalline plates (Kühne): from the ammoniacal solution it is completely precipitated by lead acetate.

Solutions of other alkalies likewise dissolve xanthine, and from these it is precipitated by acids.

Reactions of xanthine. Ammoniacal solutions of xanthine when heated with silver salts reduce the silver to the metallic state.

On heating xanthine in hot hydrochloric acid, and evaporating, microscopic crystalline masses, composed of aggregations of hexagonal plates, separate; these consist of the hydrochlorate of xanthine, $C_5H_4N_4O_2 . HCl + H_2O$. In a similar manner the nitrate is formed ($C_5H_4N_4O_2 . HNO_3$), and this crystallizes in rhombic plates arranged in clumps. The solution of the nitrate is precipitated by silver nitrate in a flocculent form, and the precipitate may be dissolved in hot nitric acid and allowed to crystallize on cooling; it has the composition ($C_5H_4N_4O_2 . AgNO_3$); this body is much more soluble in nitric acid than the corresponding hypoxanthine silver compound. It separates from its solution in nitric acid in the form of groups of fine needles, which do not resemble the hypoxanthine compound.

Xanthine (like hypoxanthine) is soluble in pure (colourless) warm nitric acid without the disengagement of gas; on *cautious* evaporation a colourless nitrate is left; the residue is not rendered purple by ammonia. By these reactions xanthine is distinguished from uric acid.

Heated with fuming nitric acid containing nitrous acid, a citron-coloured residue is left, which becomes orange or red on the addition of caustic soda, and which when heated exhibits at its margin a fine purple red colour.

Proportion of xanthine found in muscle. According to Scherer[1] the fresh muscles of the horse contain 0·0026 p. c. of xanthine.

Relations of xanthine. This body, as has been said in treating of hypoxanthine, is closely related to that body and to uric acid.

[1] Scherer, *Ann. d. Chem. u. Pharm.* Vol. CVII. (1858) p. 314.

Artificial production of xanthine from proteids. Salomon[1], and Krause[2] who worked under his direction, have shewn that both hypoxanthine and xanthine are formed in small quantities during the digestion of fibrin with trypsin and pepsin. Both bodies are likewise formed when fibrin is digested at the temperature of the body with weak hydrochloric acid.

Carnine. $C_7H_8N_4O_3 + H_2O$.

This base, discovered by Weidel[3], has only hitherto been found in Liebig's extract of meat, though doubtless it is a regular constituent of muscle; it constitutes about 1 p.c. of extract of meat.

Preparation. Liebig's extract of meat is dissolved in 6—7 times its volume of warm water, and cautiously precipitated with a strong solution of baryta water, great care being taken to avoid an excess of the precipitant. The filtrate is treated with solution of basic lead acetate, and the precipitate collected and boiled with water; the compound of carnine and lead being comparatively soluble in boiling water is extracted by repeatedly boiling the precipitate with water. The warm solution is treated with sulphuretted hydrogen, and the precipitate of lead sulphide having been separated by filtration the filtrate is concentrated and treated with solution of silver nitrate which precipitates a flocculent silver compound $(C_7H_8N_4O_3)_2 . AgNO_3$ mixed with some AgCl. The latter is separated by digesting the precipitate in ammonia. The precipitate is then suspended in water, subjected to the action of H_2S, and the filtrate being concentrated yields crude carnine, which is purified by recrystallizing and by the action of animal charcoal.

Properties. Carnine is very little soluble in cold, but easily and completely soluble in boiling water. It is insoluble in alcohol and ether. Its aqueous solution has a neutral reaction; it has a scarcely perceptible taste at first, but leaves a slight bitter after-taste.

Chemical relations. When a hot solution of carnine is treated with a saturated aqueous solution of bromine, a slight evolution of gas takes place, the solution is decolorized, and on concentration deposits crystals of the hydrobromate of hypoxanthine; at the same time bromide of ethyl is formed; thus:

$$\underbrace{C_7H_8N_4O_3}_{\text{Carnine.}} + \underbrace{2Br}_{\text{Bromine.}} = \underbrace{C_5H_4N_4O . HBr}_{\text{Hypoxanthine-hydrobromate.}} + \underbrace{CH_3Br}_{\text{Bromide of ethyl.}} + \underbrace{CO_2}_{\text{Carbonic acid.}}$$

[1] Salomon, "Bildung von Xanthinkörpern aus Eiweiss durch Pankreasverdauung." *Ber. d. deutsch. chem. Ges.* Vol. XI. p. 574.

[2] Krause, "Ueber Darstellung von Xanthinkörpern aus Eiweiss." *Inaug. Diss.* Berlin, 1878. (Abstracted in Maly's *Jahresbericht*, Vol. VIII. p. 80.)

[3] Weidel, "Carnin, eine neue Basis aus dem Fleischextract." *Ann. d. Chem. u. Pharm.* Vol. CLVIII. (1871) p. 353—368.

From this decomposition it would appear not improbable that carnine is one of the intermediate products between the proteid molecule and bodies belonging to the uric acid group.

Uric acid. $C_5H_4N_4O_3$.

It is most questionable whether uric acid exists in muscle; by Meissner it has been found in traces in the muscles of fowls[1].

Urea. CH_4N_2O.

It is yet doubtful whether urea should be reckoned amongst the constituents of normal muscle. Liebig came to the conclusion that it did not occur in that tissue, and this opinion has been generally entertained. Of late Picard[2] has stated that the muscular tissue of rabbits contains as much as 3 per cent. of urea. This statement is entirely without foundation, Picard having by the method of analysis which he employed reckoned other bodies, and especially creatine, as urea. It is probable that muscle does contain an exceedingly small quantity of urea, though its separation from the other nitrogenous constituents offers peculiar difficulties.

Inosinic acid. $C_{10}H_{14}N_4O_{11}$.

By this name Liebig[3] designated a syrupy acid which he believed to be a distinct proximate principle of muscle, though present in extremely small quantity. With bases this acid is said to form crystallizable salts. A fresh investigation is needed even to establish the existence of this body.

Taurine. $C_2H_7NSO_3$.

This body has been found in the muscles of the horse by Limpricht[4] and Jacobsen[5], and in those of fishes by Limpricht. It is, according to Valenciennes and Fremy, found in the muscles of molluscs.

Non-nitrogenous organic Constituents of Muscle.

Living muscle contains in addition to the previously described nitrogenous organic constituents, considerable quantities of non-nitrogenous organic bodies; these are *fats, glycogen, dextrin* (?), *inosit,* and perhaps small quantities of a fermentable sugar.

[1] Meissner, *Zeitschr. f. rat. Med.* Vol. xxxi. (1868) p. 144.
[2] Picard, *Comptes Rendus*, Vol. lxxxvii. (1878) No. 15 and 25.
[3] Liebig, *Ann. d. Chem. u. Pharm.* Vol. lxxii. (1847) p. 317.
[4] Limpricht, *Ann. d. Chem. u. Pharm.* Vol. cxxxiii. (1865) p. 293.
[5] Jacobsen, *ibid.* Vol. clvii. (1871) p. 227.

Fats.

The connective tissue which separates the muscular bundles always contains some fat-cells, and as it is impossible to separate the muscular fibres absolutely from these, we cannot readily determine the amount of fat which belongs to the isolated muscular fibres. There is reason to believe, however, that quite independently of the fat-cells of the connective tissue of muscle, the muscular fibres contain fat which they give up to ether; we know nothing, however, either as to its amount or composition.

In phosphorus poisoning a fatty degeneration of muscle occurs, similar to that observed to occur as an idiopathic affection, specially affecting the muscular substance of the heart. In all probability, in this as in other cases, the fatty degeneration is an evidence of impeded nutrition (probably of imperfect oxygenation) of the tissue.

Glycogen. $(C_6H_{10}O_5)_n$.

This body, which will be treated of fully in connection with the liver, is a constant ingredient of the living muscular tissue.

It was at first supposed to be only present in the muscles of the embryo[1], but it was afterwards shewn to occur in muscles of adult animals under certain conditions, and later still it was found to be constantly present by Nasse[2], Brücke, Abeles and others.

Modes of separating and determining the amount of Glycogen in muscle.

As the glycogen of muscle, on the cessation of the vitality of the tissue, is very rapidly converted into sugar, in order to separate muscle-glycogen the tissue must, whilst yet living, be placed in boiling water, with the object of destroying the amylolytic ferment which would effect the change. It is then taken out of the boiling water, reduced to a very fine state of division, and boiled again in water. From this liquid, impure glycogen may be precipitated by concentrating it and then adding an excess of alcohol. The method of effecting the purification (by Brücke's method) of the impure glycogen obtained by this method will be described in connection with liver-glycogen.

Abeles' method.

It is exceedingly difficult, indeed almost impossible, to extract the whole of the glycogen from muscles by boiling them with water. Brücke[3] suggested that the muscle should be boiled in a dilute solution of caustic potash. In this way the whole of the glycogen is extracted, but a large quantity of proteid matter passes into solution. Abeles[4] gets rid of this by boiling with zinc chloride. His method is the following:—

[1] Claude Bernard, *Comptes Rendus*, Vol. XLVIII. (1859) p. 673.

[2] Nasse, "Beiträge zur Physiologie der contractilen Substanz." Pflüger's *Archiv*, Vol. II. (1869) p. 97—121.

[3] Brücke, *Sitzungsber. d. Wien. Akad.* Vol. LXIII. p. 214.

[4] Abeles, "Beiträge zur Kenntniss des Glycogens." *Med. Jahrbücher*, 1877, p. 551.

The muscle of which the glycogen is to be separated, is subjected to long boiling in a solution of caustic potash; the solution is then *almost* neutralized with hydrochloric acid, care being taken, however, that the reaction still continues distinctly alkaline; solution of chloride of zinc is then added to it and it is boiled for a period varying between 20 and 40 minutes; the proteid bodies are precipitated and an easily filtered clear liquid is obtained. It is of importance that just enough of the zinc salt should be added to effect the precipitation. When this point has been attained and the clear liquid is no longer rendered turbid by boiling with a fresh quantity of zinc chloride, it is filtered, and the precipitate carefully washed; the filtrate and washings are concentrated on a water-bath, allowed to cool, and then treated with much alcohol which has been faintly acidulated with hydrochloric acid. Glycogen is thus precipitated; it is collected on a filter, washed with weak spirit containing about 60 p. c. of alcohol, and acidulated with hydrochloric acid, until the washings contain no zinc; the acid alcohol is then displaced by pure alcohol, and lastly the substance is dried, and weighed, or heated with dilute mineral acids for 2 or 3 hours, and the sugar formed determined.

Nasse's method[1].

Nasse scalds a known weight of muscle, then pounds it up in a mortar with a weighed quantity of quartz sand, and digests it in a beaker with water and filtered saliva for some hours. He then heats the mixture to 100° C. on a water bath, to precipitate soluble proteids, then weighs the beaker and its contents, and determines the quantity of sugar which a weighed quantity of the clear liquid contains, employing for this purpose a Fehling's solution of which 1 c.c. corresponds to 1 milligramme of dextrose. Assuming that the sugar formed from the muscle-glycogen has the same reducing power as dextrose, and that it is equally distributed throughout the scalded muscle and water, the amount of glycogen originally present can be easily calculated.

Proportion of glycogen in resting muscle.

The amount of glycogen found in different muscles of the same animal and in different individuals of the same species, varies so much that no general statement can be made. In Nasse's experiments the glycogen of resting muscles of frogs amounted, on an average, to 0·43 per cent. In rabbits the amount varied between 0·47 and 0·95 per cent. Abeles' results were decidedly higher. As yet, however, the total number of reliable determinations of the amount of glycogen in muscle is too small to allow of any statement being made as to the average amount of this constituent present.

We shall examine in a future section the changes in the amount of glycogen brought about by the passage of muscle from the state of rest into that of activity or rigor.

[1] Nasse, *Op. cit.* p. 101 and 102.

Dextrin. $(C_6H_{10}O_5)_n$.

Limpricht[1] and Scherer asserted that horse-flesh contains dextrin; the former observer obtained large quantities from the muscles of young horses.

It may be taken as certain[2] that the body was however *glycogen-dextrin*, produced after death from glycogen.

Fermentable Sugar.

It was formerly believed that muscle in a state of rest contained a small quantity of sugar. From the observations of Nasse[3] it would appear that sugar is only developed during activity or rigor and that none is actually found in muscle at rest.

Inosit. $C_6H_{12}O_6 + 2H_2O$.

This non-fermentable isomer of grape-sugar was discovered by Scherer[4] in the muscular substance of the heart, and has since been found in the voluntary muscles, of which it is said, however, not to be an invariable constituent; it is said to occur especially in the muscles of drunkards. It is likewise present in the tissues of the nervous system (Müller), and in the lungs, liver, kidneys and spleen of oxen (Cloetta[5]); it has been found in the kidneys of man, in the urine of certain cases of Bright's disease, in the urine of diabetes mellitus; in the liquid contents of hepatic hydatid cysts. Inosit is found in many plants[6], as in green kidney beans, the unripe fruit of *Phaseolus vulgaris* (by Vohl, who gave to it the name *Phaseomannite*): in the green pods and unripe seeds of the garden pea (*Pisum sativum*): in the unripe fruit of the lentil (*Ervum lens*), and of the common acacia (*Robinia pseudacacia*): in the heads of the common cabbage (*Brassica oleracea*, var. *capitata*): in the herb of foxglove (*Digitalis purpurea*); in the leaves and stem of dandelion (*Taraxacum dens leonis*), not from the flowers or roots; in the shoots of the potato; in the green herb and unripe berries of asparagus; and in two cryptogamic plants, viz. *Lactarius piperatus, L.* and *Clavaria crocea*, Pezs[7].

[1] Limpricht, *Ann. d. Chem. u. Pharm.* Vol. CXXXIII. (1865) p. 295.

[2] Nasse, "Chemie u. Stoffwechsel d. Muskeln." Hermann's *Handbuch der Physiologie*, Vol. I. part i. p. 280.

[3] Nasse, "Beiträge zur Physiologie der contractilen Substanz." Pflüger's *Archiv*, Vol. II. (1869) p. 103.

[4] Scherer, *Annal. d. Chem. u. Pharm.*, Vol. LXXIII. (1850) p. 322.

[5] Cloetta, *ibid.* Vol. XCIX. p. 289.

[6] This list of plants in which inosit occurs is copied *verbatim* from Watt's *Dictionary*, Vol. III. p. 274.

[7] As to the identity of the inosit from vegetable and animal tissues consult Jauret et Villiers, *Comptes Rendus*, Vol. LXXXVI. p. 486.

Perparation from Muscle. Boedecker's method.[1]

An aqueous extract of muscle (preferably of the muscular tissue of the heart) is prepared. This is freed from albumin by boiling &c., then treated with baryta water to free it from phosphates; it is concentrated, set aside, and the creatine is allowed to crystallize out; the mother liquor is boiled with four times its volume of alcohol; a precipitate is formed which, according as it adheres to the bottom or separates in a flocculent form, is separated by decantation or filtration. The clear liquid is set aside for 24 hours, when crystals of inosit often separate; if not, ether is added and the mixture of alcohol and ether shaken again and again; inosit then separates out gradually in the form of leaflets having the lustre of mother-of-pearl. An excess of ether does not interfere with the precipitation, but merely causes the separating crystals to be smaller (Hoppe-Seyler). The impure inosit obtained by the above methods is collected on a filter, washed with cold alcohol, and recrystallized from water.

Properties.

Inosit crystallizes in the form of large, colourless, monoclinic tables, sometimes arranged in groups like cauliflower-heads.

The crystals of inosit have a specific gravity of 1·1154 at 5°; they effloresce in dry air, or in vacuo; at 100° C. the whole of the water of

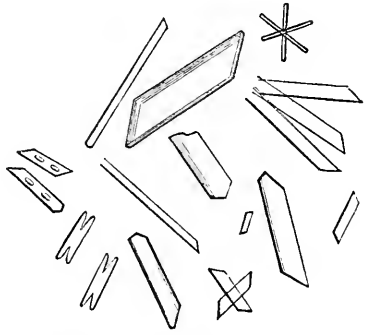


FIG. 56. INOSIT FROM THE MUSCULAR SUBSTANCE OF THE HEART OF MAN. (FREY.)

crystallization is given off, and the anhydrous inosit melts at 110°, setting, on sudden cooling, in fine needles. Inosit dissolves in 6 parts of water at 19° C.; it is insoluble in absolute alcohol and ether. Solutions of inosit when boiled with basic lead acetate yield instantly a transparent jelly (containing the compound $C_6H_{12}O_6 2Pb_2O$); this reagent has been, indeed, employed by some observers in the separation of inosit.

Inosit has a sweet, saccharine, taste; it is not fermentable; it does not rotate the plane of polarization; it does not yield a yellow or

[1] Boedecker, *Ann. d. Chem. u. Pharm.*, Vol. CXVII. p. 118.

brown colour when boiled with solutions of the caustic alkalies; it does not reduce *Fehling's* solution, but changes its colour to green.

Scherer's reaction. When inosit is treated with nitric acid, the solution evaporated nearly to dryness, then moistened with ammonia and a small quantity of calcium chloride, and again evaporated, a rose-red colouration is produced. *Scherer's test* only succeeds with nearly pure inosit.

Gallois' reaction [1]. When a solution containing inosit is evaporated, at a gentle heat, nearly to dryness, then treated with a small drop of solution of mercuric nitrate (the solution used in Liebig's method for the estimation of urea answers very well) and evaporated carefully to dryness, a yellowish white residue is obtained; on further cautiously heating, the yellow changes to a deep rose-colour, which disappears on cooling but reappears on again heating. This constitutes a delicate and characteristic reaction, helping to confirm the presence of inosit, the identification of which should however depend also upon a knowledge of the conditions under which the body was obtained, and upon such properties as crystalline form, solubility, sweetness, &c.

Derivatives of Inosit. Inosit yields when treated with nitric acid nitro-substitution compounds which are soluble in alcohol and have the composition $C_6H_6(NO_2)_6O_6$ (Hexanitroinosit) and $C_6H_9(NO_2)_3O_6$ (Trinitroinosit); these bodies explode when struck.

In the presence of decomposing proteids, inosit is decomposed, with the formation of propionic, butyric and ordinary lactic acids (Vohl).

Proportion of Inosit in Muscle. As has previously been stated, inosit is not an invariable constituent of muscle. According to Jacobsen, horse flesh contains 0·003 per cent. of inosit.

The Ferments present in Muscle.

Muscle contains a trace of *pepsin*, as was shewn by Brücke. It is perhaps in consequence of its presence that muscle so readily dissolves in very dilute hydrochloric acid. Dead muscle also contains an amylolytic ferment which readily converts the muscle-glycogen into sugar. Other hypothetical ferments have been surmised to exist in order to explain the processes going on in muscle during activity and rigor.

The Inorganic Constituents of Muscle.

Water. The proportion of water in muscle varies between 74 and 80 per cent., the average being about 75. It is said to be larger in young than in adult animals. The muscles of cold-blooded animals contain more water than those of warm-blooded animals.

[1] Gallois, *De l'Inosurie*. Paris, 1864. The Author has been unable to consult this Memoir.

The variations in the proportion of water which occur during muscular contraction will be referred to in a subsequent section.

Mineral Salts. Fresh muscle yields on ignition from 1 to 1·5 per cent. of mineral matters, containing as their principal constituent potassium and phosphoric acid. 100 parts of the mixed mineral matters contain about 36 parts of phosphoric acid. There are also small quantities of calcium and magnesium phosphates and traces of chlorine and iron. The remarkable preponderance of potassium over sodium in the ash is to be remarked.

Summary of the Quantitative Composition of Muscle.

In the following Table, which is quoted from Hofmann[1], the average amounts of the various constituents in 1000 of the muscles of vertebrates is given.

	Mammals.	Birds.	Cold-blooded animals.
Solid constituents in 1000	217—255	227—282	200
Water ,, ,, ,,	745—783	717—773	800
Organic matters ,, ,,	208—245	217—263	180—190
Inorganic ,, ,, ,,	9—10	10—19	10—20
Coagulated albumin. Sarcolemma, nuclei, vessels	145—167	150—177	(?)
Alkaline albuminate	28·5—30·1		
Creatine	2·0	3·4	2·3
Xanthine and Hypoxanthine	0·2		
Taurine	0·7 horse	0·0	1·1
Inosit	0·03		
Glycogen	4·1—5·0		30—50
Lactic acid	0·4—0·7		
Phosphoric acid	3·4—4·8		
Potash	3·0—3·9		
Soda	0·4—0·43		
Lime	0·16—0·18		
Magnesia	0·4—0·41		
Sodium Chloride	0·04—0·1		
Oxide of Iron	0·03—0·1		

Sect. 2. General Phenomena of Living Muscle.

Muscle in a state of Rest.

We have in the previous section made ourselves acquainted with the normal appearances and composition of living muscular tissue.

[1] Hofmann, *Lehrbuch der Zoochemie*, p. 104.

General Phenomena of Resting Muscle.

The maintenance of this normal—or in other words, the preservation of the life of the tissue from moment to moment—is a physiological act consisting in successive and simultaneous degenerations and regenerations of parts. The apparent changelessness of repose depends upon the regularity and equilibrium of many hidden changes. The general nature of these changes is roughly indicated by comparing the constitution of the blood flowing to and from muscles. The blood enters muscle comparatively rich in oxygen and poor in carbon dioxide; it leaves the tissue relatively poor in oxygen and rich in carbon dioxide. Therefore the changes of degeneration and regeneration proceeding within the muscle are, collectively, changes in which at least carbon and oxygen are implicated; and further, they are, at least in part, of the nature of oxidations. Hence it appears that a supply of new matter to the tissue is ultimately indispensable. The tissue is the channel of a continuous circulation or migration of matter; it is the theatre of constant material exchanges. The blood conveys to it the substances which are needed; these are elaborated, rearranged, and converted into other forms within the tissue; and are finally again cast out into the blood-current. These operations, wrought within the tissue of muscle during repose, are included in the term *Nutrition;* which may be defined, in a figure now well known to Physiology, as the sum total of the processes which maintain the 'stock' of the organism at the normal. In reality these internal processes of muscle at rest are but little understood; most of our knowledge of them is derived by inference from the processes of muscular contraction. But we may assume that they occur in at least three well-defined stages. In the first, what may be called raw material is received into the tissue and stored up in some proximate modification; in the second, this store is elaborated into an intermediate form by a process independent of the process of storing; and in the third stage this intermediate body suffers decomposition into certain ultimate products, some of which, but probably not all, are discharged into the blood.

The grounds for assuming this threefold process will be understood when the contraction of muscle has been discussed. It may be remarked that it finds an exact analogy in the processes of glandular tissues.

The transformations of matter within the substance of resting muscle, though the more obvious, are not the sole phenomena of nutrition. Running parallel with them are certain transformations of energy. The energy implied in the mutual affinities of the elements involved in the transformations of matter, undergoes conversion into energy of other forms during the various nutritive changes.

What these forms are, is again largely a matter of inference. It is unquestioned that heat is the most important. A short time ago electrical inequalities or tensions would probably have been set down as a second form; but this is no longer admissible. The various move-

ments of small masses, where they occur, such as in the fission of nuclei and of fibres, constitute a third form. A fourth, if entirely hypothetical, deserves to be mentioned. It is not indeed another form of actual energy, like heat or mechanical motion, into which the energy of chemical affinity is converted; but rather a re-distribution of the original potential energy. The elaboration of the intermediate product in the above series of tissue-changes is entirely unknown to us in its nature; but it is at least conceivable that it is not altogether a process in which stronger affinities are satisfied at every step. It may, in part, be a dissociation; in which case some of the energy set free in the chemical changes of the final stage may again at once become potential.

The intensity of the chemical and physical processes of resting muscle depends upon temperature, the supply of the necessary substances, and what may be called the nutritive instinct, or inherent capacity, of the tissue for the changes. Both the supply of matter and the capacity of the tissue for the changes in question, are exalted in the active state of muscle.

Muscle in Action.

General Phenomena of Contracting Muscle.

The phenomena of resting muscle thus consist of two parallel and associated series of transformations, one of matter, the other of energy. The same dualism is seen in active muscle. The whole phenomenon of contraction comprises (1) a sudden acceleration and extension of chemical decompositions, and (2) a sudden and extensive conversion of the potential energy of chemical affinity into actual energy of various forms. The manifestations of actual energy in the case of contracting muscle are pronounced and admit of a careful study; they assume the form of heat, electrical inequality, and mechanical motion.

Special Phenomena of Contraction.

Contraction may be started either by the normal stimulus proceeding from a nerve; or by electrical, chemical, thermal, or mechanical stimuli applied to muscle even in the absence of nerves. In its mechanical aspect contraction is a shortening and thickening both of the whole muscle and of its individual fibres, associated in the case of entire muscles with a small reduction of bulk. Contracted muscle is less elastic and more extensile than resting muscle.

Microscopic appearances.

Viewed under the microscope, the act of contraction falls into well-marked stages. In the first, the bands draw near together as the muscular fibre shortens; and the dark and light bands approximate in tint, until the whole fibre is evenly dark with little or no striation: this is called the *homogeneous stage*. As contraction proceeds, striae again appear, but this

time in a regular alternation of simple dark and simple bright bands. There is however this difference: those parts of the fibre which before were dark are now bright, and those parts which formerly were bright are now dark. The fibre has emerged from the homogeneous stage with its bands interchanged as far as regards their tint; and this, therefore, may be called the *stage of transposed bands*. Their transposition only affects the *shade* or *tint* of the stripes, the isotropous and double-refracting elements of the fibre maintaining their original relationship. This is well shewn in the figure on p. 314, where the same contracting muscle is exhibited by ordinary and by polarized light.

It may be added that Engelmann believes he has demonstrated that, in contraction, the volume of the main double-refracting zone increases at the expense of the isotropous layers.

Rate. If contraction is started at one point with a given intensity it does not instantly extend over the whole fibre; but travels along it as a wave with a velocity of 3 metres a second in frogs. In the case of excised muscles, the wave suffers in its course a diminution[1] of intensity.

Latent period. When muscle is directly stimulated the contractile force does not at once begin to develope. An interval elapses between the application of the stimulus and the beginning of contraction; this is known as the *period of latent stimulation*, or *latent period*. Its value was determined by Helmholtz to be about $\frac{1}{100}$ sec., but it is found to vary in different circumstances, and under favourable conditions it is said to become as short as $\frac{1}{300}$ or $\frac{1}{400}$ of a sec.[2]

Course, or Curve, of Contraction. When once started, the force does not spring suddenly to perfection, but developes in course of time. If a resistance be opposed to the shortening of the muscle it is clear that no contraction can occur until the contractile force has grown large enough to overbalance the resistance; hence, the greater the resistance, the longer the interval which must elapse between the moment of stimulation and the beginning of actual contraction. During contraction the contractile force does work and becomes spent. Hence a smaller resistance serves to check contraction near its end than near its beginning. The rapidity of contraction is not equal throughout its course; it first increases and then diminishes until the summit of contraction is reached, as it usually is within $\frac{4}{100}$ or $\frac{5}{100}$ of a second after stimulation. Beyond the maximum the contractile force dies gradually away[3]; this is rendered probable by the course of re-extension under the influence of a weight, which

[1] See Hermann's *Handbuch der Physiologie*, Bd. I. Abth. i. p. 55.

[2] See Hermann's *Handbuch*, Bd. I. Abth. i. p. 36.

[3] Heidenhain, "Ueber Ad. Fick's experimentellen Beweis für die Gültigkeit des Gesetzes von der Erhaltung der Kraft bei der Muskelzusammenziehung." Pflüger's *Arch.*, Vol. II. p. 426.

is not such as to admit of the assumption that gravity alone determines it; and it becomes more than probable when we learn that the heat-developing processes[1] and the chemical processes[2] are also carried over into the relaxing period. The duration of the period of relaxation is about $\frac{5}{100}$ of a second. Muscle appears to have no power of active re-extension[3].

Tetanus.

When stimuli are thrown into muscle with sufficient rapidity, contractions overtake one another, sum their effects, and maintain the muscle against extending forces, in a position more or less of maximum contraction. Such continued contraction is called a *tetanus*, the laws of which belong to the physics of muscle.

The course of contraction is not similar in all kinds of muscle; nor is the course the same in any one muscle under all circumstances. Thus the rate of contraction is much quicker in the muscles of insects than in those of frogs; and quicker in the latter again than in those of the tortoise; and in these than in the heart-muscle; and in this than in the smooth muscles of the intestines or ureter. Such differences are of the greatest interest to the physiologist as indicating either differences in the machinery for the conversion of energies, or different capacities for the chemical changes upon which contraction depends.

Red and Pale striated Muscles.

In respect of such internal machinery, or capacity, the differences of red and pale striated muscles are remarkable. The contraction of the red variety is slow and enduring, 10 stimuli a second being enough to cause almost unbroken tetanus; while of the pale variety, the contractions are short and sharp, 20—30 stimuli a second being needed for a perfect fusion of them[4]. In the former the latent period is so long as $\frac{1}{18}$ sec.; while in the latter it has the value of $\frac{1}{83}$ sec.[5].

These physiological differences are all associated with varieties of structure; but differences of a similar nature may be exhibited by the same muscle when it contracts under varied conditions. Thus cold, many poisons, and incipient exhaustion prolong contraction and diminish its amplitude. Indeed the stimulus of a sharp blow to dying muscle often produces a local contraction which may be likened to a *wheal*, and which may persist for a long time: such a contraction is described as 'idio-muscular'.

Idio-muscular Contraction.

Absolute force.

The force of muscular contraction is measured by the weight which is just sufficient to prevent the

[1] Steiner, "Ueber die Wärmeentwicklung bei der Wiederausdehnung des Muskels." Pflüger's *Arch.*, Vol. xi. p. 196.

[2] Heidenhain with Landau and Pacully, *Loc. cit.* Pflüger's *Arch.*, Vol. ii. p. 429.

[3] Kühne, *Loc. cit.* *Arch. f. Anat. Physiol. u. wiss. Med.* (Reichert u. du Bois-Reymond), 1859, p. 815.

[4] Kronecker and Stirling, "The Genesis of Tetanus." *Journal of Physiol.* (Foster), Vol. i. p. 395.

[5] Ranvier, *Loc. cit.* *Arch. de physiol. norm. et path.*, 2 sér. Vol. i. p. 5.

shortening of the muscle[1]. The force varies with the stimulus: as this gradually increases, that enlarges, quickly at first but afterwards more slowly, until a maximum is gained, which is known as the 'absolute force'. The absolute force is usually stated to be 2800—3000 grms. per sq. centimetre of tetanized frog-muscle; and between 6000 and 8000 grms. per sq. centimetre in the muscles of man voluntarily contracted[2].

These numbers cannot be taken as the direct or exact equivalent of that portion of the chemical changes which is devoted to mechanical effect. For the result so obtained is less than the true absolute force of the muscle experimented on by an amount which depends on its extensibility. If muscle were more elastic than it is, although the process of contraction with all its chemical changes remained the same, the absolute force would seem to be less. This may readily be demonstrated by interposing an elastic band between the muscle and the weight about to be raised. The absolute force of such a system is less than that of the muscle alone; whence we may conclude that the proper extensibility of muscle has a like diminishing effect[3].

If a loaded muscle be made to contract by the application of a stimulus, the height through which the load is raised is called the *lift;* and this multiplied into the load gives the value of the mechanical work actually done. As the stimulus is increased, the lift grows proportionally to the stimulus up to a maximum, beyond which it remains constant[4]. Inasmuch as muscle is extensible, and its extensibility is increased during the state of contraction, it is clear that the lift is the expression of the actual shortening of the muscle *minus* the difference between the extension of the uncontracted and the extension of the contracted muscle. For the full illustration of this the reader is referred to Ed. Weber's article 'Muskelbewegung' in Wagner's *Handwörterbuch,* and to the Textbooks of Physiology.

The lift varies also with the load, becoming smaller as the load increases; and the variation is such that the product of lift into load first of all increases and afterwards decreases as the load varies from nothing onwards. In other words, within certain limits, the more a muscle is weighted the more mechanical work will a given stimulus produce. The increased tension to which the muscle is for the time subjected converts it into a body capable of yielding a larger amount of mechanical work than the same muscle less tense. Not only does the state of tension in the very act of contraction influence the work done, but the state of tension immediately prior to contraction has the same effect: the greater the tension the greater within certain limits[5] the yield of mechanical energy.

[1] E. Weber, Wagner's *Handwörterbuch,* III. 2, p. 84. Helmholtz, *Arch. f. Anat. Physiol. u. wiss. Med.* (Müller), 1850, p. 276; 1852, p. 199.

[2] See Hermann's *Handbuch,* Bd. I. Abth. i. p. 64.

[3] See Hermann's *Handbuch,* Bd. I. Abth. i. p. 65.

[4] Fick. See Hermann's *Handbuch,* Bd. I. Abth. i. p. 108.

[5] Heidenhain, *Mechánische Leistung, Wärmeentwicklung und Stoffumsatz bei Muskelthätigkeit.* Leipzig, 1864, p. 84.

Maximum work. The maximum work done under most favourable conditions is said to vary between about 3500 and 5500 gram-meters per gram of frog-muscle[1].

Heat of Contracting Muscle. The mechanical motion of contraction is not the only exhibition of kinetic energy which accompanies the chemical changes of acting muscle. During tetanus[2] and in single contractions[3], the muscles become raised in temperature; and since this occurs in muscles removed from the circulation or even in muscles entirely removed from the body, it must be due to the heat-developing processes of the tissue itself.

Helmholtz, in a 2—3 minutes-tetanus through nerves, found the thigh of a frog raised ·14 to ·18° C.; and Heidenhain observed the temperature of the gastrocnemius to be raised ·001 to ·005° C. in a single contraction.

If the weight of the gastrocnemius be known, and also the specific heat of muscular tissue, it is possible to estimate in heat-units the amount of heat generated in one contraction. Fick[4], taking the specific heat of muscle to equal that of water, found that in one energetic contraction, under most favourable conditions for activity, every gram of the contracting muscle generates heat enough to raise 3·1 mgr. of water through 1° C. The specific heat of muscle is however stated to be ·7692 by Adamkiewicz[5], and ·825 by Rosenthal[6].

The evolution of heat in contraction is amenable to the same influences as the evolution of mechanical work; but though amenable in the same sense it is not so in the same degree. Thus, as the stimulus gains in strength, not only does the lift become higher, but the heat liberated is also increased, with this difference, that the heat evolved is increased more rapidly than the lift[7]. So also the greater the tension of a muscle, whether before or during contraction, the greater, within bounds, will be the heat evolved as well as the work done; but here again, as the tension increases, the heat evolved reaches a maximum and begins to decline sooner than the mechanical effect[8].

[1] Hermann's *Handbuch*, Bd. I. Abth. i. p. 79.

[2] Bunzen in Gilbert's *Annalen*, 1807, vol. xxv. p. 157 : quoted in Heidenhain, *Mechanische Leistung*, etc., p. 33, where also will be found an account of the earlier researches in which the heat of the body, and of muscles within the body, was found to be influenced by exercise. Helmholtz, "Ueber die Wärmeentwicklung bei der Muskelaction." *Arch. f. Anat. Physiol. u. wiss. Med.* (Müller), 1848, p. 144.

[3] Heidenhain, *Mechanische Leistung, Wärmeentwicklung und Stoffumsatz bei Muskelthätigkeit*. Leipzig, 1864, p. 73.

[4] Fick, "Ueber die Wärmeentwicklung bei der Muskelzuckung." Pflüger's *Archiv*, Vol. xvi. p. 84.

[5] Adamkiewicz, "Die Wärmeleitung des Muskels." *Arch. f. Anat. Physiol. u. wiss. Med.* (Reichert and du Bois-Reymond), 1875, p. 254.

[6] Rosenthal, "Ueber die specifische Wärme thierischer Gewebe." *Monatsber. d. Berliner Acad.*, 1878, p. 307.

[7] Nawalichin, "Myothermische Untersuchungen." Pflüger's *Archiv*, Vol. xiv. p. 295.

[8] Heidenhain, *Mechanische Leistung*, etc. p. 84 et seq.

When muscle becomes exhausted, both the work done and the heat generated decline; but the latter more quickly than the former[1]. The cause of this dissimilarity may be one or other of the two following. It may be that the heat-evolving and the work-evolving appliances in muscle are totally distinct, and variously affected by the same conditions. Or it may be that the heat and mechanical work of muscle, like the heat and mechanical work of a steam-engine, arise in a common fundamental combustion; and that the relative proportions of the two are to some extent determined by external conditions, just as some steam-engines work more economically than others, *i.e.* with a larger proportionate yield of mechanical work[2].

It is impossible to say whether heat-developing processes are occurring during the latent period[3]; but there is little doubt that they continue beyond the period of maximum contraction. It is at least certain that the heat developed in a muscle is influenced by the load which it bears during relaxation as it is by that which it bears in contraction; and such influence cannot be explained as the result of mere forcible extension[4].

The proportion of heat and work evolved in contraction has been determined by Fick[5] in the case of excised frog-muscles to vary according to the load; the greater the load the larger the proportion of the total actual energy taken up by mechanical work. Under the most favourable circumstances for the performance of mechanical work the relation of work to heat was 1 : 3·8, and in the least favourable of Fick's experiments the relation was 1 : 23·6. It is extremely uncertain how far these fractions can be applied to muscles within the body, or to the muscles of warm-blooded animals.

Fick's demonstration of this interesting relationship depends upon the fact that, when the motion of a falling body is suddenly arrested, an amount of heat appears, equivalent to the mechanical motion destroyed. By direct experiment he proved that, if a weight suspended from a muscle is raised by external means to a certain height and then let fall, the muscle suffers a heating proportionate to the fall, *i.e.* which is the precise equivalent of the work done in lifting the weight. He therefore caused a loaded muscle to contract and afterwards allowed it to re-extend under the weight which it had lifted; and then observed by how much the temperature of the muscle had been raised. From the specific heat of muscular tissue he was able to calculate the total quantity of heat gained by the muscle in the process; and by subtracting from this total the heat-equivalent of the work done in raising the weight, he was able to compare the heating of a muscle under a certain load and the work done in raising the load.

[1] Heidenhain, *Mechanische Leistung*, etc. p. 74.
[2] See Hermann's *Handbuch der Physiol.*, Bd. I. Abth. i. p. 168.
[3] Nawalichin, *Loc. cit.* Pflüger's *Archiv*, Vol. XIV. p. 311.
[4] Steiner, *Loc. cit.* Pflüger's *Archiv*, Vol. XI. p. 204. See also Heidenhain with Landau and Pacully, *Loc. cit.* Pflüger's *Archiv*, II. p. 423.
[5] Fick, *Loc. cit.* Pflüger's *Archiv*, Vol. XVI. p. 79.

Electrical tensions of contracting muscle.

The third form under which the actual energy of contracting muscle appears, is that of electrical disturbance. Muscles within the body, or absolutely uninjured muscles, are electrically homogeneous: they exhibit no current[1]. But whenever a stimulus is applied to a muscle, the spot stimulated assumes a lower, or negative, potential as compared with the rest of the muscle; so that if the two electrodes of a galvanometer were applied to an excited and a non-excited spot of muscle respectively, a current would be discovered. This *functional current* increases to a maximum very rapidly and afterwards disappears, but more slowly. It begins instantly on stimulation, *i.e.* it has no latent period; and the whole phenomenon lasts about $\frac{1}{300}$ sec. Hence it falls entirely within the latent period of contraction. It travels down excised muscles from the point of stimulation with a velocity which agrees with that of the contraction-wave, namely about 3 metres a second in the frog. In the normal muscles of the human fore-arm the velocity has been determined to lie between 10 and 13 metres per sec. Like the wave of contraction, the negative wave diminishes in intensity during its course along excised muscles; but no such diminution has been detected in the case of muscles in which the processes of restitution are active[2].

As is the case with the evolution of heat and mechanical effect, the disturbance of electrical tension which follows stimulation differs in degree according to the different conditions of stimulus, irritability and tension. It increases up to a maximum as the stimulus becomes more and more intense; it diminishes as exhaustion approaches[3]; it increases with the lift[4]; and it varies directly as the tension of the muscular fibres[5].

Rigor Mortis.

Besides the conditions of rest and activity, there is a third condition of muscular tissue with characteristic phenomena and a singular bearing on the theories of muscular function, viz. the moribund condition.

After the death of the body, or after the ligature of their tributary arteries, or on subjection to a certain temperature, muscles become *rigid*. That is to say, they become shorter and thicker, and of less

[1] Hermann, "Ueber das Fehlen des Stromes in unversehrten ruhenden Muskeln." Pflüger's *Archiv*, Vol. III. p. 35.

[2] Bernstein, "Ueber den zeitlichen Verlauf der negativen Schwankung des Muskelstromes," *Monatsber. d. Berliner Acad.*, 1867, p. 444. *Untersuchungen ü. d. Erregungsvorgang im Nerven- u. Muskelsystem.* Heidelberg, 1871. Hermann, "Ueber den Actionsstrom der Muskeln im lebenden Menschen." Pflüger's *Archiv*, Vol. XVI. p. 410.

[3] Hermann, *Handbuch der Physiol.*, Bd. I. Abth. i. p. 220.

[4] Harless, *Gel. Anz. d. bayr. Acad.*, XXXVII. p. 267, 1853; quoted by Hermann, *Handbuch*, Vol. I. Abth. i. p. 220.

[5] S. Lamansky, "Ueber die negative Stromesschwankung des arbeitenden Muskels." Pflüger's *Arch.*, Vol. III. p. 202.

bulk, as in the act of contraction. The lift of a muscle passing into rigor is greater with a small load, but less with a heavy load, than during a single contraction, and the absolute force is in the same circumstances sometimes greater and sometimes less. No similar comparisons have yet been made between rigid and tetanized muscles[1].

Rigid muscle is less extensile, as well as less elastic, than normal resting muscle, thus differing from contracted muscle, which is more extensile. It is, farther, distinguished from contracted muscle by its peculiar doughiness and opacity.

Rigor is associated with the evolution of heat—*post mortem* elevation of temperature. This is doubtless in part a mere consequence of the physical changes of density, and the transformation from the fluid to the solid state. But a physical explanation will not account entirely for the phenomenon; for no rise of temperature can be detected during the quasi-rigor—which is a simple coagulation—induced by acids or alcohol, and true rigor is unquestionably attended by chemical changes[2].

The passage into rigor is further associated with a difference of electric potential; dying muscle, like contracting muscle, is negative to normal resting muscle[3].

Thus the last event in the life-history of muscle resembles a common contraction very closely in the nature of its physical phenomena. We shall find that they are alike also in their chemical changes.

Sect. 3. Special Study of the Chemical Changes of Living Muscle.

It has been pointed out that the whole life of muscle consists of two parallel series of transformations, of constitution and of energy. The characters of one series, the transformations of energy, have been rapidly sketched; and it now remains to describe in detail the changes of the other, or chemical series. It may at once be stated that our knowledge of these two series is, and must be, of very different extent. In the case of the physical transformations we are able to study their course in time, to fix their maximum and trace their decline. In the case of the chemical series the steps are entirely hidden; we can merely compare the constitution of a muscle before and after, but not *during*, an act of contraction. We cannot say whether the chemical changes run *pari*

[1] E. Walker, "Die absolute Kraft der Erstarrung." Pflüger's *Arch.*, Vol. IV. p. 186.

[2] Hermann, *Handbuch der Physiol.*, Vol. I. Abth. i. p. 171. Schiffer, "Ueber die Wärmebildung erstarrender Muskeln." *Arch. f. Anat. Physiol. u. wiss. Med.* (Reichert and du Bois-Reymond), 1868, p. 442.

[3] For a full account of the demarcation-current and its relation to the so-called natural muscle-current of du Bois-Reymond, see Hermann's *Handbuch*, Vol. I. Abth. i. p. 173 et seq.

passu with the physical phenomena of work, heat, and electrical disturbance, which are in some fashion linked to them; or whether the contraction of a muscle is not rather like the firing of a gun, in which the progress of the bullet affords no clue whatever to the course of the explosion.

Methods of the chemistry of Muscle.

Since then the chemical history of any event in the life of muscle rests on an analysis of chemical constitution before and after the event; and since certain of the constituents of muscle may be exhaled into the surrounding medium; it is clear that the chemistry of living muscle comprises two lines of enquiry:

1. Into the chemical composition of the muscle itself.
2. Into the chemical composition of the medium surrounding the muscle.

These have for the most part been carried on independently; and the latter has, beyond question, led to the more important results. The enquiry into the chemical composition of the medium surrounding muscle has been followed under two sets of conditions, not however essentially different: the simpler, in which the muscles are exposed to the air as a medium; the more complex, in which the muscles remain in the body, or in which the blood is the surrounding medium. In the latter circumstances the enquiry is complicated by the occurrence of restitutional changes. Finally, when muscles are examined while still within the body there are two ways of obtaining a knowledge of the changes in their surrounding medium, viz. by contrasting before, during, and after a muscular act,

1. The blood of muscle,
2. The general excreta.

While this summary includes all the methods of muscular chemistry, it is necessary to state that they have not been equally applied to living muscle in each of its three possible conditions, the resting, the active and the moribund. The latter two conditions have been most freely investigated, and it will be convenient to describe the results of their investigation together, since they have much in common; and before the results of the examination of the normal state of rest are stated.

The Chemical Changes of Contraction and Rigor.

A. *Changes in the chemical composition of muscle itself.*

Changes in the gaseous constituents[1].

Apparatus.

The air pump, which has proved so valuable a means of research in the chemistry of the blood, has been also

[1] The first rudimentary attempt at the gaseous analysis of muscle which the author has met with, is described in a memoir *sur l'Irritabilité*, by Girtanner, contained

employed in the analysis of muscle, but with much greater difficulty. The difficulty is due in part to the nature of the method, for the muscles cannot be transferred to the vacuum without preliminary exposure to the contamination of air and indifferent fluids; and in part to the nature of fresh muscle, whose tissue entangles bubbles of gas, and whose gaseous contents, owing to the acidification of rigor (p. 359), and to putrefaction, rapidly undergo change even at ordinary temperatures.

For the analysis of muscle a special boiling-flask is necessary, such for example as is figured in the following diagram.

A and B represent two views of the same apparatus, and the letters are identical in their reference.

v is the froth-chamber, a globe provided with a short neck *g*, fitting on to the drying-chamber *t* but shut off from it by the stop-cock *b*. It is provided also with a longer neck, *h*, at right angles to the other, interrupted by a stop-cock *c*, and fitting into the boiling flask *f*.

f is the boiling-flask, with a rounded bottom and a wide neck; it is provided with three platinum wires melted through the sides and reaching almost to the bottom of it. It is fitted on to the neck, *h*, of the froth-chamber not quite at right-angles, as *B* shews, and in a plane at right-angles to that of the neck *g*. *f* contains the muscle to be exhausted; and at the mouth of the neck *h* is a cork *k* grooved at the sides to permit the passage of gases from *f* to *v*, while stopping any solid fragments which might do damage to the stop-cock *c*.

v serves a double purpose besides that of a froth-chamber: Firstly, any liquid which spirts over from *f* during ebullition is collected here, and may, by turning *v* round the axis of its neck *g*, be made to trickle back into *f*. Secondly, a reagent, such as an acid, may be kept in *v* during the preliminary exhaustion of a muscle in *f*, and by a similar tilting of *v*, may be brought to play on the muscle at any given moment.

t is a small drying chamber containing sulphuric acid, sufficiently large to keep the vacuum of the pump dry so long as the stop-cocks *c* and *b* are never open together; by this means the access of watery vapour to the absorption tube of the gas-analyser is prevented. The capacity of the boiling-flask *f*, and the part of the neck *h* up to the stop-cock *c*, may be about 200 c.cm.[1]

in Rozier's *Observations sur la physique*, Vol. XXXVII. 1790, p. 148. Muscle, cut into small pieces, was enclosed in a glass retort connected with a pneumatic apparatus. A very gentle heat was applied by means of a lamp for more than two hours, and the gases which passed over into the pneumatic receiver were examined at different stages of the experiment. At first atmospheric air passed over "mêlé à une très-petite quantité d'air vital, dont le gaz nitreux indiquoit la présence;" the second portions were vital air, "mêlé à du gaz acid carbonique." Girtanner very innocently remarks: "On peut retirer la même quantité de ce gaz [vital air or oxygen] plusieurs fois de suite, en exposant des substances animales alternativement à l'air atmosphérique et à une chaleur de 60 à 70 degrés du thermomètre de Réaumur." He found the exact adjustment of the temperature a matter of great difficulty: "Si l'on applique un degré de chaleur trop fort, on aura du gaz acide carbonique au lieu de gaz oxigène." The fallacies of the method lie on the surface, but do not destroy the historical interest of the experiment. Girtanner further found that he could extract almost all the oxygen which animal substances contain "par le moyen de l'eau chaude."

[1] Hermann, *Untersuchungen ü. d. Stoffwechsel der Muskeln ausgehend vom Gaswechsel derselben.* Hirschwald, Berlin, 1867, p. 4.

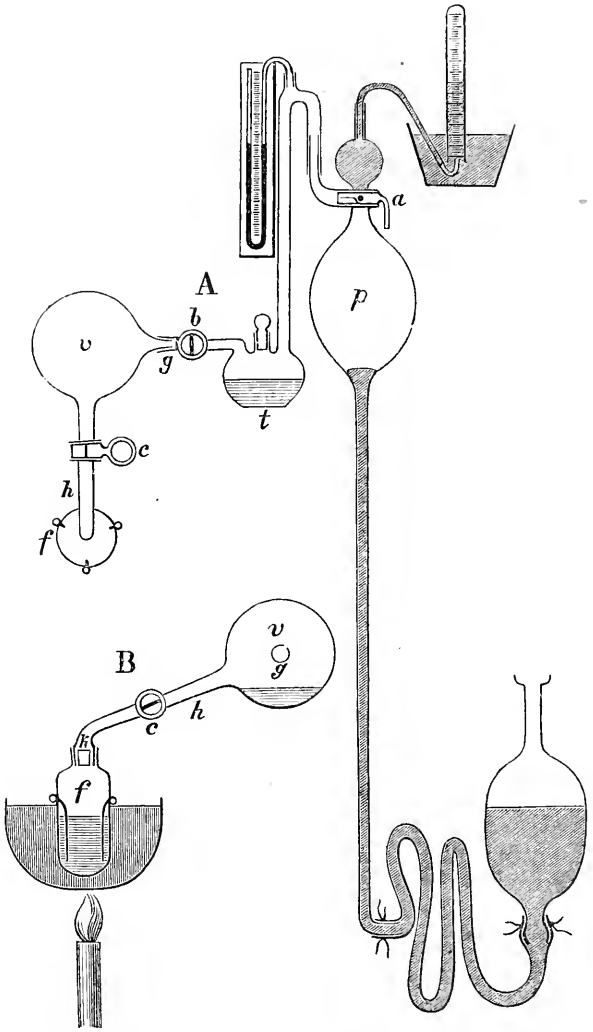


FIG. 57. HERMANN'S APPARATUS FOR THE EXTRACTION OF THE GASES OF MUSCLE.

Gaseous analysis of 'scalded' muscle.

If the muscles of a frog deprived of blood be quickly removed from the body (and frog-muscles, for obvious reasons, are made use of in these experiments); and if they be then plunged instantly into a large volume of briskly boiling salt-solution of indifferent strength, they will be coagulated at once throughout their mass, and die without preliminary rigor

and acidification. This method is technically known as *scalding*. If an inverted beaker be placed in the large dish of boiling fluid and very accurately filled with the salt-solution or its steam, to the exclusion of all air, the muscle may be thrown at once beneath it, and any gas which escapes from the muscle in the process may be collected. Under these circumstances it appears that scalded muscle, during the process of scalding, loses no appreciable quantity of gases. If the muscle is now reduced to a low temperature, and minced to prevent the mechanical entanglement of gas-bubbles; and then subjected to the influence of a vacuum; it is found to yield a small per-centage of gases. If, after a first evacuation, phosphoric acid is added to the minced muscle by tilting the froth-chamber, a further escape of gases follows. In both cases the gas is carbon dioxide. Thus scalded muscle—muscle in which the process of rigor has been circumvented, and which may therefore be regarded as presenting the gases of fresh normal muscle—contains an extremely small quantity of carbon dioxide, both free (*i.e.* capable of withdrawal by the air-pump), and fixed (*i.e.* needing an acid to drive it out). The former may amount to 2·74 per cent., and may be due, in part, to the imperfect scalding of the central portions of the muscle; for, if the temperature is not high enough, rigor follows, and not instant death; and rigor is associated with acidification and the production of carbon dioxide. The fixed carbon dioxide may amount to 1·95 per cent.[1]

Gaseous analysis of muscle passing into rigor.

If the above figures be taken as indicating the gases of fresh normal muscle, we shall observe a marked difference in muscle which is passing into rigor. A convenient method of producing the gases of rigor, and at the same time facilitating their liberation from the muscular substance, is the following. Frogs, whose blood-vessels have been well washed out with ·5 per cent. solution of NaCl, are taken into a cold atmosphere, and their belly-muscles and muscles of the hinder limbs (excluding the feet and tendons) are quickly cut away and weighed on a watch-glass. They are then placed, still on the watch-glass, over a freezing mixture until they are frozen to a firm mass; and afterwards they are minced with cold knives and rubbed up in a cooled mortar. The freezing preserves the normal composition of the muscle more or less perfectly during the mincing and trituration[2]; and these processes are devised to facilitate the disentanglement of the gas during evacuation.

The frozen and triturated muscle is introduced into the boiling-flask which is filled to the brim with normal NaCl solution at 0° C., the various portions being so quickly dropped in that a constant overflow of salt-solution is kept up. The object of this manœuvre is to wash away the air-bubbles which are carried into the salt-

[1] Hermann, *Op. cit.* pp. 115, 116. Expt. 9.

[2] Kühne, *Untersuch. ü. das Protoplasma.* Leipzig, 1864, p. 3. See Hermann, *Stoffwechsel der Muskeln*, p. 5.

solution clinging to the frozen muscle, and which, becoming disengaged, rise to the surface. This simple method is more successful in preventing the intrusion of air-bubbles than that of introducing the muscle into the boiling-flask under a surface of mercury. When all the muscle has been introduced, the boiling-flask is at once attached to the froth-chamber, the stopcock *c* being closed, and the greatest care being taken not to include bubbles of air. Salt-solution may be used either after shaking it up with the air of the room, or after exhaustion of its dissolved gases by the air-pump; in the former case it is necessary to ascertain the gaseous impurities of the salt-solution and allow for them at the close of the experiment.

If it is desired at any time during the experiment to treat the muscle with acids, the acid must be carefully placed in the froth-chamber *v*, before its neck *g* is attached to the pump. Meanwhile the boiling-flask *f* is surrounded by a freezing mixture in the position figured in B.

It will be seen that the whole object of the experiment is to boil a mixture of salt-solution and muscular tissue reduced to as fine a state of division as possible; the preparation (which may occupy about two hours) being made at a temperature least favourable to spontaneous changes of the tissue to be analysed.

The muscle having been lodged in the apparatus for collecting the gases, all that is necessary is to induce rigor; and this may most readily be done by raising the temperature of the boiling-flask. The froth-chamber *v* is then made vacuous and the gases which boil over are passed through the pump into the absorption-tube for analysis. At a temperature of 0° C. little or no gas is given off, and then only after several hours exposure. As the temperature rises to 15° C. there is an indefinite, dribbling discharge. At temperatures beyond this, up to 30° C., there is at first a large escape of gas which afterwards subsides. But it is at still higher temperatures, of 40—50° C., that the greatest discharge occurs; here, also, it is voluminous at first, becoming less and less as the exposure continues. At this time the muscles have become acid and have ceased altogether to be irritable. When the temperature has been raised beyond 70° no further yield of free gases is obtained.

If the muscle is treated with phosphoric acid at 0° C., and subsequently heated to assist the liberation of gas, a sharp evolution occurs, of short duration and yielding but a small amount. If the acid be added to a preparation subjected to a temperature of 20°—50° C., at a time when the discharge has ceased to be voluminous, a brief acceleration of the discharge will result, followed by complete and final stoppage.

Secondary, or putrefactive, discharge of gases.

If the muscle is kept in the apparatus beyond the time at which the first discharge subsides, the liberation of gases begins again, even without the addition of an acid. This constitutes a secondary discharge, and is due to putrefaction; it may begin

within a few hours of the commencement of the experiment, and must not be confused with the primary discharge. We need not here further discuss it[1].

Nature of gases liberated by muscle in rigor.

The gases of the primary discharges obtained from muscle raised from 0° to temperatures varying from 5° to 70°, added to the gases liberated by phosphoric acid, vary from 1 to 15 vols. per cent. of the muscle used. These gases contain no oxygen whatever; on the contrary, if salt-solution containing dissolved air have been used, some of the oxygen will have disappeared from solution. Nitrogen is constantly present in small amount in the portions of gas first set free; but subsequently no nitrogen can be detected until the secondary discharge begins. Carbonic anhydride is the chief, and indeed the only constituent of the gas discharged during the middle of an experiment[2].

The following experimental numbers will serve to illustrate these conclusions.

Experiment. 57·3 grms. (= 54·16 c.cm.) of frozen and triturated frog-muscle (the muscles of three or four frogs may be used) were exhausted in boiled salt-solution at a temperature of 50° C. After the cessation of the primary discharge, phosphoric acid was added, and the gases collected at a temperature of 60°, as long as they continued to be evolved.

PORTION I. BEFORE ADDITION OF ACID.

	Constituents in c.cm. (0° C. and 1 mtr.)	Per-centage of muscle.
Total gas	7·051	
CO_2 (free)	6·385	11·79
N + error	0·666	1·23
O	0·0	0·0

PORTION II. AFTER ADDITION OF ACID.

CO_2 (fixed)	1·105	2·04
Total CO_2 free and fixed	7·490	13·83

Experiment. 34·2 grms. (= 32·33 c.cm.) of frozen and triturated frog-muscle, in boiled salt-solution, exhausted at 0° C. yielded no gas. Phos-

[1] Hermann, *Op. cit.* p. 11.

[2] Hermann, *Op. cit.* p. 10.

phoric acid was then added without causing any discharge. The acidified muscle was heated to 50°C., and thereupon liberated quickly a quantity of gases[1].

	Constituents in c.cm. (0°C. and 1 mtr.)	Per-centage of muscle.
Total gases	4·025	
CO_2	2·222	6·87
N + error	1·803	

Thus the change in gaseous composition which muscle undergoes on passing into rigor may be summarized as a large increase of that carbonic anhydride which is defined as free, or capable of simple withdrawal by an air-pump.

The discharge of gases is a primary phenomenon of rigor and not due to the decomposition of carbonates already existing in the muscle, by the acid formed in the same process. For the addition of phosphoric acid at a time when the discharge is free, tends rather to diminish the total discharge than increase it, and never leads to an evolution of gas proportionate to the yield of free carbon dioxide. In other words, the carbon dioxide is formed step by step with the process of rigor; but, although it is actually formed during rigor, it may still first appear in some fixed and stable modification; and this is a possibility which we have as yet no means of testing[2].

Gaseous analysis of contracting muscle.

With very similar appliances to those just described the gaseous alterations of muscle during activity may be determined; but this research is beset with great difficulties, if a complete analysis of the gases is desired. In the first place it is indispensable to employ boiled salt-solution and to use scrupulous care in excluding air-bubbles. In the second, there is great danger of electrolytic action due to the strong currents needed to stimulate muscles immersed in salt-solution. And in the third, it is impossible to adopt wholly the method of trituration in order to facilitate the escape of gas in the vacuum; all that may be attempted for this purpose is to mince the muscle coarsely, or to select muscles of small bulk like the sartorius. But many of the difficulties of experiment may be avoided after it has once appeared that, as in the case of rigor, carbon dioxide is the only important constituent. No oxygen is ever detected; and the nitrogen evolved behaves like the nitrogen set free in the rigor of muscle. Hence it is at once possible to dispense with the troublesome salt-solution and the strong tetanizing currents; and to examine muscles

[1] Hermann, *Op. cit.* p. 114. [2] Hermann, *Op. cit.* p. 16.

stimulated in a vessel of air. The method of the experiments is to take a preparation of frog-muscles and expose it before, during, and after tetanus under exactly identical conditions, collecting the yield of gases separately, for comparison. For this purpose the pelves and hinder extremities of three or four frogs divested of their skins may be arranged to form a chain attached at each end to a platinum electrode of the boiling-flask *f*. At the bottom of this flask is a little normal salt-solution to keep the atmosphere moist; salt-solution being preferred to water in order to defend the preparation from injury during the accidental spurtings of the fluid. The temperature of the boiling-flask is carefully maintained constant throughout the experiment, at about 16—20° C. The muscles are first exposed to a vacuum for an hour and the gases (A) collected. They are then tetanized at intervals during another hour and the gases (B) again collected, care being taken not to force tetanus into rigor. And lastly, they are again allowed to rest for an hour while the escaping gases (C) are a third time collected. On analysis it appears that

A contains the least amount of CO_2.
B „ the greatest „ „
C „ somewhat less than B.

B and C may each contain more than three times as much carbon dioxide as A; and C may contain more than B, if, from any cause, rigor happen during the third hour[1].

Thus in tetanus, as in rigor, the gaseous changes consist in an increase of the carbonic anhydride capable of withdrawal by an air-pump. The increase is due to a special production of the dioxide within the muscle, and not to the decomposition of some pre-existent stable form of it, by means of the acid which appears during tetanus. This is demonstrated by comparing the gases of normal and tetanized muscles from the same animal—an experiment which is practicable from the circumstance that muscles when tetanized in the cold lose a very small quantity of gases[2]. Frogs are taken and buried in snow until almost rigid. Their vessels are then washed out with ice-cold salt-solution; and one leg from each is amputated and scalded in the manner already described: if the scalding has been perfectly done the reaction of the muscle to litmus paper is *neutral*, not acid. The scalded limbs are minced in a vessel kept cold over a freezing mixture, and put into the boiling-flask with (unboiled) salt-solution at 0°. Phosphoric acid is placed in the froth-chamber *v* ready for use. The minced muscle is evacuated at 50°; then acidified, and again evacuated. Meanwhile the rest of the cold carcases are arranged in series on a cold plate and tetanized at intervals during many hours. At the end of this time these muscles also are scalded: they should have an *acid* reaction. They are minced

[1] Hermann, *Op. cit.* pp. 116, 117. Expt. 11 and 12.
[2] Hermann, *Op. cit.* p. 25.

and exposed to the vacuum both before and after treatment with phosphoric acid.

These experiments may be illustrated by the following notes:

Experiment. Three frogs prepared as above described. Tetanus was induced at intervals during $3\frac{3}{4}$ hours[1].

MUSCLES IN REPOSE. WEIGHT 13·4 grms. (=12·67 c.cm.).

	Constituents in c.cm. (0° C. and 1 mtr.)	Per-centage of muscle used.
CO_2 (free)	0·381	3·01
CO_2 (fixed)	0·620	4·90

MUSCLES TETANIZED. WEIGHT 20·2 grms. (=19·09 c.cm.).

CO_2 (free)	1·462	7·66
CO_2 (fixed)	0·843	4·42

Whence it appears that in tetanus the carbonic anhydride which a vacuum can extract, added to that which is liberated by acids, may rise as high as 12·08 p. c. by volume of the muscle used. Further, that the carbonic anhydride set free by an acid is constant in resting and tetanized muscle.

Experiments of Pflüger and Stintzing.

From the experiments of Hermann, which have just been detailed, we may conclude that muscle contains some constituent which in the course of contraction or of rigor suffers a decomposition and yields carbon dioxide in a condition to be removed by the air-pump. Further, that after scalding (p. 352), or after acidification by phosphoric acid (p. 353), this constituent is no longer capable of decomposition by the means which commonly bring rigor about. But although it is then incapable of decomposition by a vacuum at a temperature of 50° C., it appears to yield to the prolonged action of boiling water[2], splitting up with the liberation of carbon dioxide. In the experiments in question the muscles of rabbits were used. They were deprived of blood, finely minced, and then plunged into a large volume of briskly boiling water, which was kept boiling for two or three hours. The carbon dioxide which escaped was absorbed, with every precaution to avoid losses, by means of caustic solutions, and afterwards determined both by weighing the potash bulbs and also by the gasometric analysis of the carbonate formed.

[1] Hermann, *Op. cit.* p. 118. Expt. 14.

[2] R. Stintzing, "Untersuchungen ü. die Mechanik der physiologischen Kohlensäurebildung." Pflüger's *Arch. f. d. ges. Physiol.* Vol. XVIII. 1878, p. 388.

Under these circumstances mammalian muscle yields on prolonged boiling about 100 vols. p. c. (at 0° and 760 mm.) of carbon dioxide. The source of this large volume of gas is not the decomposition of a preexistent compound of it; since, if muscle is well acidified and afterwards washed for *many hours* at an ice-cold temperature before being boiled, the yield of carbon dioxide on boiling is but little less than when acidification is omitted. There is, in short, little doubt that the constituent of mammalian muscle which liberates carbon dioxide on prolonged boiling, is the same as that which is decomposed in tetanus and rigor; for if muscles are tetanized or made rigid, while at the same time opportunity is offered for the escape of the carbon dioxide which is known to be generated in those processes, the yield of dioxide on subsequent boiling is reduced to a mean of 20 or 30 vols. p. c. instead of 100.

Relationship between the gases of rigid and contracting muscle.

If a comparison be made of the carbon dioxide produced during rigor and during tetanus, a very curious relationship will be found between them. Such a comparison should be made with the limbs of the same frog: one limb being passed into rigor by a temporary exposure to 45°C. while still in its skin; the other limb being tetanized frequently during a long interval. After this preparation each limb should be scalded and otherwise made ready for the extraction of its gases. It will be observed that during the induction of rigor by a temperature of 45° there is an opportunity for the escape of gases, which is however diminished as far as possible by preserving the skin, and making the operation as short as may be.

After this experiment it will appear that the rigid muscle contains more carbon dioxide than the tetanized. Similar experiments further shew that tetanized muscles produce less carbon dioxide on passing into rigor than muscles which have not been tetanized previously. Now, the total carbon dioxide set free by the rigor of muscle which has been tetanized is made up of

a. the amount in the muscle at the moment of bleeding it;
b. the amount produced during tetanus, *minus v*, the small amount lost to the air in tetanizing;
c. the amount produced in rigor,

while the total carbon dioxide set free by rigor in an untetanized muscle is made up of

a. the amount in the muscle at the moment of bleeding it;
d. the amount produced in rigor.

But experiment shews that the difference between

$$(a+b-v+c) \text{ and } (a+d)$$

is about 2 per cent. Hence, if we assume that $v=2$ per cent. (and such is not an improbable assumption), then

$$(a+b+c)=(a+d), \text{ and } b+c=d.$$[1]

[1] Hermann, *Op. cit.* p. 26.

Changes in the non-gaseous constituents of Muscle in the states of Activity and Rigor.

1. *Change in Reaction and its causes.*

During rigor the muscle which had been neutral or alkaline becomes acid.

The flesh of dead animals, however *fresh* in the ordinary sense of the word, has an acid reaction. Berzelius[1], who discovered this fact, concluded from his experiments that it was due to the presence in muscle of that acid which his countryman Scheele had separated from sour milk. The lactic acid of muscle was shewn, by the subsequent researches of Engelhardt, Heintz and Strecker, to differ from the common lactic acid produced by fermentation. Liebig, who at first denied the presence of lactic acid in muscle, afterwards based many ingenious hypotheses upon its supposed presence in the muscular tissue during life. All these chemists, because they had discovered lactic acid in the flesh of recently killed animals, concluded that it must have been present during life; for, at that time, the conception had not yet been formed that when a tissue dies processes set in which may give rise to new bodies—products of the decomposition. This conception was due to Du Bois-Reymond. In his papers on the reaction of the muscular tissue, and the changes which it undergoes at death[2], he established the immense importance of distinguishing between a tissue which is yet living, though it may be separated from the living body of which it once formed a part, and one which has ceased to manifest the phenomena which it possessed during life. With the cessation of these phenomena—and in warm-blooded animals that cessation follows so soon upon *somatic* death as to be almost coincident with it—there is a change in physical properties and chemical structure. Thus whilst muscle is alive and in a physiological condition it possesses a neutral reaction; so soon as it dies the reaction becomes acid. This change takes place so rapidly in warm-blooded animals as to render it almost impossible to ascertain the normal reaction; in cold-blooded animals, in which the vitality of the tissues continues long, the acidification goes on so slowly as to permit of its careful study.

It is impossible to over-estimate the importance of these, the first researches which pointed to the subtle differences which may exist, even from a chemical point of view, between living and dead tissues; the conception which guided them and which was securely based upon them, immediately led one of Du Bois-Reymond's pupils, Kühne, to the discovery of the most important points in the chemistry of living muscle; and it has since then so influenced the progress of Physiology that we can scarcely realize how much we owe to it. Our knowledge of the changes which occur in secreting glands in various conditions of functional activity; of the variations in the objective characters of

[1] Berzelius, *Lehrbuch der Chimie*, übersetzt von Wöhler, 4th ed. Vol. IX. p. 569. *Ann. d. Chem. u. Pharm.* Vol. I. p. 1.

[2] Du Bois-Reymond, "Ueber angeblich saure Reaction des Muskelfleisches." *Gesammelte Abhandlungen zur allgemeinen Muskel- u. Nervenphysik.* Leipzig, 1877. Vol. II. p. 3.

the retina, &c., has been gained by researches which prove the value of the conception of Du Bois-Reymond.

Methods of determining the reaction of muscle.

Strips of red and blue litmus paper are pinned alternately in rows to a varnished board, so that the edges of adjoining pieces are in contact. A section of the muscle of which the reaction is to be determined is then pressed firmly over the boundary of two slips. In normal muscle it is then observed that both the red paper assumes a bluish tinge and the blue paper a reddish tint. This so-called *amphichromatic* or *amphoteric* reaction, depends upon the muscle having in reality often a neutral reaction; when this is the case, though it affects both blue and red litmus paper, it does not alter the tint of violet litmus.

When a muscle passes into the state of *rigor mortis* the reaction becomes *ipso facto* decidedly acid, except in cases where the rigor is brought on by plunging the muscle into hot water, when the reaction is found to remain neutral or alkaline.

It would appear that the amount of acid which can be produced in a muscle when it passes into rigor is a definite quantity, doubtless depending upon the quantity of the body which, by decomposing, sets acid free.

Acidification of muscles, removed from the influence of the blood, when they are tetanized.

As was first shewn by Du Bois-Reymond, when a separated muscle is tetanized and its reaction is determined from time to time, it is observed to become more and more acid[1].

Heidenhain shewed that the amount of lactic acid formed during contraction increased with the resistance which the muscle had to overcome[2].

It was shewn by Ranke that in this case as in tetanus there is a maximum amount of acid which can be generated in the muscle which is cut off from the blood-stream, and then tetanized. If two muscles were taken for the determination of the amount of acid formed during *rigor mortis*, but if one were subjected to prolonged tetanus until rigor set in, whilst the other was allowed to remain at rest, the quantity of acid formed in the first case would exceed that formed in the second; there is, therefore, a consumption of acid-yielding substance during tetanus[3].

Cause of the acid reaction of muscle which is in the state of rigor.

The acidity of muscle in the state of rigor or which has been tetanized is chiefly, if not entirely, due to the liberation of lactic acid. In the very earliest stage of rigor it is probable that the acid reaction is really due to an acid potassium phosphate, produced from the alkaline phosphate by the action of lactic acid. Soon however the reaction is acid because of the presence of lactic acid.

[1] Du Bois-Reymond, *Op. cit.* p. 26.

[2] Heidenhain, *Mechanische Leistung*, p. 143 et seq.

[3] In a thesis presented to the University of Bonn on 4th June, 1880, and entitled "Beiträge zur physiologischen Chemie des Muskels," Dr Joseph Warren communicated preliminary observations tending to shew that the amount of lactic acid which can be obtained from muscles which have been tetanized is smaller than is yielded by similar muscles which have been maintained in a state of rest.

The isomeric Lactic Acids.

At least three acids are known to chemists which have the composition expressed by the formula $C_3H_6O_3$. These acids all agree in being syrupy, colourless, liquids of strongly acid reaction, soluble in water, alcohol and ether, and yielding when heated first *lactic anhydride* ($C_6H_{10}O_5$) and afterwards *lactide* ($C_3H_4O_2$). Though possessed of many common characters, a careful examination of their behaviour to polarized light, of the crystalline form and the amount of water of crystallization of their salts, and of their products of decomposition, has clearly established the existence of three perfectly distinct lactic acids.

These three acids are (1) sarcolactic or paralactic acid, the chief acid of dead muscle: (2) ordinary lactic acid: (3) ethylene-lactic acid. The two first of these acids appear to possess the same chemical constitution, and they may be spoken of as ethylidene lactic acids; still they exhibit certain well-marked differences, the first being, for instance, dextrogyrous whilst the second is optically inactive, and the salts of the two acids differing in the amount of their water of crystallization, and in their solubility.

a. *Sarcolactic Acid.* (*Optically active ethylidene lactic acid.*)

Preparation of lactic acids from muscle.

1. Liebig's extract of meat is dissolved in four parts of warm water and 8 parts of 90 per cent. alcohol are then added to the liquid which is continually stirred. The mixture is allowed to stand until the insoluble matter has subsided and a clear supernatant liquid is obtained, and the latter is then separated by decantation. In order to separate any lactic acid from the insoluble residue, the latter is mixed with twice its weight of warm water, and then precipitated with four or five times its volume of alcohol. The alcoholic fluids obtained by these two operations are then evaporated on the water-bath to the consistence of a thin syrup, and the latter is again precipitated by the addition of three or four times its volume of alcohol; the insoluble matter may be kept for the preparation of creatine, hypoxanthine, &c. The alcoholic solution is now evaporated to dryness, the residue is mixed with water, some dilute sulphuric acid added, and then shaken up several times with ether. On evaporating this liquid, a residue is obtained which consists of a mixture of sarcolactic and ethylene-lactic acids.

2. Instead of employing the above method, the syrupy liquid from which creatine has crystallized, in Liebig's method of preparing creatine, is acidulated with sulphuric acid and then shaken with ether, and the ether evaporated.

Separation of sarcolactic from ethylene-lactic acid.

Having obtained a mixture of the two acids, their separation is effected by converting them into zinc salts, and the separation of the two salts is based upon their different solubility in alcohol. With this object, the mixture of

raw acids is dissolved in water and boiled with suspended zinc carbonate or zinc oxide; the clear liquid is separated by filtration from the insoluble zinc compounds, and then evaporated until crystals commence to form. The liquid is now treated with four or five times its volume of 90 per cent. alcohol; after some time the liquid becomes turbid and deposits needle-shaped crystals, consisting of zinc sarcolactate; the ethylene-lactate, being far more soluble in alcohol, remains in solution. The crystals of the former body are then collected on a filter, washed with cold absolute alcohol; and they may with advantage be re-crystallized. From the zinc compound sarcolactic acid may be obtained by dissolving the salt in water, decomposing by means of sulphuretted hydrogen, filtering the solution, concentrating, shaking with ether, and then evaporating the ethereal solution, when the pure acid is left.

Properties of sarcolactic acid and its compounds.

Sarcolactic acid is distinguished from the two other varieties of lactic acid by its property of deviating the plane of polarization to the right. The *specific rotatory* power is greatest immediately after the acid is dissolved; it then sinks rapidly, and afterwards slowly rises again, without however again reaching its initial value. It is worthy of note that whilst free sarcolactic acid is *dextrogyrous*, its salts are *laevogyrous*.

Zinc sarcolactate has the composition $Zn(C_3H_5O_3)_2 + 2H_2O$. When heated for half an hour at 100° C. it loses the whole of its water of crystallization (12·9 per cent.). It is soluble in 17·5 parts of water at 14—15° C. It is almost insoluble in absolute alcohol, requiring 1000 parts of boiling absolute alcohol for solution.

The specific rotation of this salt is $= -7°·6$.

Calcium sarcolactate, $2[Ca(C_3H_5O_3)_2] + 9H_2O$, crystallizes in the form of tufts of microscopic needles. The specific rotation of the salt is $= -3°·8$.

b. *Ordinary Lactic Acid.* (*Optically inactive ethylidene lactic acid.*)

This acid is perhaps not present in acid muscle, though it has been stated to be so by Heintz. Its quantity is at least inferior to that of the other isomeric lactic acids.

Preparation. This acid is formed when saccharine liquids ferment in the presence of certain decomposing matters of animal origin (Milk, Cheese), which serve as vehicles for a peculiar organized ferment; to the fermentation thus induced the term *lactic acid fermentation* is applied. For this reason this variety of lactic acid is often designated 'lactic acid of fermentation.' For the details of the methods for preparing this variety of lactic acid the reader is referred to any systematic work on organic chemistry.

Properties of inactive ethylidene lactic acid and its compounds.

The acid resembles sarcolactic acid except in its not possessing the power of rotating the plane of polarization. Its salts differ in crystalline form and in the amount of water of crystallization which they contain, from those of sarcolactic acid.

Zinc lactate has the composition $Zn(C_3H_5O_3)_2 + 3H_2O$. When heated in the water-bath for half an hour it loses all its water

of crystallization (18·178 per cent.). It is insoluble in alcohol; but soluble in 6 parts of boiling water and 58—63 parts of water at 14°—15° C.

Derivatives of the ethylidene lactic acids. Both sarcolactic acid and the acid product of fermentation yield exactly the same derivatives or products of decomposition when subjected to identical processes, so that we are justified in considering them to have the same chemical constitution.

When heated for some hours in the water-bath, the lactic acids yield the body termed lactic anhydride, $C_6H_{10}O_5$; when heated at a higher temperature, 140° or 150°, lactide, $C_3H_4O_2$, is formed.

When oxidized with dilute chromic acid the ethylidene lactic acids yield acetic and formic acids *but no malonic* acid.

Synthesis of inactive ethylidene lactic acid. Many synthetic processes are known which yield the inactive ethylidene lactic acid of fermentation; the most instructive, as bearing upon the constitution of the acid, consists in heating ethyloxycyanide of ethylidene $\begin{pmatrix} C_2H_4'' \\ C_2H_5 \end{pmatrix}\begin{matrix} CN \\ O \end{matrix}\Big)$ with aqueous solutions of the alkalies, when ammonia and common lactic acid and a small quantity of ethylene-lactic acid are formed.

c. *Ethylene-lactic Acid.*

This acid undoubtedly accompanies optically active ethylidene lactic acid in the juice of flesh.

Preparation. This has been described in part under 'sarcolactic acid.' The alcoholic fluid from which sarcolactate of zinc has separated contains ethylene-lactate of zinc, which may be obtained from it by evaporation. From the zinc compound, the free acid may be liberated, by following precisely the same process as was recommended for the separation of sarcolactic acid.

Properties. Is optically inactive.

Its zinc salt has the same composition as that of sarcolactic acid, losing when heated in the water-bath 12·9 per cent. of its weight. The zinc salt, unlike that of the isomeric lactic acid, is exceedingly soluble in water; it is also much more soluble in alcohol.

Products of oxidation. When oxidized by means of chromic acid, ethylene-lactic acid yields malonic acid ($C_3H_4O_4$).

Synthesis. By heating ethylene chlorhydrate with potassium cyanide the nitrile of ethylene-lactic acid is formed; thus

$$\underbrace{\begin{matrix} CH_2Cl \\ | \\ CH_2OH \end{matrix}}_{\text{Ethylene Chlorhydrate.}} + \underbrace{\begin{matrix} CN \\ K \end{matrix}\Big\}}_{\text{Potassium cyanide.}} = \underbrace{\begin{matrix} CH_2 . CN \\ | \\ CH_2 . OH \end{matrix}}_{\text{Cyanhydrin.}} + \underbrace{\begin{matrix} Cl \\ K \end{matrix}\Big\}}_{\text{Potassium chloride.}}$$

When cyanhydrin is boiled with caustic potash, the potassium salt of ethylene-lactic acid is obtained; thus:

$$\underbrace{\begin{matrix}CH_2.CN\\|\\CH_2.OH\end{matrix}}_{\text{Cyanhydrin.}} + \underbrace{KHO + H_2O}_{\text{Potassium hydrate.}} = \underbrace{\begin{matrix}CH_2.COOK\\|\\CH_2.OH\end{matrix}}_{\text{Potassium ethylene-lactate.}} + \underbrace{NH_3}_{\text{Ammonia.}}$$

2. *Changes in the proportion of Water.*

According to Ranke[1] the amount of solid matter in muscles undergoes diminution when muscles are tetanized, so that there appears to be a relative increase of water. Further, according to this author, the proportion of water in muscle is inversely proportional to its power of doing work.

3. *Changes in the water and alcohol extractives.*

It was first shewn by Helmholtz[2] that when muscles are tetanized they yield a smaller quantity of matters soluble in water (*water-extractives*), but a larger quantity of *alcohol-extractives* than before. This has been fully confirmed by Ranke[3].

Heidenhain and his pupils Nigetiet and Hefner[4] have more recently shewn that as the resistance which an active muscle has to overcome increases, the amount of the alcoholic extract increases and that of the aqueous extract decreases.

4. *Changes in the Proteids.*

The total nitrogen of resting and tetanized muscle was found by Ranke to be the same (about 14·4 p. c.). Ranke[5] fancied that he had made out that the preparation of proteids removable by water diminishes in the tetanized muscle. We may fairly say that no trustworthy experimental results exist to prove that the proteids of muscle undergo changes during activity.

5. *Changes in the amounts of Creatin.*

According to Sarokin[6] the amount of creatin in muscle is the same whether it be in a state of rest or activity; according to this author a large production of creatinine occurs, however, during tetanus.

[1] Ranke, *Tetanus*, Chap. II. p. 63 ("Der Wassergehalt des Muskels").

[2] Helmholtz, "Ueber den Stoffverbrauch bei der Muskelaction." *Arch. f. Anat. u. Phys.* 1845, p. 72.

[3] Ranke, *Tetanus*, p. 141.

[4] Heidenhain, Nigetiet und Hefner. "Versuche über die Abhängigkeit des Stoffumsatzes in den thätigen Muskeln von ihrer Spannung." Pflüger's *Archiv*, Vol. III. (1870) p. 574.

[5] Ranke, *Tetanus*, p. 119. See also Nawrocki, *Centralblatt*, 1866, p. 385.

[6] Sarokin, Virchow's *Archiv*, Vol. XXVIII.

This statement has been contradicted by Nawrocki[1]. Voit has found the creatine to be diminished by activity[2].

6. *Changes in the proportion of Glycogen and Sugar.*

As was first pointed out by Ranke, during tetanus sugar is produced in muscle. At the same time there is a diminution in the amount of glycogen (Nasse[3]). It is quite unknown whether the sugar is produced at the expense of the glycogen.

7. *Changes in the amount of Fat and volatile fatty acids during activity.*

Ranke[4] thought he had established that the quantity of fat increases in muscle during activity, but the conclusion is probably not warranted by the experimental data[5]. The same remark applies to the statement of Sczelkow[6] that during tetanus there is a diminution of the volatile fatty acids contained in muscle[5].

8. *Oxidizing and reducing properties of Muscle during rest and tetanus.*

Grützner shewed[7] that whilst resting muscle is able to oxidize pyrogallic acid, muscle which has been tetanized fails to do so. He further shewed that solutions of sulphate of indigo undergo a change of colour when circulating through tetanized muscle, which is of such a kind as to point to the production of reducing substances[7]. Gscheidlen[8] has further shewn that during activity nitrates are converted into nitrites.

B. *Changes in the chemical composition of the medium surrounding muscle.*

a. When muscle is exposed to the air.

'Respiration' of muscle: methods of studying it. G. Liebig.

The study of 'muscular respiration,' or the study of the physiological processes of muscle by examining the air to which muscle is exposed, seems to have first been systematically practised by George Liebig[9] in 1850. His method was the simple one of enclosing the muscles in a tube inverted over a surface

[1] Nawrocki, *Centralblatt*, 1865, p. 417.
[2] Voit, *Zeitschr. f. Biol.* IV. 1868 (p. 77).
[3] Nasse, Pflüger's *Archiv*, Vol. II. (1869) p. 97.
[4] Ranke, *Tetanus*, p. 190.
[5] See Hermann's criticism, *Untersuchungen über den Stoffwechsel*, &c., p. 86 and 87.
[6] Sczelkow, "Die flüchtigen Fettsäuren des Muskels und ihre Veränderung während des Muskeltetanus." *Archiv f. Anat. u. Phys.* 1864, p. 672.
[7] Grützner, "Ueber einige chemische Reactionen der thätigen und unthätigen Muskeln." Pflüger's *Archiv*, Vol. VII. (1873) p. 255.
[8] Gscheidlen, "Ueber das Reductionsvermögen des thätigen Muskels." Pflüger's *Archiv*, Vol. VIII. (1873) p. 506.
[9] Georg Liebig, "Ueber die Respiration der Muskeln." *Archiv für Anat. Phys. u. wiss. Med.* (J. Müller), 1850, p. 393. The method was first suggested by Du Bois-Reymond.

of mercury. On the top of the mercury in the tube, floated a caustic solution to absorb the carbonic anhydride. A rise of the surface of mercury betokened absorption of oxygen; for Liebig paid no regard to the nitrogen, and assumed that the oxygen and the carbonic anhydride were interchanged volume for volume. He stated that excised frog-muscles, whether bloodless or unbled, on exposure to an atmosphere of common air or of oxygen, absorb oxygen and excrete carbonic anhydride. He made out also that the excretion of carbon dioxide may occur into an atmosphere containing no oxygen[1].

Valentin. After Liebig, in 1855, Valentin[2] took up the same question of the influence of excised muscle on its surrounding medium, with a view to discover differences of composition between irritable and non-irritable muscle. The muscular hind limbs of frogs were exposed to air in closed tubes for 1—6 days, and the air examined at intervals. Irritability was abolished in various ways, as by spontaneous death, by subjection to high temperatures, or by beating to death; and comparisons were established between the gaseous exchanges of normal muscle, of non-irritable muscle, and of various tissues, such as the skin and bones of the frog's body. He discovered that other organs besides muscle abstract oxygen and excrete carbonic anhydride; and, which was more important, that the gaseous exchanges of muscle continue uninterruptedly after the death of the muscle. In a word, not only living muscle, but skin and even dead muscle have a 'respiration.' The gaseous exchanges of dead muscle are indeed different from those of the yet living; and the kind of exchange which is characteristic of the dead state is established as soon as ever death of the muscle intervenes, whether it be suddenly induced or whether it be reached by a protracted decline. Active muscle does not appreciably affect the nitrogen of the surrounding air; but after irritability is lost, nitrogen escapes from the muscle. This difference in the gaseous exchanges of the living and dead state was considered by Valentin to betray some difference of constitution so subtle as to escape chemical analysis or electrical tests.

Matteucci. One year later, in 1856, Matteucci published a paper in which the same subject of muscular respiration is treated of[3]; but especially the respiration of muscles during contraction. He divested frogs of their skin, took the hinder extremities and freed them roughly from blood with filter-paper, and arranged them in a closed air-space over mercury, for electrical stimulation. The air-space measured about 70—80 c.cm. Stimulation was carried on at intervals so as not to fatigue the muscles.

[1] *Op. cit.* p. 408.

[2] G. Valentin, "Ueber die Wechselwirkung der Muskeln und der sie umgebenden Atmosphäre." *Arch. f. physiol. Heilkunde*, 14th year, 1855, p. 431. His methods of analysis are explained in Valentin's treatise on Physiology.

[3] Ch. Matteucci, "Recherches sur les phénomènes physiques et chimiques de la contraction musculaire." *Ann. de chimie et de physique*, 3 série, Vol. XLVII. 1856, p. 129.

The air was analysed by absorbing the carbon dioxide with caustic solutions and the oxygen with phosphorus. He found that muscle, whether at rest or in contraction, caused a diminution of oxygen and an increase of carbon dioxide, and usually of nitrogen also, in the surrounding air—changes which were greater during contraction than during repose of the muscle. The oxygen absorbed was greater than the carbon dioxide exhaled. He exposed muscles to a vacuum, then to pure hydrogen for two or three hours, then to an exhausted receiver, which was subsequently filled with pure hydrogen. Notwithstanding this careful removal of oxygen from about the muscles, carbon dioxide was yielded up by them, especially on stimulation. Hence Matteucci concluded that the oxygen which, in muscular respiration, helps to form the carbon dioxide is not the oxygen of the air, but oxygen which exists in muscle in a state of chemical combination[1].

Valentin.

In 1857 Valentin published researches on the effect of contracting frog-muscles upon the atmosphere[2]. His apparatus consisted of a glass cylinder, *abcd*, 2 decimetres high and between 2 and 3 centimetres bore. The bottom was closed by an iron plate, *f*, with a hook, *e*, externally for the attachment of a battery wire. The top was provided with a short iron flange, *gh*, which supported an iron plate or lid, *ik*, capable of being hermetically fixed to the cylinder by means of an interposed washer and screws. This lid was provided with an exit-pipe, *x*, guarded by a stopcock, as well as with a thermometer, *l*, and a gauge, *nrt*, open to the air, all being securely fixed in an air-tight fashion.

In order to determine exactly the volume of air in the cylinder after everything had been arranged for an experiment, the following preliminary calculations were made.

Let v = cubic contents of the glass cylinder and the proximal limb of the gauge when all is screwed up and the gauge stands at zero (*qr*, Fig. 58).

Let h be the height of mercury which must be poured into the distal limb of the gauge to raise the mercury in the proximal limb up to *o*: *i.e.* to diminish the volume v by the volume between *q* and *o*, or μ.

Then, if b equals the barometric pressure, it follows that

$$v : (v - \mu) = (b + h) : b,$$

$$v = \mu \cdot \frac{b + h}{h},$$

$$= \mu \left(\frac{b}{h} + 1\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(i)}.$$

Now suppose that a volume, p, of mercury be poured into the glass cylinder to the level *vw*, before the lid is screwed up; and let the gauge again stand

[1] Matteucci, *Op. cit.* p. 138.

[2] G. Valentin, "Die Wirkung der zusammengezogenen Muskeln auf die sie umgebenden Luftmassen." *Arch. f. physiol. Heilkunde.* New Series, Vol. I. 1857, p. 283.

at zero. The cubic contents of the cylinder and proximal limb of the gauge now $=(v-p)$. And let h' be the height of mercury which must be

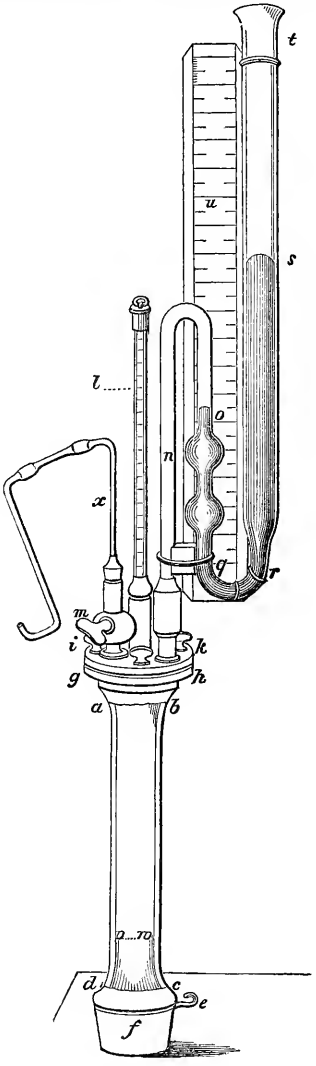


FIG. 58. APPARATUS FOR DEMONSTRATING THE RESPIRATORY EXCHANGES OF LIVING MUSCLE. (VALENTIN.)

introduced into the distal limb of the gauge to reduce the new volume $(v-p)$ by the same volume μ; *i.e.* to raise the proximal mercury again from q to o.

Then, by (i),

$$v = \mu\left(\frac{b}{h} + 1\right) \quad \text{.....(ii),}$$

and

$$(v - p) = \mu\left(\frac{b}{h'} + 1\right) \quad \text{..... (iii);}$$

whence

$$\mu = \frac{p}{b\left(\frac{1}{h} - \frac{1}{h'}\right)} \quad \text{..... (iv).}$$

This gives the value of μ, which is the distance between two fixed points q and o of the proximal limb of the manometer.

Having determined μ carefully, once for all, it is clearly easy to determine the volume of air under observation, diminished as it is by a muscle preparation or any apparatus of unknown volume which may, in an experiment, be introduced into the cylinder. It is only necessary to ascertain the height of mercury which must be poured into the distal limb to raise the proximal surface of mercury from q to o, and apply the formula

$$V \text{ (the unknown volume)} = \mu \cdot \left(\frac{b}{h} + 1\right).$$

By means of this apparatus Valentin was able to confirm Matteucci's statements; but he pointed out that comparable results could only be obtained by employing small preparations and by restricting the experiment to the first half-hour. He found that the relative amount of absorbed oxygen, which Matteucci had discovered to be greater than the exhaled carbonic anhydride, became less during contraction: that is to say, during contraction *more* carbonic anhydride is exhaled than oxygen is absorbed. He noticed also that muscles which have been fatigued by prolonged tetanus yield more carbon dioxide and absorb more oxygen absolutely (though less relatively to the carbon dioxide) than sound and vigorous muscles; and this he thought to be due to an enfeebled power of resisting the disintegrating action of the atmosphere, owing to some altered constitution of the tissue which is characteristic of the state of exhaustion.

To Valentin, therefore, belongs the credit of pointing out that the so-called 'respiration' of muscular tissue is in part a phenomenon of putrefaction; but it was Hermann who clearly enunciated this fact and perfectly discriminated the living and the dead factors in the process.

Hermann. The method adopted by Hermann was extremely simple[1]: The muscle-preparation was suspended by a platinum wire in a wide absorption tube, which was then inverted over a mercury bath in such a manner that the open mouth dipped below the surface, and the mercury stood at the same level outside and inside the tube. The tube thus enclosed an unmeasured volume of air at

[1] Hermann, *Stoffwechsel der Muskeln*, p. 32.

the atmospheric pressure, together with the muscle, and usually a little moisture floating on the surface of the mercury; while the muscle was kept within reach by means of the attached wire. If stimulation of the muscle were desired, it is easy to see how it would be accomplished. At the close of the experiment the muscle was dragged down through the mercury, an operation which was found to be attended by no loss of gases whatever. The gases left in the tube were then passed into several dry absorption tubes in succession, in order to get rid of the moisture on the surface of the mercury; and finally they were ready for analysis. The carbon dioxide was directly absorbed by caustics; the oxygen was exploded, and the nitrogen read off. The oxygen of the original air was estimated from the nitrogen left behind. All deviations from this in the final analysis were considered to be due to the absorption of oxygen by the muscles during the experiment. The method is open to three trivial objections: The small amount of carbon dioxide normally present in the air (4 parts in 10,000) is neglected; the atmosphere is not *absolutely* constant in its composition as regards oxygen and nitrogen; muscle itself yields up nitrogen on exposure, but only in excessively small amounts, and during the earlier parts of an experiment (Hermann).

Resting muscles absorb O.

In this apparatus, by comparing during 15 or 20 hours the gaseous exchanges of bloodless muscles, some of which were rigid while others were still living, it appeared that the absorption of oxygen is practically equal in the case of both living and rigid muscles; and therefore is probably not connected with the functional mechanisms of the tissue. It is, in short,

Absorption of O is in part putrefactive.

dependent upon a process of putrefaction which is accelerated according to the extent of muscular surface exposed to the action of the air, and which begins at a very early period of exposure.

And in part physiological.

But while the absorption of oxygen, as determined by an analysis of the surrounding gaseous medium, cannot be ascribed to any but putrefactive causes, we must hasten to explain that other and more delicate tests would lead us to infer an extremely slight but constant employment of oxygen which is truly *physiological*.

Influence of medium upon irritability of muscle.

As early as 1795 Humboldt[1] observed that muscles preserved their irritability longer in oxygen than in air or gases containing no oxygen—an observation which has since been confirmed by Georg Liebig[2] and others, and with especial accuracy by Prof. Hermann. In Hermann's experiments a muscle was suspended in an absorption-tube by means

[1] Al. Humboldt, *Versuche über die gereizte Muskel- und Nerven-faser*, 1797, Vol. II. p. 282.

[2] Georg Liebig, *Op. cit.*

of platinum wires melted through the sides of the tube, the wires being adapted to an electrical apparatus for stimulation. The absorption-tube was filled with salt solution after the muscle had been fixed within it; and then inverted over a vessel containing a lower stratum of mercury and an upper one of salt solution, in such a manner that the open mouth of the tube passed through the upper layer to the mercury. A bent tube proceeding from a reservoir of electrolytic hydrogen, or pure nitrogen, or air, or detonating gas, served to introduce the gas into the absorption-tube, and at the same time to drive out the salt solution. Muscles of different degrees of thickness were kept under observation; but the sartorius of the frog was found to be peculiarly favourable for these experiments. The results were not modified by previously curarizing the muscles.

Tested in this manner, it appeared that the thickness of the muscles had a singular influence on the result. The sartorius, of large surface and small bulk, lived longer in hydrogen than in gases containing oxygen; while thicker muscles agreed with the muscles observed by Humboldt, in retaining their irritability longer in oxygen. Another form of this experiment led to the same conclusions. If muscles were enclosed in tubes which were then made as vacuous as possible, until nothing remained in them but traces of carbon dioxide, the sartorius was found to live longer in the vacuum, in the absence of oxygen, than in air; while thicker muscles lost their irritability sooner in the vacuum. It should be noticed that all muscles exhibit an exalted irritability when first the vacuum is produced.

This influence of oxygen upon the irritability of thick and thin muscles seems to admit of but one explanation. There are two concurrent processes in muscles exposed to the air, in which oxygen plays a part: one tends to destroy, the other to preserve irritability. The former is, beyond doubt, the putrefactive process already demonstrated in living and rigid muscle, which spreads the more rapidly the greater the surface exposed. Hence in the thin sartorius the process invades all parts of the tissue within a short time, and death results: to defend the muscle from oxygen is to preserve it alive. On the other hand, the second process implicating oxygen is a true physiological process of revival. In the thicker kinds of muscles, whose internal mass is long shielded from the putrefactive action of oxygen, this process of revival is a marked benefit; and hence the muscle removed from the influence of oxygen by enclosure in a vacuum more rapidly becomes enfeebled than one exposed to the air.

Of the nature of this functional absorption of oxygen and process of revival we have as yet no exact conception. The process itself is but of small value in prolonging the normal irritability of frog-muscles exposed to the air and of no appreciable moment in the function of contraction; but, as will be hereafter explained, it is extremely potent in restoring irritability to mammalian muscles exhausted by interruption of their blood current (p. 380). It is

probably not effected by hæmoglobin but by the tissue juices; for many invertebrates have no hæmoglobin which yet have muscles not essentially different from those of the frog. It may be simply that the presence of oxygen assists the escape of the deleterious carbon dioxide better than hydrogen or nitrogen, as was found to be the case with the gases of the blood by Ludwig[1].

It may, therefore, be said respecting the oxygen of the surrounding medium, that while an appreciable amount is abstracted and absorbed in the inevitable putrefaction of exposed muscle, a small portion, altogether too slight to affect a gas-analysis, is taken up to preserve irritability.

Resting muscles exhale CO_2.

The atmosphere surrounding exposed muscles, besides losing oxygen, suffers an increase of its carbon dioxide. As was pointed out by Valentin, this is not wholly an exchange of a functional character; but is common to living and dead muscular tissue. In other words, it is one of the early phenomena of putrefaction, together with the absorption of oxygen. But a comparison of the exhalations of living muscle and of muscle made rigid, discloses that less carbon dioxide escapes from the normal, on mere exposure, than from the rigid. Since putrefaction is more, and not less, rapid in normal muscle than in muscle made rigid by heat (the method of inducing rigor usually adopted in these experiments), it is clear that putrefactive changes cannot be called in to explain this difference. There can be little doubt that it is due to the increased amount of carbon dioxide which rigor is known to generate in muscle (*vide supra*). The amount of carbon dioxide given off is very irregular and has no relation to the oxygen at the same time absorbed.

Contracting muscles absorb more O and exhale more CO_2 than resting muscles.

Hitherto we have considered the case of muscles in repose; and we have come to the conclusion that, apart from an inappreciable quantity of oxygen, absorbed or otherwise employed, the exchanges of the 'respiration' of exposed muscle are not functional, but putrefactive, and are shared alike by muscle, skin, and other tissues. When we turn to the case of muscles in tetanus we find that the gaseous exchanges have a greater value, and especially as regards the carbon dioxide excreted. The increase in the amount of oxygen absorbed is indeed slight, and is due to the agitation of the tissue during tetanus; for if the air about an unstimulated muscle be mechanically kept in motion, a similar increase of the oxygen absorbed is found to occur. It is not due to any increased activity of the putrefactive processes brought about in the passage of the electrical currents, since such currents have no influence over the putrefactive absorption of oxygen by rigid muscles. The more remarkable increase of the carbon

[1] Hermann, *Op. cit.* p. 52.

dioxide exhaled is due, in the first place, to the increased production of it within the muscle during tetanus[1]; and in the second to the increased facility for its escape offered in the agitation of the muscle.

The following experimental figures will serve to illustrate the extent of the gaseous exchanges of muscle[2].

Experiment. Comparison of gaseous exchanges of living muscle, and muscle made rigid by exposure to a temperature of 45° C.

Duration of experiment 19 h. 15 m. Temp. 14—17° C. Gases estimated at 0° and 1 mtr.

1. Living muscle = 7·352 grms. = 6·948 c.cm.
2. Rigid muscle = 7·631 grms. = 7·213 c.cm.

	Oxygen absorbed.		Carbon dioxide exhaled.	
	c.cm.	p. c.	c.cm.	p. c.
Living muscle (6·948 c.cm.)	1·277	18·37	0·605	8·70
Rigid muscle (7·213 c.cm.)	1·127	15·62	1·184	16·41

Experiment. Comparison of the gaseous exchanges of muscle in repose and in tetanus.

Tetanus induced at intervals during the experiment.

Duration of the experiment 3 h. 10 m. Temp. 15—16° C. Gases estimated at 0° and 1 mtr.

1. Resting muscle = 9·468 grms. = 8·949 c.cm.
2. Tetanized muscle = 9·480 grms. = 8·960 c.cm.

	Oxygen absorbed.		Carbon dioxide exhaled.	
	c.cm.	p. c.	c.cm.	p. c.
Resting muscle (8·949 c.cm.)	0·548	6·12	0·128	1·43
Tetanized muscle (8·960 c.cm.)	0·746	8·33	0·836	9·33

B. *Changes in the chemical composition of the medium surrounding muscle.*

β. When muscle is still in the body.

In the previous section, we have discussed the so-called 'respiration' of muscles removed from the body, or the gaseous exchanges between excised muscles and their surrounding medium;

[1] See the preceding section.

[2] Hermann, *Op. cit.* pp. 123, 125: Expts. 23 and 27.

and we have tacitly assumed that the medium in question is the air. This is not strictly true. The medium enclosing the elementary parts of contractile substance consists of the tissue juices, which come into contact with the air only at the surface of the muscle: the tissue juices mediate between the muscular substance and the air. Now the fluid which bears to muscle within the body the same relation which air has to excised muscle, is the blood; and as in the former case, the tissue juices are those which deal directly with the muscle-substance, mediating between this and the blood. By the ramification of blood-capillaries between the fibres of muscle, the opposed surfaces of the muscle and its medium become enormously more extended than in the case of air; and by how much the more extensively the muscle is presented to the medium, by so much the more readily will exchanges be effected. This is a circumstance favourable to exchanges between muscle and blood apart from any peculiar fitness or endowment of blood for the work of exchange, which are matters for discussion in the Chapters on Respiration.

In consequence of the organization of the body, and the necessity which it is under of preserving a normal standard or equilibrium, there are two methods of determining the influence which muscles exert upon the blood. We may, in the first place, contrast the blood flowing to and that flowing from the muscle, while the muscle is left in repose, or is thrown into activity. This is the direct method, and is equivalent to exposing muscles to an atmosphere of known constitution and afterwards analysing the atmosphere. In the second place, we may observe the changes of the general ingesta and excreta of the body which are brought about when muscle is converted from one state to the other. This is essentially an indirect method. Hitherto it has only been employed to ascertain the chemical processes of muscle on passing from the state of rest to that of activity, when the *same* animal is compared, in respect of its ingesta and excreta, during repose and during exertion. But a simultaneous comparison of the daily food and excreta on the one hand, and the proportion of muscular and non-muscular elements of the body on the other, in *different kinds* of animals enjoying the same conditions of rest, might be employed to ascertain the normal exchanges between muscle and its medium when the former is at rest.

It is only necessary here to point out in general terms the uncertainty of the indirect method of analysis. Everything which is given up by muscle to blood is not of necessity given up by the blood to the general excreta. The method of excretion is only one of the means employed by the body to preserve its equilibrium. Some part of the substances cast by muscle into the blood may be appropriated by other organs or tissues, and never appear at the surface of the body; and some part of the substances excreted, though brought to the surface during muscular activity, may not have arisen within the active muscle.

Changes of the medium surrounding muscle as shewn in an analysis of the blood of muscle.

Analysis of the blood flowing to and from muscle. Analysis of the gases by the method of Ludwig and Sczelkow.

The general nature of the exchanges between muscle and blood has been long known or inferred from the physical character of the blood flowing out of the muscles. This blood is defined in general terms as venous; and the distinction between arterial and venous blood is one of the most obvious and interesting problems of the physiologist. But it is to Professor Ludwig that we owe the first accurate examination and account of the exchanges. Assisted by Sczelkow[1], and subsequently by A. Schmidt[2], he determined by means of the air-pump the composition of the inflowing and outflowing blood of mammalian muscle. The blood was collected from the muscles with as little disturbance to the general circulation as possible, by the following means. A cannula was inserted into the *femoral vein*, below the opening of the *vena profunda*, with its mouth towards the heart, and a loop of ligature was slipped beneath the *femoral vein* above the opening of the *profunda*. When the ligature was tightened, the normal current of blood from the *profunda* into the *femoral* towards the heart was at once turned from its course and flowed without obstruction through the cannula; when it was slackened again, the current at once resumed its original channel, without the tension having for a moment been raised. In this manner a supply of venous blood from resting muscle was obtained. Arterial blood was at the same time drawn through a cannula in the carotid artery. If it were desired to stimulate the muscles of the leg, electrodes tipped with moistened sponges were applied, one in the inguinal hollow and the other behind the sacrum, opposite the origin of the sciatic plexus. The extraction of the gases was at once undertaken in a Ludwig's blood-pump. As a rule the bloods were examined in the following order: (1) the arterial blood; (2) the venous blood from stimulated muscle; (3) the venous blood from resting muscles. Very frequently the examination of the last had to be postponed until the following day; sometimes both the second and third kinds were examined on the day after their withdrawal. In this case the blood was kept, surrounded by ice, in the tube into which it had been drawn.

The analysis of the gases was made by Bunsen's method.

On examination it was found that:

1. The colour of venous blood from active muscle is sometimes brighter and sometimes darker than the colour of venous blood from

[1] Sczelkow, "Zur Lehre von Gasumtausch in verschiedenen Organen:" presented by Prof. Ludwig. *Sitzungsber. d. k. Akad. Wien*, Vol. XLV. Abth. i. 1862. Second series of experiments.

[2] A. Schmidt, "Das Verhalten der Gase, welche mit dem Blut durch den reizbaren Säugethiermuskel strömen." *Sitzungsber. der math.-phys. Classe der k. s. Gesellsch. der Wissensch.* Vol. XX. p. 12. See also *Arbeiten aus der physiol. Anstalt zu Leipzig*, 3rd year, 1868 (Leipzig, 1869), p. 1.

muscle in repose. It may be brighter even when the blood contains less oxygen.

2. Blood streams more rapidly out of contracting, than out of resting, muscle.

3. Taking arterial blood as the standard, the following table represents the condition of venous blood from resting and from active muscle.

Venous blood :—	O, *less* than arterial blood.	CO_2, *more* than arterial blood.
of resting muscle	9 p. c.	6·71 p. c.
of active muscle	12·26 p. c.	10·79 p. c.

Since blood streams more rapidly from active muscle than from muscle at rest, these differences of the blood in the two cases are really much more considerable than the table shews; since in a given interval of time more blood, with its reduced oxygen and increased carbon dioxide, flows from active than from passive muscle.

4. If Q represent the numerical relation between the increase of carbon dioxide and the decrease of oxygen as blood is converted from the arterial into the venous state; that is to say,

$$\text{if } Q = \frac{\text{difference between } CO_2 \text{ of arterial and venous blood}}{\text{difference between O of arterial and venous blood}};$$

then this quotient Q is found in most instances to be greater during contraction of muscle than during repose. This might be due to the fact that, in contraction, more carbon dioxide is generated for every volume of oxygen absorbed, than in repose; but since the precise seat of the production of carbon dioxide is as yet but a matter of hypothesis, we cannot at once draw this conclusion from the above experiments. It may be merely that the forces which determine the *diffusion* of carbon dioxide and oxygen respectively, are differently affected by the condition of contraction; whence the change in the relationship Q would be brought about, not by an increased generation of carbon dioxide, but by an increased elimination.

Method of Ludwig and A. Schmidt.

The method of experiment which has just been described is not free from objection. The uncontrollable changes of the blood current in the course of an experiment introduce a variable element which deprives the results of all exact quantitative value. To meet this objection, and to obtain results which should be strictly comparable, Ludwig and Alex. Schmidt[1] devised a method of investigating the changes which

[1] Ludwig and A. Schmidt, *loc. cit.*

defibrinated blood undergoes as it is artificially forced in a constant stream through separated though still living muscles. For this purpose the biceps and semitendinosus muscles of the dog's hind-limb may be employed. These muscles are supplied with blood by a branch of the hypogastric artery, and by three or four branches indirectly from the

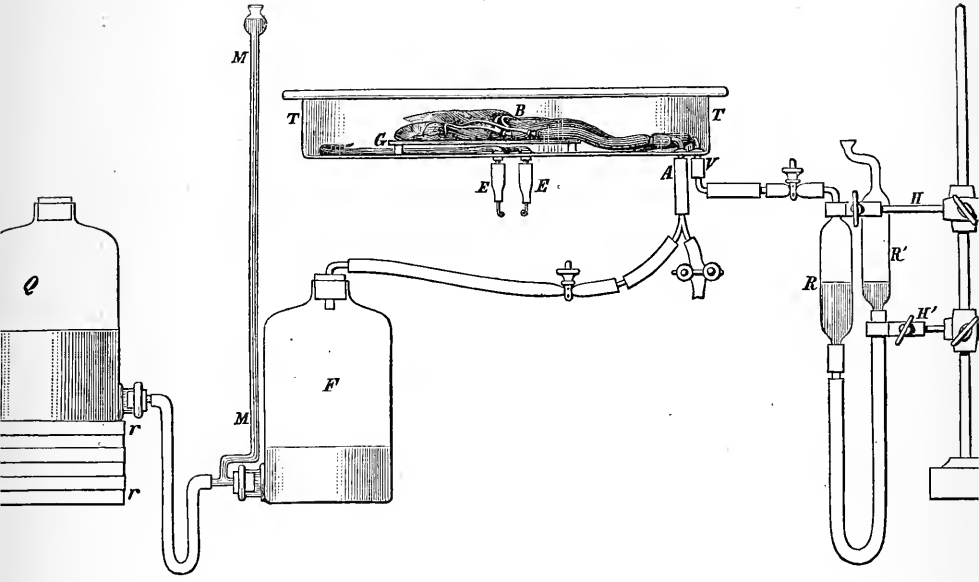


FIG. 59. APPARATUS OF LUDWIG AND A. SCHMIDT.

TT is a glass vessel containing the muscles *B*, resting upon a support *G*; the upper edge is ground, and smeared with grease, to permit the hermetical closure of the vessel by means of a glass plate.

Q is a glass vessel containing mercury, resting on blocks *r*, *r*: by raising *Q* the blood in *F* may be driven through the vessels of the muscles in *T*.

F is a vessel containing blood.

A connects the vessel *F* with the blood-vessels of the muscles under experiment.

V conducts the venous blood from the muscle.

R, *R'* are two graduated pipette-like vessels connected by means of a flexible tube and containing mercury. Venous blood flows into *R*, displacing the mercury.

E, *E*, electrodes.

M, *M*, mercurial manometer.

femoral artery. Cannulae should be tied into the hypogastric vessel and into the main branch from the femoral vessel, and all the arterial twigs going to neighbouring parts should afterwards be carefully ligatured. A corresponding number of cannulae should be introduced into the chief veins. The muscles may then be separated from the surrounding tissues, a portion of the *tuber ischii* being sawn off with their origin. The two arterial cannulae are connected with the two limbs of a T-tube of glass, the third limb leading to the reservoir which contains the blood. A similar arrangement connects the two veins with a vessel

into which the venous blood may flow, and where it may be collected for analysis.

It is convenient and advisable to take the blood which is needed in the experiment, from the dog whose muscles are examined. The dog should be first bled nearly to death, and its blood then defibrinated and made ready for the experiment.

Various conditions of blood must be used for comparison; arterialized blood, or blood perfectly reduced, or asphyxiated blood, or asphyxiated blood restored by oxygen[1].

After its blood has been prepared, the dog should be killed and its muscles separated in the manner already described. They are then to be transferred to a glass vessel, *T*, *T*, such as is figured in Fig. 59, and covered over by a glass plate. It will be observed that the vessel is perforated so as to allow the tubes conveying arterial and venous blood, *A*, *V*, to pass into and out of it, and to permit the passage of wires, *E*, *E*, connecting the muscles with an induction coil by which tetanus may be induced. If it is thought necessary, the muscles may be attached to a lever so arranged as to record its movements upon a revolving cylinder. The blood may be forced into the arteries by means of a column of mercury, *M*, the pressure of which admits of careful regulation.

It will be found necessary to increase the pressure of mercury in the course of an experiment in order to maintain a constant flow of blood. The pressure of mercury (40—60 mm.) which, at the beginning of an experiment, serves to drive 2·5 to 3 c.cm. of blood per minute through a biceps muscle of 150—200 grms. weight, will have to be more than doubled (100—150 mm.) after four hours in order to do the same amount of work. It must not be supposed that the resistance to flow suffers a regular increase during this time; on the contrary, the gradual increase is interrupted by frequent variations to and fro, for which there is no assignable cause. The observer must pay unremitting attention to the rate of outflow, if he wishes to maintain it constant even for a few minutes[2].

The cause of these variations in the rate of outflow is left obscure by Ludwig and Schmidt; but it is extremely probable that part of the obstruction is due to the gradual death and contraction of the smaller arteries. When the driving pressure is raised, the constricted vessels will again be opened for the passage of blood, and the original rate of flow will be restored. If this cause of obstruction is admitted, it follows that the more rapid outflow of blood from a muscle which is brought about by raising the driving pressure, may be due not so much to accelerating the current of blood, as to enlarging the number of channels for it. In other words, raising the pressure of injection does not bring a larger volume of blood to play upon the same amount of muscular tissue, but rather brings more muscle under the influence of the blood[3].

[1] Ludwig and Schmidt used small pieces of iron wire to effect the reduction.

[2] Ludwig and A. Schmidt, *Op. cit.* p. 27.

[3] Pflüger, "Ueber die physiologische Verbrennung in den lebendigen Organismen." Pflüger's *Arch.* Vol. x. 1875, p. 350.

It will not have escaped the attention of the reader that these experiments are complicated by the exposure of the muscle to what is practically an enclosed space of air. In other words, two methods of experiment are being employed side by side—the method of exposure to air as a medium and the method of exposure to blood as a medium. And, as a matter of fact, Ludwig and Schmidt determined that the air in the glass vessel, after an experiment of some hours' duration, had lost some of its oxygen and gained in carbon dioxide. The value of this exchange is, however, relatively slight. Another defect in the method of experiment is also deserving of mention. The blood as it flows into and out of the muscle is necessarily exposed to the air of the glass chamber through the membranous walls of the arteries and veins into which the cannulae are inserted. This possible source of error was determined by Ludwig and Schmidt[1] to have no effect upon the analyses as regarded the oxygen which the blood might take up from the air. While to counteract the error as regarded the carbon dioxide which the blood might yield up to the air in the same manner, only those experiments were compared in which the facilities for the escape of it were approximately the same in the rate of flow, and amount of carbon dioxide contained in the blood.

Although in these experiments the authors above referred to succeeded in imitating to a great extent the changes which go on in the blood in its circulation through muscles, they found that in separated muscles the gaseous exchanges were not so great as in muscles connected with the body, the latter appearing to act more energetically upon the oxygen of the blood. In fact, the conditions of temperature adopted by Ludwig and Schmidt but little favour the diffusion of oxygen amongst the tissues and the dissociation of oxy-haemoglobin[2].

From the experiments which have been made in the manner described it may be concluded that:

1. When a muscle through which an artificial stream of blood has been circulating, is deprived of blood, the capacity for doing work is not immediately lost. In the first stages of bloodlessness the irritability increases; but soon it sinks, at first with rapidity, then more slowly.

The circulation of blood freed from oxygen, or of the blood obtained from asphyxiated animals, exerts the same action on the irritability of muscles as the absence of blood.

2. As regards the oxygen absorbed:

a. The quantity of oxygen taken up by muscle increases directly with the increase in the rate of flow, apart from contraction. Hence the greater proportion of oxygen absorbed in contraction is, in

[1] *Op. cit.* p. 41.
[2] Pflüger, *Op. cit.*, Pflüger's *Arch.* Vol. x. p. 354.

part at least, accounted for by the greater rapidity of blood current which then occurs.

This is only true under the conditions of Ludwig and Schmidt's experiments, in which an increased flow of blood through the muscle was probably due to the blood being driven over a wider capillary area. More O was taken up under the circumstances because more muscular substance was brought to act upon the blood. It does not imply that the assumption of O is dependent upon the rapidity of the blood stream, which is expressly denied by Pflüger[1] and Finkler[2].

b. The more oxygen is contained in the blood flowing through muscle, the greater is the ease with which the muscle takes up oxygen from the blood.

c. The amount of oxygen consumed by muscle in activity, or by muscle exhausted by doing work, is usually perceptibly greater than that consumed during rest. But the oxygen consumed bears no definite relation to the work done.

3. As regards the carbon dioxide excreted :

a. In most cases, but not in all, the venous blood flowing from contracting or exhausted muscle contains an increased amount of carbon dioxide. The exact cause of the less usual condition, in which the carbon dioxide of the blood is diminished, is not clear.

b. The relationship between the carbon dioxide excreted and the oxygen absorbed, or the quotient $\frac{CO_2 \text{ excreted}}{O \text{ absorbed}}$, in these experiments underwent no constant variation as the muscle passed from the resting to the active condition.

Dependence of muscular irritability upon a supply of O.

The value of oxygen in preserving the irritability of excised mammalian muscles may be readily demonstrated. The circulation of a stream of oxygenated blood through muscle prolongs its life 17 or 20 hours beyond the time when it would have died if left bloodless. Hence Ludwig and Schmidt[3] concluded, in opposition to the doctrine then current, that a peculiar respiration goes on within muscle which proceeds independently of the so-called vital properties of the contractile matter. Furthermore, irritability may not only be preserved in muscle by means of oxygenated blood, it may also be restored after it has become lost by exhaustion of the tissue. For the purposes of such restoration of muscle extremely minute quantities of oxygen are sufficient. In one experiment, when a muscle had completely lost its irritability owing to the interruption of its blood current for 128 minutes, and when for 38 minutes more a stream of *reduced* blood had been let flow through the muscle without beneficial effect, the passage of 13·5 c.c. of *arterialized* blood through it, occupying the

[1] Pflüger, "Ueber die Diffusion des Sauerstoffs, den Ort und die Gesetze der Oxidationsprocesse im thierischen Organismus." Pflüger's *Arch.* Vol. VI. p. 48.

[2] Finkler, "Ueber den Einfluss der Strömungsgeschwindigkeit und Menge des Blutes auf die thierische Verbrennung." Pflüger's *Arch.* Vol. X. p. 368.

[3] *Op. cit.* p. 46.

three succeeding minutes, restored the muscle almost perfectly. It would seem that so little as 1·8 mgr. of oxygen is sufficient to restore the irritability of a muscle weighing 209 grms.[1]

Analysis of the non-gaseous constituents of the blood of muscle.

As compared with the changes wrought in the gaseous constituents of the blood by the exercise of muscle, the changes in the non-gaseous constituents due to the same circumstance are small and less certain.

It has been stated that during muscular activity, the amount of the aqueous extractive matters removable from muscle diminishes, whilst the alcoholic extractives increase: that whilst glycogen diminishes, sugar increases and lactic acid makes its appearance; further that tetanized muscle possesses considerable reducing powers, which we may surmise to be associated with the production of new substances within the muscle.

Were our knowledge of the chemical composition of the blood complete, we should expect to find variations in the composition of that fluid, after it has passed through a muscle, which should be the correlatives of the changes which occur in the muscle itself. In so far as the gases are concerned this has been shewn to be the case. In reference to non-gaseous constituents, our information is, however, of the scantiest character; it indeed is limited to the two following statements.

1. During tetanus, blood circulating through muscle becomes charged with reducing substances.

Alexander Schmidt passed two different quantities of blood free from oxygen through muscle at rest, and through muscle which was tetanized, and then agitated the two specimens of blood with oxygen. He found that the blood which had traversed tetanized muscle took up more oxygen than that which had traversed resting muscle, and from this he concluded that tetanized muscle gives up reducing substances to blood.

2. During tetanus blood acquires sarcolactic acid (Spiro[2]). We yet possess very slight information on this point.

Changes in the medium surrounding muscle as shewn in the analyses of the general excreta of the body.

The excretions which are modified by muscular exercise are those of the lungs and kidneys. The description of the methods of collecting and examining these excretions properly belongs to the Chapters on Respiration and the Urine. It will, therefore, merely be necessary here to speak of the methods of experiment having a peculiar bearing on the question of muscular work.

Effects of muscular exercise on the pulmonary exchanges.

As regards the excretion of the lungs, it has long been known that the volume of respired air is increased during muscular exertion, and that the proportion of carbon dioxide and oxygen involved in the process of

[1] Ludwig and A. Schmidt, *Op. cit.* pp. 58 and 61.

[2] Spiro, "Beiträge zur Physiologie der Milchsäure." *Zeitschrift f. phys. Chemie*, Vol. I. (1877—78) p. 111.

respiration is enlarged both during and immediately after the period of exercise[1].

But the variations of the carbon dioxide exhaled and the oxygen absorbed do not occur *pari passu;* the relationship of rest is different from that of activity. The exact determination of this relationship, although theoretically very simple, is a matter of considerable practical difficulty; and the gradual improvement of the apparatus employed may be traced in the series of papers already referred to. No attempt will be made here to explain the practical methods, inasmuch as the classical apparatus of Regnault and Reiset[2], Sczelkow[3] and Pettenkofer and Voit[4], will be described in the Chapter on Respiration. The object of all the improved appliances is to exactly estimate the oxygen absorbed, and the carbon dioxide excreted, while the air entering the animal's lungs is of fairly normal constitution and pressure.

Methods of Experiment. To effect this there are three chief methods. The animal may be enclosed in an air-space disconnected from the external air, the carbon dioxide being removed, and the oxygen being replaced, as they are formed and consumed respectively (Regnault et Reiset). Or the animal may be made to breathe out of one vessel and into another, the loss and gain respectively being accurately measured, while the pressure in each vessel is maintained, by suitable apparatus, the equal of that of the atmosphere (Sczelkow). Or the animal may be kept in a space through which air is continually

[1] Among those who discovered and investigated the influence of muscular exercise upon the exchanges of respiration may be mentioned the following: Jurine, quoted in the *Encyclopédie méthodique*, Art. "Médecine," Vol. I. p. 494: ed. by Vicq. D'Azyr, 1787. Seguin et Lavoisier, "Premier Mémoire sur la Respiration des animaux." *Mém. Acad.* 1789, p. 575. Other researches of Lavoisier will be referred to under 'Respiration.' W. Prout, "Observations on the quantity of carbonic acid gas emitted from the lungs during respiration." Thompson's *Annals of Philosophy*, Vol. II. 1813, p. 328. Translated into Schweigger's *Journal für Chemie u. Physik*, 1815, Vol. XV. p. 47. E. A. Scharling, "Dritte Reihe der Versuche um die Menge der Kohlensäure zu bestimmen welche vom Menschen in einen gewissen Zeit ausgeathmet wird." *Journal für prakt. Chemie*, Vol. XLVIII. 1849, p. 440. Vierordt, *Physiologie des Athmens.* Andral et Gavarret, "Recherches sur la quantité d'acid carbonique exhalé par le poumon dans l'espèce humaine." *Ann. de Chimie et de Physique*, Sér. III. Vol. VIII. 1843, p. 129. Regnault et Reiset, "Recherches chimiques sur la Respiration des animaux des diverses classes." *Ann. de Chimie et de Physique*, Sér. III. Vol. XXVI. 1849, p. 299. Ed. Smith, "Experimental Inquiries into the phenomena of Respiration." *Trans. Roy. Soc. Lond.* 1859, Vol. CXLIX. pt. ii. p. 681. Pettenkofer und Voit, "CO_2-Ausscheidung u. O-Aufnahme während des Wachens u. Schlafens." *Sitzungsber. der k. bayer. Akad. d. Wissensch. zu München*, 1866, Vol. II. p. 236. *Ibid.* 1867, Vol. I. p. 255. Speck, *Schriften d. Gesellsch. z. Beförder. d. ges. Naturwissensch. zu Marburg*, Vol. X. p. 3, 1871. Röhrig und Zuntz, "Zur Theorie der Wärmeregulation und Balneotherapie" Pflüger's *Archiv f. d. ges. Physiol.* Vol. IV. p. 57. Zuntz, "Ueber den Einfluss der Curarevergiftung auf den thierischen Stoffwechsel." Pfluger's *Arch.* Vol. XII. p. 522.

[2] Regnault et Reiset, "Recherches chimiques sur la Respiration des animaux des diverses classes." *Ann. de Chimie et de Physique*, 3rd series, XXVI. p. 299, 1849.

[3] Sczelkow, *Op. cit.* 1862.

[4] Pettenkofer, "Ueber einen neuen Respirations-Apparat." *Abhandlungen der math.-phys. Classe d. k. bayer. Akad. d. Wiss.* Vol. IX. München, 1863, p. 229. Voit, "Beschreibung eines Apparates zur Untersuchung der gasförmigen Ausscheidungen des Thierkörpers." *Ibid.* Vol. XII. München, 1876, Abth. I. p. 219.

being drawn, and the air analysed as it emerges either in whole or in sample (E. A. Scharling[1], Pettenkofer and Voit).

In a fourth plan (Röhrig and Zuntz)[2], oxygen is respired instead of air. A rabbit whose lungs have been cleared of nitrogen by the free respiration of pure oxygen for some time, is made to breathe into and out of the same gasometer of oxygen, the bell of which is carefully counterpoised. The oxygen passes from the gasometer to the rabbit, and back again, through water-valves which contain a caustic solution instead of water. In this manner the carbon dioxide formed in respiration is completely absorbed. As the oxygen is used up and the gasometer sinks, the counterpoise is adjusted from time to time in order to maintain the pressure within the apparatus equal to that of the atmosphere. A pump for artificial respiration may be readily adapted to this apparatus.

Effect of exercise on the gases of respiration.

In whichever way the experiment is made, the fact is clearly elicited that muscular exertion increases both the oxygen absorbed and the carbon dioxide excreted, but in no ratio of equivalency. To be precise we may take the experiments of Sczelkow, inasmuch as they were specially devised to demonstrate this fact.

Rabbits were the animals employed. They were fed on a diet of wheat and milk; and the gaseous exchanges of the whole body were determined during rest, and during tetanus of the hind limbs brought on in the manner already described. A study of the numerical results shews that

1. Much more carbon dioxide is excreted during tetanus.

2. Usually, but not always, more oxygen is absorbed; but never so much as corresponds with the carbon dioxide at the same time exhaled. In other words, the quotient $\frac{CO_2 \text{ exhaled}}{O \text{ absorbed}}$ is increased during tetanus.

It should be observed that all the other conditions of the animal besides those of movement specially contrasted, should be taken into account in these comparisons; and particularly the condition of food. According to the food the relation of carbon dioxide exhaled and oxygen absorbed is found to vary. This most probably explains the different values assigned to the relationship $\frac{CO_2}{O}$ during a period of repose by different observers[3].

The above conclusions are illustrated in the following table of three experiments. Q indicates the quotient $\frac{CO_2 \text{ excreted}}{O \text{ absorbed}}$. The numbers in the last column ("experimental errors + N in c.c.") are found by subtracting

[1] E. A. Scharling, "Versuche ü. die Quantität der von einem Menschen in 24 Stunden ausgeathmeten Kohlensäure." *Ann. der Chemie u. Pharm.* Vol. XLV. 1843. Heft ii. p. 214.

[2] *Op. cit.* Note, p. 382.

[3] See Regnault and Reiset, and Sczelkow, *Op. cit.*

the N of the *inspired* air (estimated by the method of difference) from the N of the *expired* air; a + sign indicates that the N of the expired is greater than that of the inspired. It is evident that all experimental errors, *i.e.* errors in reading off measurements, etc., will sum themselves algebraically to the N so determined.

The duration of the experiments was recorded on a revolving cylinder; and the gases were analysed by Bunsen's method.

The amounts of CO_2 and O, reduced to the standard temperature and pressure, are averaged per minute of the experiment.

R = repose: T = tetanus.

	Duration of experiment in minutes.	Respirations.	C.c. in one minute. CO_2.	C.c. in one minute. O.	Q.	Experimental errors + N: total in c.c.
i. R.	7·6	92	4·97	12·29	·404	+ 13·54
T.	6·5	82	13·69	12·11	1·13	+ 31·74
ii. R.	9·2	80	7·85	12·76	·615	− 18·19
T.	5·1	106	17·62	19·02	·927	+ 11·73
v. R.	9·2	140	6·99	17·47	·400	− 5·3
T.	5·1	130	19·61	30·35	·646	+ 16·4

Cause of the increase of CO_2 excreted.

A simple consideration of the amount of carbon dioxide excreted, serves to shew that the increase during tetanus is not due simply to favoured elimination. A very large rabbit rarely weighs more than 2 kilogs., of which $\frac{1}{19}$, or 105·23 grms., may be considered to be the weight of the blood. Taking 30 vols. per cent. as the proportion of carbon dioxide in it, this weight of blood includes about 31·6 c.c. of carbon dioxide—the total carbon dioxide in the blood of a very large rabbit at a given moment. Now, even if we make the large assumption that the carbon dioxide of the blood is, by rapid elimination, reduced to one half in the course of an experiment, we shall still be quite unable to account for the extraordinary excretion of carbon dioxide in tetanus; for if this enormous reduction were supposed to be effected during a short experiment like Exp. ii. of the above Table, it would only give an excretion of about 3 c.c. $\left(\text{or } \frac{31·6}{2} \div 5·1\right)$ of carbon dioxide per minute—a quantity far less than the observed. Hence the excess of carbon dioxide excreted by the lungs during tetanus must be due to an increased production of it within the body.

What part of this production has its seat in the tetanized limbs, and what part in the rest of the body, these experiments fail to discover. It is more than probable that the material exchanges of the body at large do not preserve their equilibrium during the manifold

disturbances of tetanus; but in what direction they are influenced, whether they are checked or accelerated, is not clear. During tetanus the blood becomes deprived of oxygen and charged with carbon dioxide. Both these circumstances are unfavourable to the gaseous exchanges of tissues generally; nor are they compensated by an increased respiratory and circulatory activity, for on prolonged tetanus general asphyxia may arise[1].

These considerations do not, however, entirely make clear the origin of the carbon dioxide; and the uncertainty must always be kept in mind when conclusions obtained in a study of the general exchanges of the body are applied to muscles alone.

The absorption of O discussed.

A small correction is necessary in respect to the oxygen absorbed. After tetanus, the blood generally contains a smaller proportionate quantity of oxygen. This defect is to be ascribed to tetanus just as much as the defect of oxygen from the air inhaled; and hence it must be added to the latter before the oxygen absorbed during tetanus can be compared with the carbon dioxide excreted. The effect of this addition is to make the quotient $\frac{CO_2}{O}$ somewhat smaller than before; but in no remarkable degree. For, assuming the blood to contain 13 per cent. of oxygen, and half of it to be found wanting at the close of a short experiment, it would merely raise the oxygen absorbed by about 1—2 c.c. per minute of the experiment, in the case of a rabbit of 2 kilogs.

Effects of muscular exercise on the urinary secretion.

While the changes of respired air which are brought about by muscular exertion are so pronounced as to have been remarked by the earliest observers, and moreover give a decisive indication of the changes which muscular exercise works in the blood, the changes of the urinary excretion under the same circumstances have been a matter of continual uncertainty. An examination of the methods of the earlier investigators discloses the causes of this uncertainty. The urine—the great drain of effete nitrogenous subtances—although it is beyond doubt affected by the activity of the eminently nitrogenous substance of muscle, is affected to an unexpectedly small degree. In a word, the urine, in so far as it represents the tissue-waste of muscle, is dependent upon the nutritional rather than upon the functional processes of muscular tissue. Moreover nitrogenous muscle, although an important source of the nitrogenous excreta of urine, is by no means the only source. The character of the food, its composition, the proportion of its elements, the time of its ingestion, are all conditions which exert a large influence over the constitution of the urine. It is to these two circumstances, viz. to the smallness of the change which muscular exercise effects in the urine, and above all to the omission from the calculation of the

[1] Sczelkow, *Op. cit.*

various simultaneous modifying agents, that the discrepancies in the statements of different observers are due.

Statements of early observers.

In order that the reader may have some idea of the extent of these discrepancies, the results of some of the earlier observers may be here stated briefly. In most cases, for the reasons already given, they cannot be considered either to support or to confute the results of later experiments.

C. G. Lehmann[1] found that the excretion of urea in man was raised from 32 grams per diem to 36—37 grams during exercise.

J. Fr. Simon[2] also found an increase during exertion.

Mossler[3] failed to discover any marked increase immediately after exercise.

H. Beigel[4] made experiments on six men under conditions of spare diet and rich diet, water being taken at pleasure. With the former diet the excretion of urea was raised from 31·86 grms. to 33·32 grms. per diem, on a day of labour; with the rich diet, from 46·10 grms. to 52·26 grms. per diem.

W. Hammond[5] found that the excretion of urea, which during 24 hours of rest was 31·51 grms. (487 grains), rose to 42·4 grms. (682·09 grains) during a working day, and to 56 grms. (864·97 grains), during a day of hard labour.

Genth[6] observed that the urea excreted during prolonged labour was increased beyond the normal.

Beneke[7] found an increase on exertion.

Franque[8] found an increase during exertion.

J. C. Draper[9] found that a powerful man kept perfectly quiet in bed for a long period, excreted on an average 26·47 grams of urea daily, a quantity which did not differ much from the normal excretion in health.

L. Lehmann[10] found a very small, or no increase in the excretion of urea, on excessive exertion.

C. Speck[11] found on exertion only a slight daily increase of urea, viz. 8 grms., with a rich nitrogenous diet, and 4 grms. with a poor nitrogenous diet.

[1] C. G. Lehmann, *Wagner's Handwörterbuch*, Vol. II. p. 21. *Physiological Chemistry* (Cavendish Soc. Trans.), 1851, Vol. I. p. 163.

[2] J. Fr. Simon, *Animal Chemistry* (Sydenham Soc. Trans.), 1846, Vol. II. pp. 144 and 168.

[3] Mossler, "Beiträge zur Kenntniss der Urinabsonderung." *Diss. inaug. Giessen*, 1853. Quoted by Voit, *op. cit. infra*.

[4] H. Beigel, "Untersuch. ü. den Harn- u. Harnstoff-mengen." *Verhandl. der k. Leopold. Akad. d. Naturforsch.*, Vol. XXV. pt. i., 1855, p. 477. Quoted by Voit, *op. cit. infra*.

[5] W. Hammond, "Relation existing between urea and uric acid." *American Journal of Med. Sci.*, new series, Vol. XXIX., 1855, p. 119.

[6] Genth, *Untersuch. ü. den Einfluss' des Wassertrinkens auf dem Stoffwechsel.* Wisbaden, 1856. Quoted by Voit, *op. cit. infra*.

[7] Beneke, *Nord. See Bad.*, 1855, p. 83. (Quoted by Playfair, *Food and Useful Work*, p. 46.)

[8] O. von Franque: Extract in *Schmidt's Jahrbuch*, 1856, Vol. XCII. p. 9.

[9] J. C. Draper, *New York Journal*, March, 1856. See Voit, *infra*.

[10] L. Lehmann, *Arch. f. wissensch. Heilkunde*, Vol. IV. pt. iv., 1860. See Voit, *infra*.

[11] C. Speck, *Arch. f. wissensch. Heilkunde*, Vol. IV. See Voit, *infra*.

Experiments of Voit. Voit[1] seems to have been the first to examine very carefully this question of the influence of exertion upon nitrogenous excreta. He selected the dog, as being better fitted for the conditions of rigorous experiment than men, who had been previously observed. The dog underwent severe exertion under two sets of conditions: (1) when fasting from all food except water, and (2) when on a diet just enough to cover all loss of weight *when no work was done.* In each set of conditions the average excretions of resting days and working days were compared. Sometimes resting days and working days alternated; but usually two or three days in succession were devoted to rest, and two or three to labour. In this way there was less danger of urine being retained in the bladder over the period of a working day and expelled during the following resting day. In all the experiments the dog was allowed to drink as much water as he desired. Work was done in turning a tread-wheel, the number of turns being registered, and the work carefully calculated. The dog was taught to drive the wheel with great rapidity, encouraged by his master's voice; and it was found that 10 minutes at a time was sufficient to thoroughly fatigue the animal. A working day consisted of about six periods, of 10 minutes each, spent in the wheel, with about an hour's rest between each period. The greatest care was taken to obviate loss of excretions; thus the dog was taught to micturate at a given spot and at regular intervals. The weight of the dog, the water ingested, the urine, the urea, and the faeces were all carefully determined from day to day. The dog weighed about 33 kilograms.

Conclusions. A study of Voit's data conclusively shews us that during the days of exertion the excretion of urea is increased; but it discovers also that,

1. The increase is absolutely very small; viz. an increase of about 1—5 grams in the fasting experiments when the excretion of repose was 10—15 grams a day; and an increase of 5—10 grams in the experiments with food, when the excretion of repose was about 110 grams a day.

2. The increase has no constant relationship to the work done.

3. The increase is evidently more influenced by the diet (viz. by the amount of water ingested; by the fact of food being taken; &c.) than by the circumstance of work.

Voit's discussion of his experiments. Impressed by the smallness of the increase of nitrogenous excreta during severe labour, Voit endeavoured to discredit the inference that some part of it, however small, is directly owing to the activity of muscles. He dwelt upon the circumstance that the free ingestion of water is, of itself, enough to raise the excretion of urea by the urine. He pointed out that the

[1] C. Voit, *Untersuchungen ü. den Einfluss des Kochsalzes, des Kaffee's und der Muskelbewegungen auf den Stoffwechsel.* München, 1860.

circulation is much stimulated by muscular exercise, and that respiration and the consequent ingestion of oxygen are enlarged by the same means; all of which conditions, apart from the fact of muscular contraction, favour the flow of parenchymatous juices and the manufacture of urea. He concluded, very justly, that the nitrogenous waste was wholly incompetent to account for the mechanical work done. He believed that the oxygen imported so largely into the body during exercise served to burn up fat in extraordinary quantity and repair the losses of heat which occurred by evaporation and radiation; and he formed the opinion that the small increase of urea excreted was due to some of the nitrogenous tissues falling a prey to the oxygen during this increased activity of combustion. In short, the increased oxidation of nitrogenous matter was an accident of the general extension of processes of combustion within the body, which would probably have been avoided by a larger ingestion of fats.

These experiments have been since repeated by Voit[1] with a view to determine not merely the urea, but the total excretion of nitrogen in the urine; and similar experiments have been made by Pettenkofer and Voit[2] on men; with confirmatory results in each case.

It must ever be borne in mind, in following the early discussion of the influence of muscular exercise upon the excretion of nitrogen, that the question which divided observers was not whether exercise produces any increase of nitrogenous excretion, but whether it produces an increase corresponding with the mechanical effect of exercise. In respect of the former question, a much greater unanimity would probably be found than is commonly supposed to exist among those who have entered into the discussion.

Experiments of Fick and Wislicenus.

Although Voit selected the lower animals as best fitted for exact experiment, yet it was by a very simple observation conducted upon men that the question in one of its phases was finally laid at rest. In the summer of 1865 Professors Fick and Wislicenus[3], while abstaining from all nitrogenous food, performed a definite amount of muscular work; and, having estimated the destruction of albuminous matters in the body from the nitrogen excreted during the same time, they discovered that the combustion of so much albuminous material was quite inadequate to account for the mechanical effect. The work done was an ascent of the Faulhorn to a height of 1956 metres. Therefore the mere mechanical lifting of their bodies through this height was, in the case of

Fick (wt. 66 kilog.).....129096 kilogm.
Wislicenus (wt. 76 kilog.)...148656 kilogm.

[1] Voit, *Zeitsch. für Biologie*, Vol. II. p. 307. 1866.
[2] Pettenkofer u. Voit, *Zeitsch. für Biologie*, Vol. II. p. 459. 1866.
[3] Fick und Wislicenus, *Vierteljahresschrift d. naturf. Gesellsch. in Zürich*, X., 1865. *Lond., Edin. and Dub. Phil. Mag.*, Ser. 4, Vol. xxxi. p. 485. Suppl. number, 1866. This important paper is also translated in the *Ann. des Sci. Nat.*, Sér. v. Vol. x., 1868, p. 257.

This by no means represents the total work done during the ascent; for there is omitted the muscular work of circulation and respiration; the muscular work of maintaining the upright posture; the accidental, adventitious movements of the arms, &c.; none of which assisted directly in raising the experimenters to the top of the Faulhorn, but all of which would swell the products of muscular tissue-waste. For 17 hours before the work began no albuminous food was taken; during this period, during the period of the ascent, and for some hours afterwards, the solid food consisted of rice, fat, and sugar, taken in the form of cakes, with beer, tea and wine as fluid food.

The following diary serves to shew the relation of the urine as it was collected, to the various parts of the experiment:

Aug. 29.	12 A.M.	Ceased to take albuminous food.	
	6.15 P.M.	Evacuated bladder. Slept.	Urine of night before labour (A.)
Aug. 30.	5.10 A.M.	Started; made the ascent.	Urine of labour (B.)
	1.20 P.M.	Reached summit; perfect rest.	Urine after labour (C.)
	7 P.M.	Partook freely of meat, etc. Slept.	Urine of night after labour (D).
Aug. 31.	5 A.M.	Arose.	

The urine was analysed at the summit for urea &c.; and sealed samples of each period were again examined for the total N, in the laboratory on descending. Neubauer's method was used to determine urea; and Liebig's method for chlorine.

The total N was determined by heating the urine with soda-lime, collecting the NH_3 as chloride, and determining as usual with $PtCl_4$.

From the analyses it appeared that the total elimination of nitrogen by the urine during the different periods was as follows:—

	Fick.	Wislicenus.
A.	6·9153	A. 6·6841
B.	3·3130	B. 3·1336
C.	2·4293	C. 2·4165
D.	4·8167	D. 5·3462

That is to say, reducing the numbers to a comparable form, the excretion of nitrogen per hour was as follows:

	Fick.	Wislicenus.
A.	0·63	A. 0·61
B.	0·41	B. 0·39
C.	0·40	C. 0·40
D.	0·45	D. 0·51

It will be observed that, in both cases, the nitrogen actually excreted during and immediately after exercise appears to diminish from the standard of A. This fact will be afterwards discussed. It is not so much the relative as the absolute elimination of nitrogen which we are now considering.

Conclusions. Having determined the excretion of nitrogen for some hours before, during, and after a period of muscular exertion, Fick and Wislicenus had a key to the amount of albuminous matter which had in the same interval of time been decomposed within the body; but one which required certain assumptions for its use.

In the first place it was assumed that all the nitrogen escapes by the urine. This is not strictly true, since some is removed in the faeces and some in the sweat; both these sources of loss were neglected.

In the second place it was assumed that the nitrogen excreted during labour (B), together with that excreted during the six hours succeeding labour (C), fully represented all the albuminous matter decomposed during the period of labour. This is the most vulnerable point of the argument, and deserves some consideration.

It is clearly improbable that the nitrogen eliminated in the urine (B) emitted during the occurrence of labour exactly represents the albuminous decomposition during the same period. The act of decomposition may not—and probably does not—occur at one step. Intermediate stages between proteid and the ultimate form in which the nitrogen escapes, are first formed and may possibly remain at the seat of manufacture, or in some other organ, until long after the period of exercise. Such intermediate stages would therefore have no representative in the urine excreted during labour. But this lagging of elimination behind the time of formation, as the observers themselves pointed out, is true also of the nitrogenous products of the period *before* labour. If the excretions of the period B lack some of the decomposition-products proper to that period, it is also true that they possess some which are proper to A. In order amply to cover the effects of this retardation of excretion, Fick and Wislicenus added to the nitrogen excreted during the eight hours of the ascent the whole of that which was excreted during the six hours following.

In the third place an assumption was necessary in order to convert the nitrogen excreted into terms of albuminous substance. The albuminous substances differ in constitution among themselves; and it is impossible to say which kind is chiefly taxed to supply the nitrogen excreted during exercise. But all albuminous bodies except permanent cartilage, contain more than 15 p.c. of nitrogen. If, therefore, we assume that every 15 parts of nitrogen excreted represent 100 parts of albuminous substance decomposed, we shall obtain a quantity of albuminous matter greater than could possibly have been destroyed in the body within the given time

If now we turn to the first table of the total nitrogen excreted, and add the quantities B and C together, we find that the nitrogen which Fick and Wislicenus agreed to take as representing the albuminous decomposition of the period of exercise, is as follows :—

Fick 5·7423 grms.
Wislicenus 5·5501 grms.

And assuming that this represents an albuminous substance containing 15 p.c. of nitrogen, we get as the quantities of nitrogenous matter decomposed during the time of exercise, by

Fick 38·282 grms.
Wislicenus 37·000 grms.

Owing to a slight mistake fully explained in the original memoir, this number in the case of Fick had to be reduced to 37·17 grams.

Thus were obtained numbers representing all the albuminous matter which could possibly have been consumed during the period of the ascent. Knowing this, we can easily calculate the kilogram-metres of work which the burning of so much matter would represent. According to Frankland[1] the burning of 1 grm. of beef-lean dried yields 2161 kilogram-metres: whence, if the material consumed in the body of each experimenter had been wholly burnt up, the oxidation would have corresponded

In Fick to (37·17 × 2161) or 80324·37 kilog.-met.
In Wislicenus (to 37 × 2161) or 79957 kilog.-met.

As a fact the oxidation is not carried to its ultimate conclusion in the body, since urea is the product. Hence the mechanical equivalent of each combustion is really considerably less than that stated.

If we refer to the calculation of the work done in the mere ascent of the Faulhorn, without taking into account for a moment the large circulatory and respiratory work, we shall at once see what a large proportion of the mechanical effect of muscular exercise is wholly uncovered by the combustion of albuminous material.

Fick and Wislicenus were not acquainted with the heat of combustion of dried albumin as directly obtained; they therefore made an approximation which now appears to have been rather in excess of the truth. Neglecting the N altogether, they assumed that the heat of combustion of albumin would not be greater than the sum of the heats of combustion of the C and H which it contained.

1 grm. of albumin contains ·535 grm. C.
·07 grm. H.

But the value of the heats of combustion are,

of C ..... 8080 grm.-degrees.
of H ..... 34462 grm.-degrees.

And

of ·535 grms. C ... 4320 grm.-degrees.
of ·07 grms. H ... 2410 grm.-degrees.

[1] Frankland, "Origin of Muscular Power." *Lond., Edin. and Dub. Phil. Mag.*, 4th Ser., Vol. xxxii., 1866, p. 187.

Therefore that of the gram of albumin was assumed to be
6730 grm.-degrees.

Hence the heat of combustion of the decomposed albumin amounted,
In Fick to (37·17 × 6730) or about 250000 grm.-degrees.
In Wislicenus to (37 × 6730) or about 249000 grm.-degrees.

This converted into mechanical equivalents of that day, gave
For Fick, 106250 kilog.-metres.
For Wislicenus, 103825 kilog.-metres.

What Fick and Wislicenus' experiments shew beyond all doubt is, that during and after muscular contraction no quantity of effete nitrogenous material passes out of the body which is at all adequate to the mechanical work done in contraction. What they do not shew is whether or not *any* nitrogenous waste occurs in muscle during activity.

It will have been observed that the experiments of Fick and Wislicenus do not afford a comparison of the same organism during repose and during activity, *while all the other conditions are rigorously the same.* It is true that the food during the period immediately before and immediately after exercise was non-nitrogenous, and so far identical with that of the time of exercise itself. Nevertheless the experimenters were not under precisely similar conditions in the three periods named, because these periods were not equally remote from the last ingestion of nitrogenous food. When the supply of nitrogenous food is suddenly stopped, it is well known that the excretion of nitrogen sinks to the starvation-standard with diminishing velocity. Unless we make ourselves acquainted with the rate of this descent, we cannot estimate the effect upon the excretion of nitrogen of other circumstances or conditions.

Experiments of E. A. Parkes.

In order to furnish such a comparison, and at the same time to control the observations of Fick and Wislicenus, Parkes[1] undertook some experiments upon soldiers at the Victoria Hospital, Netley. Two sets of experiments were carried on. In one, work and repose were contrasted, in respect of their influence upon nitrogenous excreta, on an unrestricted diet containing no nitrogen; and in the other, on a normal diet including nitrogen, which was maintained practically constant during the whole time of the experiment. We will confine ourselves in the first instance to the experiments with a non-nitrogenous diet.

I. The two soldiers were kept for four days at light employment, with a normal temperate diet including meat, bread, ale, etc.

II. During two days they were put on a non-nitrogenous diet of arrow-root, sugar and fat, and kept in perfect repose.

[1] E. A. Parkes, "On the Elimination of Nitrogen during Rest and Exercise." *Proceed. Roy. Soc. Lond.*, Vol. xv. p. 339; Vol. xvi. p. 44, 1867.

III. For four days they returned to the normal occupation and diet of period I.

IV. During two days they were again put on a non-nitrogenous diet, and made to perform a long march each day, with intervals of rest.

V. They again returned to their normal occupation and diet.

The diet was not limited; the men took what they needed. The nitrogenous excreta of urine and faeces were carefully determined; and periods II. and IV. were then compared as to the nitrogen excreted.

We may illustrate this comparison by the numbers relating to one of the soldiers experimented upon.

TOTAL DRY FOOD FOR THE TWO DAYS OF THE RESTING AND WORKING PERIOD, IN KILOG.

R.	W.
1·0044	1·3066

WATER, IN KILOG.

4·592	5·1595

TOTAL EXCRETION OF NITROGEN, IN GRMS.

	Urine.		Intestines.	
Hours.	R.	W.	R.	W.
1—24	9·33	10·048		
24—36	4·005	4·533	·3875	·5318
36—48	3·017	3·361		
Totals	16·352	17·942	·3875	·5318

Conclusions. From these tables it appears that there is a slight total increase of nitrogen eliminated during muscular exertion. It must be remembered that more non-nitrogenous food was at the same time taken into the system, and more fluids were drunk. Both these circumstances, apart from muscular exercise, imply the use of digestive and assimilative apparatus which like muscles are mainly nitrogenous in constitution.

In the second set of experiments the fluid and solid diet (including 19·61 grms. of nitrogen) was constant during the whole time.

The alternation of light labour, with repose or long marches, was conducted as in the first experiment, viz.:

I. Four days of ordinary occupation.
II. Two days of perfect repose.
III. Four days of ordinary occupation.
IV. Two days of hard marching.
V. Four days of ordinary occupation.

Again we may take the case of one of the soldiers observed,—viz. the one whose data served us in the first experiment.

COMPARISON OF TOTAL EXCRETION OF NITROGEN DURING REST AND HARD LABOUR, IN GRMS.

	Urine.		Intestines.	
Hours.	R.	W.	R.	W.
1—24	20·094	18·478		
24—36	9·855	7·357	1·486	2·138
36—48	8·315	13·457		
Totals	38·264	39·292	1·486	2·138
Means	19·132	19·646		

The above table shews us that there is a slight increase in the total amount of nitrogen excreted during labour as compared with a time of perfect repose. This agrees with the facts elicited during the first set of experiments. But it further appears that during the first 36 hours of the period of labour the excretion of nitrogen is actually *less* than during the corresponding period of rest. This fact is not indeed supported by the data from the same man in the former experiment when the diet was non-nitrogenous; but in the case of his fellow in the previous experiment, the excretion of nitrogen passed through the same phases; that is to say, in the case of the other soldier on a non-nitrogenous diet just as in the case of this one on a diet including nitrogen, the immediate effect of hard muscular exercise was to diminish the excretion of nitrogen: it is not until the night of the second 24 hours that we observe such an out-pouring of nitrogen as to raise the total excretion of the two days of labour above that of the two days of rest. This curious circumstance of the elimination of nitrogen will at once remind us of the case of Fick and Wislicenus, in both of whom, on a non-nitrogenous diet, muscular exercise seemed at first to diminish the excretion of nitrogen.

If the above table be compared with the following one shewing the nitrogenous excretions in the four days preceding rest, in the four days following rest and preceding work, and in the four days following work, a very interesting contrast will arise.

TOTAL EXCRETION OF NITROGEN BY THE URINE IN THE PERIODS INDICATED, IN GRMS.

	Normals before Rest.	After Rest.	After Work.
1st day	17·886	15·920	21·25
2nd ,,	16·810	17·608	19·942
3rd ,,	19·212	19·382	23·488
4th ,,	17·520	17·54	19·530
Means	17·857	17·612	21·054

Conclusions. It will be seen that there is (1) a slight augmentation of the nitrogen excreted during perfect repose as compared with periods of light occupation (*i. e.* 19·132 grms. per diem, as against 17·857 and 17·612 grms.); and (2) that, after the period of hard labour there is an enlarged excretion of nitrogen in the urine which may continue for several days.

Experiments of Edward Smith. The observations of Edward Smith[1] upon prisoners at hard labour, which were published shortly after the experiments of Voit, and before those of Fick and Parkes, tend to the same conclusions.

In an alternating series of days of hard and light labour, with a fixed diet, the *average* excretion of urea on the days of labour was not indeed markedly greater than on the days of comparative rest. Nevertheless, the excretion of urea underwent from day to day a succession of oscillations which beyond a doubt had reference to the character of the daily labour. In most cases the total excretion of nitrogen during a day of hard labour was somewhat greater than that of the days of light labour just before and just after it; but in some cases it was apparent that the elimination of nitrogen had been held over until the day after labour, making the excretion of that day unusually large, and destroying the value of the averages.

Experiments of Flint and Pavy. The general fact of an increased excretion of nitrogen during periods of hard labour is also supported by the observations of Austin Flint, Jun.[2], and of Pavy[3], made upon the celebrated pedestrian Weston. This man was kept under observation on two separate occasions during the performance of extraordinary feats of walking, lasting for five or six days; and not only was he observed during the days devoted to walking, but for five or six days before and after. The ingested food in these periods was accurately weighed, and the nitrogen estimated from the tables of Payen[4]

[1] Ed. Smith, "On the Elimination of Urea and Urinary Water." *Phil. Trans. Roy. Soc. Lond.*, vol. cli. pt. iii. p. 747. 1862.

[2] Austin Flint, Jun., *New York Medical Journal*, June, 1871. "The source of muscular power, as deduced from observations upon the Human Subject under conditions of Rest and of Muscular Exercise." *Journal of Anat. and Physiol.*, Vol. xii. p. 91, 1878 (contains the same facts as the former article).

[3] F. W. Pavy, "The effect of prolonged muscular exercise on the system," *Lancet*, London, 1876, Vol. i. pp. 319, 353, 392, 429, 465; Vol. ii. pp. 741, 815, 848, 887.

[4] Payen, *Substances Alimentaires*. Paris.

or from original determinations. The urine, and in Flint's experiment the faeces also, were collected, and their nitrogen determined; in the case of the urine, only the nitrogen of the urea and uric acid was taken. In Flint's experiments the weight of Weston fell from an average of 119 or 120 lbs. to 116·5 lbs. on the first day, and gradually to about 115·75 lbs. on the fifth day: it quickly regained the normal during the rest succeeding the march. In Pavy's experiment there are no data for exactly comparing Weston's weight before, during and after the walk; but during the six days of exercise the weight fell from $134\frac{13}{16}$ lbs. on the first day to $130\frac{5}{16}$ lbs. on the sixth.

The following tables will sufficiently bear out the general conclusion of these experiments. The numbers in the case of Flint's observations are the revised ones published by him in the *Journal of Anatomy and Physiology*, after Pavy's observations had appeared.

OBSERVATIONS OF PROF. FLINT.

	Nitrogen ingested, in grains.	Nitrogen eliminated as urea and uric acid, in grains.	Relation of Nitrogen ingested and Nitrogen eliminated.
BEFORE THE WALK.			
First 24 hours	361·22	304·55	
Second ,,	288·35	276·84	
Third ,,	272·27	305·08	
Fourth ,,	335·01	283·87	
Fifth ,,	440·43	299·31	
Average	339·46	293·93	1 : ·8658
DURING THE WALK.			
First 24 hours (80 m.)	151·55	331·44	
Second ,, (48 m.)	265·92	328·05	
Third ,, (92 m.)	228·61	399·16	
Fourth ,, (57 m.)	144·70	324·59	
Fifth ,, ($40\frac{1}{2}$ m.)	383·04	306·08	
Average	234·76	338·01	1 : 1·439
AFTER THE WALK.			
First 24 hours	385·68	277·00	
Second ,,	499·10	334·44	
Third ,,	394·83	358·78	
Fourth ,,	641·71	348·19	
Fifth ,,	283·35	379·79	
Average	440·93	339·64	1 : ·7702

OBSERVATIONS OF DR PAVY.

	Nitrogen ingested, in grains.	Nitrogen eliminated as urea and uric acid, in grains.	Relation of Nitrogen ingested and Nitrogen eliminated.
BEFORE THE WALK.			
First 24 hours	378·29	292·70	
Second ,,	451·14	301·75	
Third ,,	472·29	231·54	
Fourth ,,	539·95	363·71	
Fifth ,,	441·15	342·59	
Sixth ,,	582·80	387·17	
Average	477·60	319·92	1 : ·6698
DURING THE WALK.			
First 24 hours (96 m.)	491·80	524·59	
Second ,, (77 m.)	826·43	582·42	
Third ,, (70½ m.)	759·15	600·29	
Fourth ,, (76½ m.)	547·57	503·21	
Fifth ,, (67 m.)	790·78	450·06	
Sixth ,, (63 m.)	614·61	468·70	
Average	671·72	521·54	1 : ·7764
AFTER THE WALK.			
First 24 hours		384·40	
Second ,,	(not determined).	238·39	
Third ,,		381·22	
Fourth ,,		278·46	
Fifth ,,		299·19	
Sixth ,,		268·17	
Average		306·64	

Before these figures can be compared it is necessary to remark that the tables do not include the nitrogen eliminated in the faeces, or that eliminated in the urine otherwise than as urea and uric acid. Compared with the nitrogen of the urea and uric acid, the nitrogen of the other egesta named is indeed small and unimportant; still inasmuch as the nitrogen eliminated as urea and uric acid does not bear a constant relationship to that otherwise excreted, no comparison is perfect which does not include the latter. Unfortunately the experiments of Flint and Pavy do not, together, furnish us with the necessary data for a perfect comparison.

Conclusions. If now we contrast the experiments of Flint and Pavy we observe in the first place that both shew an increase of nitrogen eliminated during exercise: the proportion of nitrogen ingested to nitrogen excreted is, during the days before the walk,

in Flint's case 1 : ·8658
in Pavy's case 1 : ·6698

while during the walk it is

in Flint's case 1 : 1·439
in Pavy's case 1 : ·7764

But in the second place, we are struck by the remarkable difference in the degree of the increase. This discrepancy, which has led Prof. Flint into a long discussion of the question of muscular power, it is not necessary to examine fully here. Assuming for the moment that the calculations of both observers are well-founded, it is sufficiently clear that some circumstance must have existed in one or the other experiment to destroy their precise analogy. For example, in Flint's case the food of Weston fell off considerably during the period of exercise; while in Pavy's it increased. Moreover, in Flint's experiment Weston urged himself to the very extreme of endurance. "The most notable event in the course of the five days' walk was what appeared to be a total collapse of muscular and nervous power. * * * * At 10.30 P. M. on this (the fourth) day, Mr Weston broke down completely. He could not see the track, and was taken staggering to his room, having reached apparently the limit of his endurance. * * * The calculations as well as the general condition of the system, shew that the period had probably arrived when repair of the muscular system had become absolutely necessary[1]." If we may suppose that over-exertion brings about a condition of muscular tissue in which disintegration proceeds with unusual ease[2], the very marked increase of urea and uric acid in Prof. Flint's case admits of a simple explanation; and especially if we may further suppose that over-exertion, in certain extreme cases, leads to the absolute rigor of individual fibres, as it does in the case of muscles out of the body. In this condition the rigid albuminous fibres would rapidly degenerate and serve to increase the common nitrogenous excretions of the body[3].

In addition to demonstrating a slight increase of nitrogenous excreta during exercise, Dr Pavy endeavours to shew that the nitrogenous waste during the walking period is wholly incompetent to account for the mechanical work done. His argument is similar to that of Fick and Wislicenus.

Experiments of W. North. Still more recently W. North[4] in experiments upon himself, has arrived at confirmatory results. He determined the urea excreted and, by Payen's Tables, the nitrogen ingested during eight days, beginning on Monday, Sept. 3. On Monday, Tuesday and Wednesday, nitrogenous food was taken *ad libitum:*

[1] Flint, *Journ. of Anat. and Physiol.*, Vol. XII. p. 134.
[2] T. R. Noyes, "Experimental Researches on the excretion of urea." *American Journal of the Med. Sci.*, New Series, Vol. LIV., 1867, p. 354.
[3] Hermann, *Stoffwechsel der Muskeln*, p. 100.
[4] W. North, "An Account of two Experiments illustrating the effects of Starvation with and without severe Labour, on the Elimination of Urea from the Body." *Journal of Physiology* (ed. by M. Foster), Vol. I. p. 171.

on Thursday the nitrogenous ingesta were reduced from an average of about 15·5 grams per diem to 4·228 grams; on Friday to 1·365 grams; and on Saturday to ·399 grams. On Saturday, Sept. 8, Mr North underwent severe exertion on the tread-wheel, the previous days having been days of comparative rest. On Sunday nitrogenous food was again taken *ad libitum*. Mr North first makes the assumption that the nitrogen excreted as urea is influenced by the nitrogen ingested on the previous day, rather than by that ingested on the same day; and then finds that the total nitrogen excreted as urea from Tuesday to Sunday is indeed greater than the total nitrogen ingested from Monday to Saturday (the work-day), but only by 1·176 grams, which is wholly insufficient to account for the loss of weight ($3\frac{1}{2}$ lbs.) sustained during the experiment, or for the work done.

Later experiments have led to a like result, in a majority of cases. But whilst as a rule the nitrogen appears to be practically unaffected by exercise, occasionally the excretion of nitrogen is decidedly increased. This phenomenon, in Mr North's as in other cases, may, we think, depend upon a temporary condition resembling fever engendered by the exercise, when it is not due to causes already referred to.

General Effect of Muscular Contraction upon the Nitrogen of the Urine.

It may, therefore, be regarded as established that muscular exercise somewhat enlarges the total excretion of nitrogen. There is no reason to doubt that this enlarged excretion is due, in the last instance, to the degradation of the nitrogenous tissues of muscle; but the degradation is far too small to account, by mechanical equivalence, for the work done in contraction. Moreover it appears that the actual elimination of waste nitrogenous matters does not coincide with, or very closely follow, the period of muscular contraction. Sometimes, perhaps most frequently, the immediate effect of exercise is rather to diminish the elimination of nitrogen, and to postpone the enlargement of excretion for some hours, or even days. This fact is well illustrated in the experiments of Parkes; by whom it was thought to be of such essential importance as to warrant the hypothesis that muscle, in activity, gains rather than loses nitrogen. "When a voluntary muscle is brought into action by the influence of the will, it appropriates nitrogen and grows..... A state of rest ensues, during which time the effete products are removed, the muscle loses nitrogen, and can again be called into action by its stimulus[1]." And this also, according to Parkes, is the explanation why the elimination of urea is greater during absolute rest than during light and regular labour.

Such a hypothesis is not, however, necessary. The formation of effete nitrogenous matters in muscle, and their elimination at the kidneys, are separate operations conducted by different protoplasmic structures. The conditions favourable to one are not necessarily favourable to the other; blood, for example, is received into muscles in large quantity during contraction, and at the same moment is

[1] Parkes, "On the Elimination of Nitrogen." *Proc. Roy. Soc. Lond.*, Vol. XVI., 1867-68, p. 58.

diverted from the kidneys. It is therefore as probable that the kidneys act ill during excessive muscular exertion, as that digestion is imperfectly performed in the same circumstances. Further, the formation of urea, the end-product of nitrogenous waste, takes place in all probability in several stages, of which the earlier only have their seat in muscle itself. This much at least is certain, that muscle contains little or no urea, either at rest or after contraction; whence it must probably be concluded that if the proteids of muscle contribute to the urea excreted normally, their contribution takes the immediate form, not of urea, but of some antecedent of urea. It is not necessary to suppose that this antecedent form is creatine or any body like creatine: indeed, as will be urged elsewhere, the tendency of the experimental evidence is to render it very improbable that any of the urea excreted passes through a preliminary creatine-stage; for when creatine is introduced artificially into the blood it is invariably excreted not as urea, but as creatine. The form in which muscle-proteid leaves the muscles, after having become effete as contractile matter, may still be proteid; in which case the whole oxidation of muscle-proteid to the urea-form would occur altogether outside the muscular tissues. But, if urea is not at once formed in muscle—if the nitrogenous waste of muscle escapes into the blood in a proteid (or other) form—the elaboration of the waste material into the form in which it is actually excreted must go on elsewhere; and in whatever organ this elaboration has its seat, it is very probable that the action of the organ is hampered during prolonged or excessive muscular exercise.

The manufacture of urea in two stages also fully explains the other fact elicited in Parkes' experiments, viz. that during absolute rest the elimination of nitrogen is slightly increased, if the diet remains the same. Of the nitrogen ingested as food, part is decomposed more or less directly and appears in the urine at once as urea, and part serves to repair the nitrogenous waste of muscles, reaching the urine by a circuitous path through muscular tissue; the latter portion appearing in the urine at a later date than the former. If anything occurs to diminish the wasting of muscle, less nitrogen is yielded up by muscle to the urine, but at the same time less is called upon to repair waste, and more, therefore, passes directly into the urine from the food. Thus the same quantity of nitrogen should appear in the urine whether muscles be exerted or not, so long as the food remains constant. But this is only true if the nitrogenous waste products of muscle pass *at once* from muscle into the urine; which *ex hypothesi* is not the case. On the contrary, they are intercepted by some other organ and delayed. This organ, therefore, at any given moment, contains waste products derived from muscle, in course of preparation for excretion as urea. If, now, muscles are suddenly thrown out of employment, less nitrogen of the food is called upon to repair waste of tissue and more passes directly into the urine; but at the same time the waste nitrogenous matters which happen

to be in the intermediate preparatory organ as the consequence of the preceding day's exertions, are perfected into urea and excreted. Thus the urine receives not only the nitrogen corresponding to its proper day; but also some which should have formed part of muscle, and have been excreted on the morrow.

Effect of muscular contraction on the non-nitrogenous excreta of the urine.

The nitrogenous constituents of the urine are not the only constituents which suffer change during muscular exercise; but the non-nitrogenous elements have excited less attention and still remain an object for exact research. Klüpfel found that the acidity of the urine as estimated by titration with soda solution was sometimes increased and sometimes diminished during muscular exercise, even when the food remained constant. The experiments of Sawicki gave the same results, and disclosed also that the acidity was influenced by the quality and quantity of food taken far more markedly than by the circumstance of muscular exercise. On the other hand, Pavy noticed that the acidity of the urine was increased during severe exercise; and Janowski seems to have come to a similar conclusion. Klüpfel has surmised that the diminution of acidity which is sometimes observed after muscular exertion may coincide with an abnormally large excretion of acid sweat; but no one has yet established this[1]. In the experiments of both Flint[2] and Pavy[3] the proportion of sulphuric acid and phosphoric acid excreted during labour was greater than during rest; while that of sodium chloride was less under the same circumstances. In Sawicki's experiments, referred to above, the phosphoric acid suffered no constant variation from rest to labour.

The Chemical Changes of Living Muscle when at Rest.

Many of the chemical changes of normal resting muscle have been already described or implied in the Section on Muscle in action. The "respiration" of excised muscles and the preservation of irritability by means of oxygen, were among the earliest discoveries of muscular chemistry, and have been stated at length in the account of the researches of Humboldt, Georg Liebig, Valentin, Matteucci and Hermann. The general nature of the material exchanges of muscles which are still in the circulation, is indicated in the conversion of arterial into venous blood by muscles at rest; but a point of special interest is this, that the blood flowing from muscles paralysed by

[1] Klüpfel, "Ueber die Acidität des Harnes bei Ruhe u. bei Arbeit." Hoppe-Seyler's *Med. chem. Untersuch.*, IV. p. 412, Berlin, 1871. Pavy, *Lancet*, London, 1876, Vol. II. p. 888. Sawicki, "Säuregehalt der Harnmenge in Arbeit und Ruhe." Pflüger's *Archiv*, 1872, Vol. V. p. 285. Janowski, "Säuremenge des Harnes in Verhältniss zur Muskelarbeit." *Dis. inaug.*, Moscow, 1876. (The original is in Russian, it is extracted into Hofmann and Schwalbe's *Jahresberichte*, Vol. V. pt. ii. p. 274.)

[2] Flint, *New York Medical Journal*, June, 1871.

[3] Pavy, *Lancet*, London, 1876, Vol. II. p. 881.

section of their nerves is less venous than that flowing from quite normal muscles[1]. We are naturally led to compare this with the fact which we already know, that the blood from contracting muscles is far more venous than that from the same muscles in repose, and to ask whether the cause is not the same in both cases. In short, do the nervous centres exert a tonic influence, automatic or reflex, over the muscles, keeping them constantly in a state of partial contraction at the expense of certain chemical decompositions? Such a tonus has, for physiological reasons, been ascribed to voluntary muscles; but not, as yet, upon grounds which are absolutely beyond question (see the various Text Books of Physiology). While, then, we may bear in mind this tonic contraction of voluntary muscle, as a possible or, it may be, a partial explanation, the fact itself must be taken as established, that separation of a muscle from its nervous centres is followed by a diminution of the normal chemical changes of repose. Whether or not the nervous centres induce a constant contraction of voluntary muscles, they certainly bring about a constant *chemical* tonus (as it has been called) in the same tissues[2].

The method of investigating this chemical tonus has hitherto been that of comparing the general excreta of the body before and after the separation of large tracts of voluntary muscles from the central nervous system. The excretion of the lungs was collected in an apparatus for the respiration of a definite amount of gases at an unvarying pressure. This apparatus, which differs from that of Ludwig and Sczelkow devised for a similar purpose, will be found described in the original memoirs. By means of it the oxygen consumed and the carbon dioxide excreted by an animal could be measured with considerable accuracy, while the arrangements were such as not sensibly to impede the normal respiratory movements of the animal. The apparatus was fitted with appliances for artificial respiration. The separation of the muscles from the central nervous system was brought about in one of two ways: by curare-poisoning[3], or by division of the spinal cord between the cervical and dorsal regions[4].

A rabbit was attached to the apparatus and its respiratory exchanges determined, respiration being carried on artificially to such an extent as to leave the rabbit apnoeic for 3 to 4 seconds on stopping the injection of air. This degree of apnoea is known not to affect the material exchanges of the body[5]. The rabbit was then curarized with 2—3 mgr. of curare subcutaneously injected, and its respiratory exchanges again determined.

[1] Claude Bernard, *Leçons sur les propriétés des tissus vivants*, p. 221. Paris, 1857.

[2] Röhrig und Zuntz, "Zur Theorie der Wärmeregulation und der Balneotherapie." Pflüger's *Arch. f. d. ges. Physiol.*, Vol. IV., 1871, p. 57.

[3] Röhrig und Zuntz, *Op. cit.* Zuntz, "Ueber den Einfluss der Curarevergiftung auf den thierischen Stoffwechsel." Pflüger's *Archiv f. d. ges. Physiol.*, Vol. XII., 1876, p. 522. Pflüger, "Ueber Wärme und Oxydation der lebendigen Materie." *Arch. f. d. ges. Physiol.*, Vol. XVIII., 1878, p. 247.

[4] Pflüger, *Op. cit.*, p. 305.

[5] Finkler und Oertmann, "Ueber den Einfluss der Athemmechanik auf den Stoffwechsel." Pflüger's *Arch. f. d. ges. Physiol.*, Vol. XIV., 1877, p. 38.

During this time the animal was kept wrapped up in wadding to prevent such an excessive cooling as might of itself depress the tissue-changes. Artificial respiration was carried on at the same rate as before. Under these circumstances both the consumption of O and the excretion of CO_2 were diminished by more than one-half in the course of an hour or two. Thus [1] in one case the consumption of O fell

from 1740 cm. to 750 cm. per hour;

the excretion of CO_2 fell

from 1560 cm. to 591 cm. per hour.

The diminution observed by Pflüger and others [2] working in his laboratory, as the mean of many experiments, was somewhat less than this, viz.

	O consumed per kilog. per hour in c.cm. (at 0° C., and 760 mm.)	CO_2 excreted per kilog. per hour in c.cm. (at 0° C., and 760 mm.)
Normal rabbit	673·21	570·41
Curarized rabbit	436·20	356·9

that is to say,

in the amount of O 35·2 p.c.
in the amount of CO_2 37·4 p.c.

This diminution of the gases interchanged in respiration is not due to a deficient circulation; for both the blood-pressure and the heart were observed in similar experiments to be unhampered and normal. Nor can it be set down to the cessation of the ordinary muscular contractions of repose, viz. those of respiration and those which serve to maintain the upright position; unless, indeed, they are assumed to have unexpected proportions. It might be caused by some direct action of the curare upon the tissues which give rise to carbon dioxide in the body; and experiments were undertaken by Colasanti [3] to test this supposition. Curarized and non-curarized blood was made to traverse, under precisely similar conditions, the right and left hind limbs respectively of a recently killed muscular dog. On comparing the outflowing blood from the two limbs it appeared that there was no difference in the relative proportions of oxygen and carbon dioxide contained in them. Hence the diminution must be due to some influence of the central nervous system which is cut off when the animals are paralysed by curare.

The experiments in which the spinal cord was divided shewed that, even when the respiratory muscles were left freely acting, the separation

[1] Zuntz, *Op. cit.*, p. 527.

[2] Pflüger, *Op. cit. Archiv f. d. ges. Physiol.*, Vol. XVIII., 1878, p. 302. Finkler und Oertmann, *Archiv f. d. ges. Physiol.*, Vol. XIV. p. 62.

[3] Giuseppe Colasanti, "Zur Kenntniss der physiologischen Wirkungen des Curaregiftes." Pflüger's *Arch. f. d. ges. Physiol.*, Vol. XVI., 1878, p. 157.

of the rest of the muscles from the nervous centres was followed by a diminution

of O consumed	37·1 p.c.
of CO_2 produced	29·92 p.c.

but here the heart was weak and the circulation disturbed[1].

Whatever may be the nature of the influence exerted by the nervous system, it is probably reflex in its origin, and excited by the difference of temperature between the skin and the external medium. Liebermeister and Gildermeister[2] shewed in the case of men that the production of heat and the formation of carbon dioxide increase on the application of cold to the surface of the body; and Röhrig and Zuntz confirmed this in the case of rabbits, by immersing them in cold baths. If, however, the animals be first curarized, immersion in a cold bath no longer stimulates the interchange of oxygen and carbon dioxide, but rather tends to diminish the amount of both[3]. In other words, the reflex mechanism is in abeyance, and the bath, by directly cooling the tissues, renders their various processes more sluggish.

Curare-poisoning seems to have no diminishing influence over the nitrogenous excretions of the urine[4].

With regard to the constitution of muscle itself after separation from its cerebro-spinal centres, it is said that it contains less creatine[5] but more glycogen[6].

Sect. 4. Fatigue, Exhaustion and Revival.

Signs of Fatigue. Muscles are incapable of contracting continuously for an indefinite time. They become fatigued more or less quickly, and are finally exhausted, when the most powerful stimulus fails to cause a contraction. The evidence of fatigue is a slow contraction of small amplitude. The muscle contracts slowly to its maximum, which is abnormally small; but especially does it elongate more slowly and less perfectly than usual, approximating the condition of the 'idiomuscular contraction' (p. 343)[7]. The rate of transmission of the wave of excitation is also probably diminished during fatigue[8].

[1] Pflüger, *Op. cit.*, p. 320.

[2] Quoted by Röhrig und Zuntz, *Op. cit.* Pflüger's *Arch. f. d. ges. Physiol.*, Vol. IV., 1871.

[3] Pflüger, *Op. cit.*, p. 303.

[4] Voit, *Zeitsch. für Biol.*, XIV. p. 57, 1878. See Hofmann and Schwalbe's *Jahresberichte*, Vol. VII. pt. iii. p. 272.

[5] Sczelkow, *Centralblt. f. d. med. Wiss.*, 1866, p. 481 (*Original not seen*).

[6] Macdonnel, "On the formation of Sugar and Amyloid substance in the Animal Economy," *Proceed. Roy. Irish Acad.*, Vol. VII. p. 276, 1860. Also *Observations on the Functions of the Liver*, Dublin, 1865, p. 23. Ogle, "A Hypothesis as to the ultimate destination of Glycogen," *St George's Hospital Reports*, III. p. 149, 1868. Chandelon, "Ueber die Einwirkung der Arterienunterbindung u. der Nervendurchschneidung auf den Glycogengehalt der Muskeln." Pflüger's *Arch. f. d. ges. Physiol.*, Vol. XIII. p. 626, 1878.

[7] See also the condition known as "Contractur, or remnant of contraction," which follows powerful direct stimuli: Tiegel, Pflüger's *Arch.*, Vol. XIII. p. 71, 1876, and Hermann, Pflüger's *Arch.*, Vol. XIII. p. 370.

[8] See Hermann's *Handbuch der Physiologie*, Vol. I. Abth. i. p. 58.

Measure of Fatigue. Experiments of H. Kronecker.

The progress of fatigue may be gauged by the effects produced in a muscle on applying at intervals a constant stimulus. When a muscle is tetanized the course of fatigue is indicated by the gradual extension of the tetanized muscle, which takes place at first with accelerating, but afterwards with diminishing velocity, until the original length is almost attained. When the muscle (of a frog), which is moderately weighted, is stimulated at regular intervals of 2—12 seconds with a constant (maximal) stimulus from an induction-machine, the heights through which the weight is lifted diminish regularly in an arithmetical series; *i. e.* the curve of fatigue forms a straight descending line[1].

Such a series of lifts may be called a fatigue-series, the members of which exhibit a constant difference D. The value of D diminishes as the intervals of stimulation increase; but with constant intervals it is independent of the load which the muscle is made to lift. That is to say, the curves of fatigue with different loads are a parallel series of descending lines so long as the intervals of stimulation remain the same for all the loads.

Causes of Fatigue and Exhaustion.

The causes of fatigue and exhaustion are very obscure; but since the fatigue of muscles in which the circulation has ceased may be readily removed by renewing the current of blood or even by washing out the blood-vessels with indifferent salt solutions, especially such as contain permanganate of potash (·05 per cent.)[2], we may suppose them to be due either to the accumulation of the products of contraction, or to the defect of constituents, such as oxygen, which the blood can supply, or to both these causes combined. It is at least certain that carbon dioxide has an injurious influence upon muscles[3], which is shared by the lactic acid arising during contraction[4]; while it has been observed that the addition of ·05 to ·1 p. c. of sodium carbonate to a ·6 p. c. salt-solution enhances its power of maintaining the activity of a frog's heart fed with such a solution[5]: the beneficial effect of the addition is gradually lost as the salt-solution continues to be used; but it may be regained by adding a fresh supply of the carbonate, or by shaking up the old solution in the air. Ranke found that all acids have a diminishing influence on irritability. It is said that lactic acid diminishes also the electromotive force of muscle[6]. The accumulation of these products, therefore, could not fail to promote exhaustion. It is also certain that the renewal of the blood current through muscle is swiftly followed by the revival of fatigued muscles;

[1] H. Kronecker, "Ueber die Ermüdung u. Erholung der Muskeln." *Ber. der math-phys. Classe der k. sächs. Gesell. der Wissensch.*, 1871, p. 690.

[2] H. Kronecker, *Loc. cit.*, p. 694.

[3] Georg Liebig, Hermann, *Op. cit.*

[4] J. Ranke, *Tetanus.* Leipzig, 1865, p. 350.

[5] Stiénon, "Die Betheiligung der einzelnen Stoffe des Serums an der Erzeugung des Herzschlages." *Arch. f.* (*Anat. u.*) *Physiol.*, 1878, p. 263.

[6] Ranke, *Op. cit.* Roeber, *Arch. f. Anat. u. Physiol.*, 1870, p. 615. (*Original not seen.*)

and that this is largely due to the presence of oxygen is shewn in the experiments of Ludwig and A. Schmidt already described (p. 380) (Kronecker). But how far the accumulation of the products of contraction, or the defect of oxygen, and probably of other constituents of normal muscle, can be trusted to explain the fatigue of muscles which are still within the body, it is impossible to say.

Stenson's Experiment. It is well known[1] that ligature of the blood-vessels supplying muscles is followed by paralysis and rigor of the muscles: and the same consequence follows any stoppage whatever of the blood-current. Renewal of the current, on the other hand, is followed by a restoration of irritability provided that rigor is not complete. There can be little doubt in this case, that the paralysis is in part due to an interruption of the normal exchanges between muscles and the blood, *i. e.* to the accumulation of carbon dioxide and the acid of rigor, and to the defect of oxygen, etc.

Among the mechanisms which contribute to the revival of fatigued muscles or the prevention of exhaustion, must be mentioned the vaso-motor nerves. Ludwig and Sczelkow[2] discovered that venous blood flows more rapidly from contracting than from resting muscles,—a phenomenon which has been investigated by the pupils of Ludwig and traced to the vaso-motor system. The dilatation of the blood-vessels of muscle, upon which the accelerated outflow seems to depend, also accompanies the reflex stimulation of muscles, and is said to be visible under the microscope in the case of frog-muscles, even when the circulation is stopped and the blood-pressure abolished[3].

It will be remembered that the first effect of subjecting a muscle to a vacuum was to *increase* its irritability (p. 371): and that the same result followed the withdrawal of blood from muscles in the experiments of Ludwig and A. Schmidt (p. 379). No explanation of this has yet been given.

Sect. 5. The Theory of Muscular Activity.

The archives of Physiology contain many curious speculations as to the nature of muscular motion; nor is this surprising when we reflect that the power of self-movement must ever have appeared the chief and most characteristic attribute of animals. Many of the hypotheses are extremely fanciful, and most of them are incomplete, offering to explain certain elements only of the complicated act of contraction. Indeed few theories are burdened with a heavier task than that which comprehends all the phenomena of a muscular act. It must explain how nervous stimuli affect muscle, and how contraction is transmitted

[1] Steno, quoted by Schwammerdam (*de Respiratione*, Leyden, 1679, p. 61), and by Haller (*Elementa Physiol.*, IV. p. 544. 1762).

[2] Ludwig and Sczelkow, *Op. cit.* See p. 375.

[3] See *inter alia* the researches of Gaskell, *Studies from the Physiol. Lab. of Cambridge*, III. 1877 (*Journal of Anat. and Physiol.* Vol. XI.); *Journal of Physiol.*, ed. by M. Foster, Vol. I., 1878.

along the fibres; it must satisfy us as to the actual method of shortening—as to the momentary disturbance of the structural elements of the fibre; and, finally, it must account for the origin of the heat, electrical disturbance and mechanical motion which are characteristic of muscular contraction, and suggest some relationship among them. Most theories have been content to explain one or other of these points without attempting the whole.

The point which most nearly concerns the physiological chemist is the last mentioned, viz. the origin and interdependence of the heat, electrical tension and mechanical motion of contraction, and this alone will be considered in the present chapter. The probable nature of nervous action will receive consideration elsewhere in this book; and the views of observers as to the appearances of contracting muscle beneath the microscope will be found in Manuals of Histology.

John Mayow.

One of the most remarkable anticipations of modern discovery and opinion occurs in the works of John Mayow, published in 1668—1674[1]. Exactly one hundred years before the discovery of oxygen, Mayow demonstrated by a series of conclusive experiments, that some portion or constituent of the air is necessary to combustion, and that the same substance is equally indispensable to living animals. To this portion of the air he gave the name of *particulae igneo-aëreae*. The same substance enters into the composition of nitre; since, when nitre is present, combustible bodies can inflame and burn even *in vacuo* or beneath water. Hence Mayow called the igneo-aërial particles, *nitro-aërial particles* and *nitro-aërial spirit*. This substance, though indispensable to combustion, does not itself burn; but when antimony is calcined in the focus of a burning-glass, or by exposure to the flame of nitre, the antimony becomes not a little increased in weight—a circumstance which Mayow could only explain by supposing the fixation of igneo-aërial particles in the calcined antimony[2]. Respiration introduces igneo-aërial particles into the blood, where they meet with salino-sulphureous (*i.e.* combustible) particles and produce the animal heat[3]. From the blood the igneo-aerial particles are conveyed to the muscles[4], where they meet with

[1] John Mayow, *Tractatus quinque Medico-physici*. Oxford, 1674.

[2] J. Mayow, *de Sal-nitro et spiritu nitro-aëreo*, Chap. iii. p. 28. "Hinc etiam facit, quod Antimonium non tantum à Spiritu Nitri, radiisque solaribus, sed etiam à flamma nitri, in qua particulae nitro-aëreae densius agglomerantur, virtutem Diaphoreticam acquirit. Neque illud praetereundum est, quod Antimonium, radiis solaribus calcinatum, haud parum in pondere augetur; uti experientiâ compertum est: quippe vix concipi potest, unde augmentum illud Antimonii, nisi à particulis nitro-aëreis, igneisque ei inter calcinandum infixis, procedat."

[3] *Ibid.*, Chap. viii. p. 151.

[4] J. Mayow, *de Motu Musculari et spiritibus animalibus*, *Op. cit.*, part ii. p. 3. "Spiritum nitro-aëreum respirationis ope in Cruoris massam transmitti, Sanguinisque Fermentationem, et Incalescentiam ab eodem provenire, alibi à nobis ostensum est. Iam vero circa usum Spiritûs istius inspirati addo insuper, quòd idem in Motibus Animalibus instituendis partes primarias sortiatur: quam quidem opinionem à me jam olim in medium prolatam, etiamnum firmitèr retineo, non quòd praeconceptae Hypothesi mancipatus, eam, uti moris est, mordicùs defendere constitui, sed quòd eandem rationi maxime consentaneam arbitror."

other salino-sulphureous particles secreted from the mass of the blood, and by union with these cause an effervescing which produces muscular motion. How the nitro-aerial or igneo-aerial particles reached the muscles, Mayow did not feel quite certain. He was sometimes inclined to think that they proceeded directly to the muscles from the blood; but it appeared more probable on further reflection, that the salino-sulphureous or *motive* particles alone were supplied directly by the blood; while the nitro-aerial particles approached the muscles through the brain and nerves, being the same, in short, as the *animal spirits*. Why, asked Mayow, should not the animal spirits be derived from the air rather than from the food? Indeed, it seemed to him impossible that the immense waste of animal spirits could be supplied from any other source. The salino-sulphureous or combustible nature of the *motive* particles was thought to be shewn by this, that in violent exercise no small loss of fat occurs, and if exercise is long continued fat almost disappears: on the contrary, animals leading an easy inactive life grow fat, and fat appears in large quantity in the muscles. A supply both of igneo-aerial and of salino-sulphureous particles is indispensable to continued animal motion. As movement is increased and more of each sort of particles is wasted, more of them must be added to the body. Not only must respiration be enlarged, but more food containing salino-sulphureous parts must be taken. Hence those substances which contain much volatile salts and sulphur (*i.e.* combustible matter) are best fitted to recruit the frame worn out by protracted labour. Finally, Mayow clearly recognized that the animal heat arises, not solely in the union of nitro-aerial and combustible particles in the blood generally, but in that special union which is accomplished in muscles during muscular contraction: part of the heat of an animal in violent exertion arises in the union of nitro-aerial and salino-sulphureous particles in muscle[1].

This was in 1674. When we remember that it was nearly two hundred years before physiological science fully overtook the speculations of Mayow,—that, although oxygen was discovered in 1774, it was not until 1861 that Moritz Traube definitely announced that muscular contraction depends upon the combustion of *non-nitrogenous* matters in muscles themselves—we shall feel no surprise that Mayow's work was so speedily forgotten. Scientific judgment must have been strangely uneducated to have allowed the experimenters of that day to read and lose sight of observations which seem to us now so exact and suggestive. It has been the fate of Mayow, which his genius little merited, instead of leading science, to be twice revived by antiquarian zeal, at the very moment when his discoveries had been made over again by independent observers. After the researches of Priestley, Scheele and Lavoisier had made brilliant

[1] *Op. cit.*, part i., *de sal-nitro et spiritu nitro-aereo*, p. 152. "Quanquam calor iste in animalibus, per exercitia violenta excitatus, etiam ab effervescentiâ particularum nitro-aërearum et salino sulphurearum in partibus motricibus ortâ, partim provenit, ut alibi ostendetur.

the close of the last century, the importance of Mayow's work was proclaimed by the enthusiasm of Dr Beddoes[1] and Dr Yeats[2]; and in 1864, at a time when the theory of muscular activity had already received its present bent, the acute speculations of Mayow in reference to it were again most honourably made known by Professor Heidenhain[3].

It would be pushing literary justice to the extreme verge of pedantry to pretend to find in authors earlier than Mayow the germs of a theory which they were not in a position even to comprehend; still it is interesting to observe that the general idea of a combustion of matters within the body, upon which the powers of life depend, is to be found in a book with which Mayow was probably familiar. Francis Bacon, in his *Historia Vitae et Mortis*, taught that all living beings contained two kinds of spirits, *spiritus mortuales* which fill inanimate objects, and *spiritus vitalis* which confers life. The doctrine of a vital principle stirring and regulating the members of living creatures had existed, in one form or another, from the earliest times; but more than this Bacon taught that the *spiritus vitalis* exhibited a certain *incensio*, or combustion, which gave rise to peculiar motions and powers. "In omnibus animatis duo sunt genera spirituum : spiritus mortuales, quales insunt inanimatis; et superadditus spiritus vitalis.....Sunt autem duo discrimina praecipua inter spiritus mortuales et spiritus vitales.....Alterum discrimen inter spiritus est; quod spiritus vitalis nonnullam habeat incensionem, atque sit tanquam aura composita ex flamma et aëre; quemadmodum succi animalium habeant et oleum et aquam. At illa incensio peculiares praebet motus et facultates; etenim et fumus inflammabilis, etiam ante flammam conceptam, calidus est, tenuis, mobilis; et tamen alia res est, postquam facta sit flamma; at incensio spirituum vitalium multis partibus lenior est quam mollissima flamma, ex spiritu vini, aut alias; atque insuper mixta est, ex magna parte, cum substantia aërea; ut sit et flammae et aëreae naturae mysterium.....Neque tamen ulla ex ipsis actionibus unquam actuata foret (*i. e.* of the stomach, liver, heart, brain etc.), nisi ex vigore et praesentia spiritus vitalis et caloris ejus[4]."

The obvious and extreme importance of air for the support of life, and the muscular weakness which follows excessive bleeding, did not escape the earliest observers, and were the foundation of hypotheses which have been thought to foreshadow the modern view[5].

Glisson. Haller.

After the time of Mayow, the doctrine of muscle was mainly given over to the Stahlists. Armed with his conception of an immaterial and rational *anima* endowed with unlimited spontaneous powers over matter, Stahl explained nearly all things with equal facility, and among them muscular

[1] Thomas Beddoes, *Chemical Experiments and Opinions extracted from a work published in the last century.* Oxford, 1790.

[2] G. D. Yeats, *Observations on the claims of the Moderns to some discoveries in Chemistry and Physiology.* London, 1793.

[3] Heidenhain, *Mechanische Leistung, etc.* Leipzig, 1864.

[4] Francis Bacon, *Historia Vitae et Mortis*, 1623, Can. IV. and V. "Works," by Spedding, Ellis and Heath, 1857, Vol. II. p. 214.

[5] Al. von Humboldt, *Versuche ü. die gereizte Muskel- und Nervenfaser*, Vol. II. pp. 91, 93. 1797.

motion[1]. His opinion generally prevailed until the time of Haller, whose doctrine of the independent irritability of muscle marks the next advance in the theory of muscular contraction. The term Irritability was not indeed new to Physiology. The name, and in a certain sense the notion also, was introduced by Glisson in the latter half of the seventeenth century. He taught that irritability was a property of the elements of our bodies, even of the bones and juices, which was to be attributed to a *natural perception* unaccompanied by any sensation whatever. It was supposed to depend upon 'Archaeus who is the framer of his own body'; and it could be demonstrated after death by the application of acid and pungent liquors[2]. But it was Haller who first gave the idea a firm foundation in experiment. Resting on the experiments of Haller and his pupils, this important doctrine was definitely formulated in a Treatise on the Sensible and Irritable parts of Animals[3]. Irritability was defined as the property, possessed by muscular fibres alone in the body, of shortening when they are touched; while those parts were called sensible which, when handled, transmit the impression of the touch to the soul, or, in animals, lead to evident signs of pain and disquiet. Irritability is distinct from sensibility, since nerves, the most sensitive of structures, are absolutely devoid of irritability. Stimuli applied to nerves, however, lead to convulsions and palpitations of neighbouring muscles, *but only in such as are directly supplied by the nerve stimulated.* Muscles contract after separation from the brain, after their nerves are all cut away, and even after removal from the body. Hence irritability is a property quite apart from the soul and the nerves. Haller thought it probable that, some time or other, the use of the nerves with regard to the muscles would be reduced to conveying to them the commands of the soul, and to increasing and exciting that natural tendency which the fibres have of themselves to contract[4]. The property of producing motion is different from all other properties of bodies, and it probably resides in the glutinous mucus rather than in the earthy parts of muscles. It is a property of muscles as gravity is a property of matter generally, and it is doubtless owing to a physical cause depending on the arrangement of ultimate particles. It is destroyed by drying the fibre, as well as by opium.

Whytt.

The most active opponent of Haller in this country was Robert Whytt[5], Professor of Medicine in the Univer-

[1] Georg. Ern. Stahlius, *Theoria Medica Vera*, 1708. Ed. Lud. Choulant. Lips., 1831. Tom. I. sec. vi. p. 466. *Georg Ernst Stahl's Theorie der Heilkunde.* Dargestellt von Wendelin Ruf. Halle, 1802, p. 206.

[2] Francis Glisson, *de Ventriculo et Intestinis*, c. vii. Quoted by Haller, *Op. cit., infra.*

[3] Haller, "de Partibus corporis humani sensilibus et irritabilibus." *Commentarii Soc. reg. Scientiarum Gotting.* Tom. II. 1752, p. 114. *A Dissertation on the Sensible and Irritable parts of Animals.* Translated from the Latin. London, 1755.

[4] Haller, *Loc. cit.*, p. 139.

[5] Robert Whytt, *Physiological Essays*, Edinburgh, 1766. Third Edition. *On the Vital and other Involuntary Motions of Animals*, Edinburgh, 1763, Second Edition.

sity of Edinburgh, whose criticism of Haller displays the greatest ingenuity and address. This acute observer was not a disciple of Stahl; indeed his doctrine was disclaimed by the purer Stahlists; but it forms a link between the teaching of Stahl and the doctrine of vital force of the next generation. According to him all parts of the body are pervaded by a *Sentient Principle* which is affected more or less acutely by stimuli or irritants; and the motions which invariably follow irritation *and are always in proportion to the strength of it*, are the endeavour of the pervading principle to remove the part from the source of irritation; it acts upon the muscles through their nerves, but in a manner altogether obscure. Whytt's sentient principle is the soul of the Stahlists shorn of its rationality and spontaneity, and bound by an original decree to the task of responding by movements to every stimulus impressed upon the body. This principle remains for a time in parts amputated from the body; hence such parts are capable of contractions when touched. It is the merit of Whytt to have insisted upon the importance of the stimulus in all involuntary actions, and the invariableness of the motions excited by it.

John Hunter.

The experiments of Haller dealt a fatal blow to Stahlism and the like. Already in the writings of Whytt we see this doctrine subsiding into the simpler one of vital force, as it is implicitly adopted, for instance, in the pages of John Hunter. Hunter[1] was content to classify muscular motion as one of the forms of the movement of matter; of which the attraction of masses owing to gravitation was another form and the elective attraction of chemical substances was a third. He thought that it most probably arose from construction: but it was a principle in action very different from the attractions in common matter, and equally unintelligible with gravity and chemical attraction. In short, the current view of the cause of muscular motion was that it was original, a *vis insita*, a vital power peculiar to living tissue during its life.

Fothergill, and Girtanner.

It was not long, however, before the doctrine of vital force began to be expanded. The re-discovery of oxygen had quickened philosophical speculation, and seemed to have placed in the hands of physicians a remedy of the greatest promise. One of the first methods of treatment to be benefited by the new chemical discoveries was the art of restoring suspended animation. Inflation of the lungs was empirically known to be extremely useful in such cases; but it was Dr. A. Fothergill[2] who first suggested an explanation of its value which, if not wholly true, was true in the greater part. "In all cases of suspended animation the grand intention ought to be, to excite the latent principle of irritability on which the motion of the vital organs immediately depends." And how, asks he, can this be better done than by

[1] Croonian Lecture, 1781. *Works*, Ed. by Palmer, Vol. IV. p. 255.

[2] A. Fothergill, *Hints for improving the art of restoring Suspended Animation*, 1782, pp. 15, 17, 18.

inflating the lungs, not with common air used again and again, but with fresh supplies of *dephlogisticated air?* If dephlogisticated air supports the flame of a taper with a splendour hardly credible by those who have not witnessed the experiment, is it not clearly indicated as peculiarly fitted for restoring the vital spark when nearly extinguished?

The theory thus suggested soon received a remarkable amplification in the hands of Girtanner[1]. In an essay, exhibiting great research (but at the same time shewing that a restless desire to have an answer of some kind to his questions had led its author to content himself with mere specious explanations), the principle of irritability is identified with oxygen. All organized parts, whether of the animal or vegetable kingdom, are considered to be capable of irritability, and the irritable fibre is one and the same in nature wherever found. Even the fluids of the body exhibit an irritable contraction (*i.e.* coagulation) and obey the general laws of irritability. The principle of irritability, or oxygen, is received by the blood in the lungs, and conveyed to all parts of the body, where it is stored up in the irritable fibres. There is a normal quantity for each fibre, upon which its *tone* depends; and the normal is preserved by the ceaseless action of habitual stimuli, such as heat, light, nourishment, circulation of the juices, etc. which *withdraw* the surplus. Thus the health of the fibre depends upon an even balance of gains and losses of oxygen. If the gains are excessive, irritability becomes increased. If the losses are excessive through the extraordinary action of the habitual stimuli, irritability sinks or disappears altogether. All reagents which are thought to be capable of acting upon irritable fibres are divided into three classes according as their affinity for oxygen is greater than, equal to, or less than, that of the fibres. The former abstract oxygen from the fibres and depress their irritability: such are opium, alcohol, fat. The latter impart oxygen to the fibres, producing a super-irritability which is often extremely fatal: white oxide of arsenic, *l'acide muriatique oxigéné*, are eminent examples of this class. The intermediate class behave as neutral bodies, until change of temperature, or some other condition, removes them into the first class or the third. Thus irritability is always in proportion to the oxygen which an irritable organ or fibre contains; and whatever augments or diminishes the oxygen of the body likewise augments or diminishes its irritability.

Whether Girtanner had ever read Fothergill's *Hints* does not appear, nor is it of importance to enquire; but it is certain that Fothergill, four years after the publication of Girtanner's Memoirs, expanded his original conception into a theory of irritability which is practically identical with Girtanner's[2]. Vitality, in his view, consists

[1] Girtanner, "Mémoires sur l'Irritabilité, considérée comme principe de vie dans la Nature organisée." *Observations sur la Physique*, ed. by Rozier and de la Métherie. 1790, Vol. xxxvi. p. 422; Vol. xxxvii. p. 139.

[2] A. Fothergill, *On the Suspension of Vital Action*, Bath, 1795, p. 67 and elsewhere. This Essay gained the Gold Medal of the Royal Humane Society in 1794.

in action and reaction between the vital organs and their respective (habitual) stimuli, exactly in the sense of Girtanner. Irritability co-exists with animal heat and keeps pace with it through life: hence it probably has a similar origin. But, inasmuch as animal heat can be shewn to be dependent upon vital air (oxygen), whose latent heat, in short, is the source of the animal heat, may not vital air be also the *proximate cause of irritability?*

Beddoes. The above view was received with much favour, and therapeutical use was freely made of it. Dr Beddoes discussed it in his *Remarks on Girtanner's Essay,* and is stated to have specially pointed its reference to the case of muscle by the question, Does muscular action or intumescence really depend upon the combination of oxygen with hydrogen or azote (separately and combined in various proportions) in consequence of a sort of explosion produced by the nervous electricity[1]? The advances which scientific theory makes are often so insidious that we are apt to underrate their importance. It is hardly too much to say that this question of Beddoes marks the real point of departure of the modern views of muscular irritability. Previously, the prevailing tone of thought had been semi-metaphysical. Oxygen had been regarded as a *principle,* the *presence* of which conferred irritability upon tissues, and the withdrawal of which from the organs and fibres happened to be effected by its union with other elements. Now attention was concentrated upon the union itself rather than on the uniting bodies, and irritability was regarded as the result of a process, and not the attribute of a substance. A taper, a lamp, a fire, became the fashionable metaphors wherewith to illustrate various physiological acts. Food was not useful food if it had no affinity to oxygen. Life itself was but the burning of a lamp of which the body is the wick and food the oil.

Brandis. Illustrations like these did not go uncriticised. "Such metaphors," remarked Brandis[2], the German translator of Darwin's *Zoonomia,* "are apt to cast shadows where we would fain have light." It is indeed certain, argues this author, that phlogistic processes occur in the body as in the combustion of other substances: carbon is united to oxygen and expired as carbonic acid gas; phosphorus appears to become acidified in the body and is excreted in the urine in combination with lime; and such probably is the case with other constituents of the fibres. But there is as yet nothing to shew that these bodies can excite themselves to union. Some external force is needed to start the combustion, and to determine the intensity of it in any particular act. This is the unknown and subtle *vital force,* which is as indispensable to the vital processes as the spark is to the kindling of a fire.

[1] Yeats, *Op. cit.*, p. 171. Al. von Humboldt, *Op. cit.*, Vol. II. p. 105. The original *Remarks* seems to be somewhat rare, as neither Prof. Rudolf Heidenhain nor the author has succeeded in obtaining a copy.

[2] J. D. Brandis, *Versuch. ü. die Lebenskraft.* Hannover, 1795.

Reil and von Madai. On the other hand, Reil and his brilliant pupil von Madai[1] avoided even the semblance of a vital or irritable principle by referring to the structure and chemico-physical changes of living matter all the peculiar phenomena of life. As to the nature of the changes Reil was not explicit; and, although von Madai adopted provisionally the term *phlogistic processes*, because carbon and oxygen play so important a part in them, it was with a wise reservation as to their exact nature and method (*loc. cit.* p. 101).

Humboldt. It was, however, Humboldt[2] who first denied with special emphasis the exclusive importance of oxygen in the vital processes. Many observations had disclosed to him vital processes in which oxygen takes none but a subordinate part. To speak of life as an oxidation is to take a one-sided and distorted view of vital phenomena. Oxygen in his opinion is a most important stimulus, but not the common basis, of irritability. It is true that many phlogistic processes occur in the performance of vital functions: but how many other chemical decompositions go forward which do not so much express the affinity of oxygen for phosphorus, azote, hydrogen and carbon, as the affinities of these for one another? Thus physiology became accustomed to the absence of a particular contractile or irritable principle. First its office and dignity were conferred upon oxygen, and then oxygen was reduced to the rank and privileges of a common chemical element.

J. Liebig. The doctrine of vital force in muscular action by no means at once gave place to the views of the physico-chemical school. It remained indeed the prevalent doctrine until the time of Baron Liebig[3], who attempted to bring it into relation with the most recent discoveries of Physiological Chemistry. The vital force, resident in animals and plants, finds its scope of action in the presence of a certain structure and organization of parts. It is governed by laws in harmony with the universal laws of resistance and motion; nevertheless it is independent of the matter in which vitality is manifested. It is this vital force which keeps living matter from decomposition even in the presence of oxygen, which determines its growth, and which also causes the movements of animals. Besides a special organization of substance, a certain temperature and a constant supply of food are indispensable conditions of its activity. A muscle therefore is an organ endowed, by virtue of its vital properties, with certain powers of self-preservation, growth and motion. While it is at rest, and exposed to the influence of oxygenated blood, the vital force is absorbed in restraining the natural tendencies to

[1] J. C. Reil, "Ueber die Lebenskraft," *Arch. für die Physiologie* (Reil), Vol. I. Heft i. p. 8. 1796. D. von Madai, "Ueber die Wirkungsart der Reize und der thierischen Organe." *Ibid.*, Vol. I. Heft iii. p. 68. 1796.

[2] Al. von Humboldt, *Versuche ü. die gereizte Muskel- u. Nervenfaser.* Vol. II. p. 106 *et seq.*

[3] J. Liebig, *Animal Chemistry, or Organic Chemistry in its applications to Physiology and Pathology.* Translated by W. Gregory. London, 1842.

disintegration and union with oxygen. But if a portion of the vital energy is diverted for the purposes of contraction, the natural inclination of the muscle to change is, in part, unchecked, and a certain portion of the tissue becomes oxidized and dead. For every motion of contraction there is a material exchange, with an absorption of oxygen, and a certain amount of tissue cast off. The relationship among these is invariable: for every portion of force expended in motion there is a definite proportion of tissue wasted and oxygen absorbed.

Besides attacking living tissues which are for the moment left unprotected by the vital force, the oxygen which is absorbed into the body attacks those also which are already lifeless; whence arises the heat and the proper temperature of the body which is so important a condition of vitality. The production of heat and force in the body are indeed closely related; but heat can be produced without any change in the *living* elements of the body, while mechanical effect is always proportional to the amount of living matter which loses the condition of life. However closely the conditions of this twofold production seem to be connected in regard to mechanical effects, yet the disengagement of heat can in no way be considered as in itself the cause of these effects. All experience proves that there is in the organism but one source of mechanical power, the conversion of living into lifeless compounds.

Thus for every portion of oxygen taken into the body there is a corresponding proportion of heat and mechanical force produced. Further, the amount of tissue metamorphosed in a given time is measured by the nitrogen in the urine; and the sum of the mechanical effects produced in each of two individuals at the same temperature, is proportional to the nitrogen excreted by each.

J. R. Mayer.

The views of Liebig were not left unchallenged. J. R. Mayer[1], the early apostle of the conservation of energy, in his treatise on organic movement in relation to material exchange, exposed the inconsistencies of the doctrine of vital force as expounded by Liebig, and stated that not only the heat, as Liebig admitted, but also the muscular motions, of animals had but one source in the oxidation of combustible matters. He calculated from the combustion-heat of carbon, that the extraordinary consumption of combustibles by a labouring animal, bearing in mind the enlarged production of heat during labour, is fully competent to account in a natural way for the work done. But the combustions in which the animal movements take their origin are not combustions of the substance of muscle itself. To account for the production of motion in this way, we should have to assume a rapid destruction and restoration of muscular tissue, of which there are no sufficient histological and physiological signs. The oxidations take place rather in the blood, which is the true oil for the flame of life.

[1] J. R. Mayer, *Die organische Bewegung in ihrem Zusammenhang mit dem Stoffwechsel. Ein Beitrag zur Naturkunde.* Heilbronn, 1845.

Thus the oxidations of the body result in the generation of heat and of motion, which therefore, within limits, are complementary to each other. With a certain chemical combustion, the greater the mechanical effects produced, the less the amount of heat which appears; and conversely. During the performance of mechanical work a proportion of the heat which would otherwise have been sensible becomes 'latent,'—this proportion being equivalent to the work done.

In this manner J. R. Mayer emancipated muscle from the doctrine of vital force, and taught the true source of muscular power in the chemical union of substances. Muscles, according to him, are an apparatus for the conversion of chemical difference into mechanical effect, just as plants are an apparatus for converting light into chemical difference; and this power of living muscle is what constitutes *irritability*. At the same time experiment was surely establishing the other opinion which Mayer had, on theoretical grounds, opposed to Liebig's teaching, viz. that muscular exercise is not associated with an extraordinary destruction of the nitrogenous substance of muscle.

Voit. The experiments of Voit[1] on dogs, which have already been described, may be said to have effected the final overthrow of the older views. Voit himself seems to have mistaken the meaning of these experiments. He was compelled to admit that no more nitrogenous waste occurred in muscular exercise than in muscular rest; but he appears to have taken no account of the well-marked increase of respiratory products in the same circumstances. He drew the conclusion that no more energy is expended during exercise than during rest, but the same energy takes another form; and as he found no evidence that this transformation was one of heat into mechanical motion, he supposed that it was a conversion of electrical energy. This view was never much encouraged.

M. Traube. Moritz Traube, on the other hand, who was investigating the subject when Voit published his researches, recognized at once the great importance of the experiments, and explicitly formulated the view that no albuminous body is used up in muscular contraction. On the contrary, muscles contribute rather to the non-nitrogenous respiratory excretions. They are a chief seat of the oxidations of the body, and by means of their nerves the oxidations which occur in them are made to yield mechanical energy.

Matteucci. A still more interesting advance in the theory of muscular contraction concerns the oxygen which serves the oxidations of muscle. Already in 1856 Matteucci[2] had remarked

[1] C. Voit, *Untersuch. ü. den Einfluss des Kochsalzes, etc., auf den Stoffwechsel.* München, 1860.

[2] Ch. Matteucci, "Recherches sur les phénomènes physiques et chimiques de la contraction musculaire." *Ann. de Chimie et de Physique*, 3e Série, Vol. XLVII., 1856, p. 129.

that muscles which for some hours had been rigorously excluded from contact with oxygen gas, were yet capable of yielding carbon dioxide, especially when in the act of contraction; and he had concluded that the oxygen which in 'muscular respiration' forms the carbon dioxide is not the oxygen of the air but oxygen which exists in muscle in a state of chemical combination. The same circumstance attracted the attention of Traube. In his view, oxygen enters muscle from the blood and unites there in some loose chemical combination, from which it is readily abstracted by the oxidizable bodies of the muscle juices. Muscular substance reacts to oxygen and reducing substances like indigo, cupric hydrate or the vinegar ferment, and its action is more perfect and rapid at the higher temperatures. Complete deoxidation of a muscular fibre brings with it *death-rigor*, while complete saturation with oxygen implies a perfect irritability.

Heidenhain. In this manner the doctrine of muscle was beginning to assume its present outlines when Heidenhain[1] demonstrated that the heat and mechanical work produced in contraction are not complementary—that, in short, they vary in a similar although not quite identical manner, when subjected to the same conditions of tension, etc. The hypothesis of J. R. Mayer that mechanical work arises at the expense of heat in muscle, which many observers had endeavoured to sustain, became finally untenable; and it was now necessary to assume that the heat-evolving and work-evolving processes of muscle were in some degree independent of one another.

Hermann. At this point Hermann[2] began his well-known examination of 'muscular respiration,' most of the results of which have already been presented to the reader. Although it was granted that the oxygen made use of in the formation of carbon dioxide was not taken from the blood at the moment of formation, but was rather stored up in muscle at some time beforehand, yet it seems to have been assumed that the act of formation of carbon dioxide was a true oxidation; and for this reason it had been found necessary to suppose the existence of some body with affinities for oxygen intermediate between those of haemoglobin and the oxidizable matters of muscle. The great and peculiar stability of this hypothetical oxygen-carrier, which could, while easily parting with its oxygen to the oxidizable portions of muscle in contraction, yet steadily resist the action of a vacuum even at high temperatures, was however always a point of great difficulty; and, to avoid it, Hermann surmised that the chemical operation in contracting muscle is not a true oxidation, but rather the splitting up of some complex body with the formation of simpler, more stable, substances. Such decompositions were already known to be capable of yielding energy, and especially heat; as, for instance, when the complex molecule of sugar, in the process of fermentation, splits up into alcohol, carbon dioxide, etc., without the help of oxygen from the air.

[1] Heidenhain, *Mechanische Leistungen, etc.*
[2] Hermann, *Stoffwechsel der Muskeln.*

As to the supposititious substance itself, all that direct observation suggested was that it must be of such a nature as to yield carbon dioxide and some free acid, probably lactic; but by reflecting upon the analogies of contraction and rigor, Hermann was led to assign to it a very complicated structure. The resemblances of contraction and rigor are manifold. In each there occurs a shortening, thickening, and small reduction of bulk of the muscle; and a mechanical force is developed to each, although at very different rates. Both processes are associated with an evolution of heat; and we may now add that contracting muscle and muscle becoming rigid assume the same electric potential in reference to living and resting muscle. With regard to their chemical changes, both processes are independent of the oxygen of the surrounding medium, and both are followed by the appearance of a free acid and the formation of carbon dioxide. Further, there is this relationship between the two in the case of excised muscles, that the more free acid and carbon dioxide are produced by the previous tetanus of the muscle the less are generated on subsequently passing into rigor. Moreover, phenomena are known which seem to be most naturally regarded as intermediate states between contraction and rigor. If a fatigued muscle receives a sharp stimulus, as from a sudden blow, a local (idio-muscular) contraction is produced which lasts for a long time; and if such an exhausted muscle be repeatedly stimulated it may pass at once into true rigor.

If, then, contraction so closely resembles rigor, may we not consider it as a transitory form of rigor, and assume that we have in contraction what undoubtedly occurs in rigor, viz. the separation of a coagulum of myosin? In rigor the coagulum at once passes to a condition of contracted clot: here, therefore, the analogy must end; for in normal irritable muscle the clot never goes beyond the gelatinous stage.

Hermann's hypothesis may thus be summed up: The chemical substratum of muscular activity is the falling to pieces of a complex nitrogenous body, which has been called *Inogène substance*. The products of the decomposition include carbon dioxide, a fixed acid, and a gelatinous albuminous body, of which the first is cast into the blood-current, while the last, and possibly the second also, help to build up again the original compound. The decomposition is constantly occurring, even during the repose of muscle; but in such circumstances restoration keeps pace with destruction. In contraction, on the contrary, destruction largely exceeds restoration. The blood supplies to muscle the non-nitrogenous matter and the oxygen needed for the reproduction of Inogene substance.

Along with the chemical changes of Inogene substance, other changes occur in the regeneration of muscle itself. These affect the nitrogenous as well as the non-nitrogenous elements of the tissue, and help to swell the nitrogenous excreta. In cases of severe exertion it is not improbable that these changes may be unusually large; and this especially would be the case were individual muscular fibres to become rigid and stand in need of absorption and removal.

In this manner the theory of Hermann brings together all the chemical facts of muscular contraction : but it has the further merit of attempting to solve the electrical facts also. It is entirely beyond the scope of this work to explain in detail how this is accomplished. It will be sufficient to state that the key to the most complicated electrical phenomena of resting and acting muscle is to be found in the contact of heterogeneous substances; and that the heterogeneity required by the theory is supplied by the chemical difference which undoubtedly exists between resting muscle on the one hand, and dying or contracting muscle on the other. But, while the theory is so far satisfactory, we must not blind ourselves to the capital imperfection of it, that it does not represent to us how contraction itself takes place. To suppose that in the hypothetical formation of gelatinous myosin the physical particles of muscle are drawn together or suffer a rearrangement, is but to support one hypothesis by advancing another; the explanation of muscular contraction on the view that it is due to the shortening of gelatinous myosin has always appeared to us improbable, and it is certainly not countenanced by any known facts.

CHAPTER X.

THE NERVOUS TISSUES.

Sect. 1. Introductory.

Classification of nerve-organs.

The organs which compose the nervous system of the higher animals may be classed as 1st central organs, such as the brain and spinal cord and the various peripheral or sporadic ganglia; 2nd conducting structures or nerves which are engaged in bringing into communication the central organs with, 3rd, end-organs wherein the fibres of certain of the nerves (afferent) commence, and those of certain others (efferent) terminate.

Grey and white matter of the nervous system.

The large nerve-centres are composed of *grey* and *white matter*, properly so called. In the grey matter reside the nerve-cells which are the characteristic elements of the central organs, and which for the most part certainly have a connection direct or indirect with nerve-fibres.

The white matter is composed of nerve-fibres, making their way to and from the grey matter, and only very exceptionally contains nerve-cells. Both grey and white matter are supported by a connective-tissue framework termed the *neuroglia*; both are supplied with blood-vessels which penetrate from the surrounding membranes, though the grey matter is much more vascular than the white; in both we can trace the commencement of lymphatic vessels.

Nerve-cells.

Nerve-cells are irregular masses of protoplasm, possessed of a well-marked nucleus and nucleolus, and sending out one or more processes. The protoplasm of the cell is often somewhat pigmented (greyish); in the nerve-cells of the ganglia of *Aphrodite aculeata* it has been shewn to be tinged of a red colour, due to the presence of haemoglobin. That part of the protoplasm which immediately surrounds the nucleus is granular, while, in most cases, that which is disposed at the periphery of the cell exhibits a striated appearance which seems to be similar to, and indeed continuous with, that often exhibited by the *axis cylinders* of the nerves. It is beyond the province of this book to

describe all the various forms which nerve-cells present; and mention will merely be made of certain observations which refer to the most marked and readily investigated of these structures.

The processes which are given off by many nerve-cells, as by the nerve-cells of the grey matter of the spinal cord, are numerous, and such cells are often spoken of as *multipolar*. These processes are extremely fragile, but under favourable circumstances they may be observed to give off a number of fine branches. In addition, it has been maintained (Deiters) that one process which is usually distinguished from the rest by its much greater thickness and length becomes continuous with the so-called axis cylinder of a medullated nerve-fibre; such a process would, on this view, place the nerve-cell in *direct* communication with a nerve-fibre.

The other finely ramifying processes anastomose with similar processes from other nerve-cells, giving rise to a *reticulum* from which probably arise the axis cylinders of other nerve-fibres. Such a fine reticulum can readily be seen in the grey matter, though it is sometimes difficult to establish which parts of it are purely nervous and which belong to the connective tissue. Nerve-cells may or may not have a sheath or investment.

We are acquainted with very few facts relating to the microchemistry of the nerve-cells; they are doubtless in the main protoplasmic in composition, and are therefore specially rich in proteid substances. From the analysis of the grey matter as compared with the white, we conclude that the nerve-cells are comparatively poor in the complex phosphorized constituents, and in other bodies, such for instance as cholesterin, which are found in large quantities in nervous organs as a whole. From the abundant supply of blood to the grey matter as compared with the white we may assume that respiratory exchanges go on much more actively in nerve-cells than in nerve-fibres, a conclusion strongly borne out by the previously mentioned discovery of haemoglobin in the nerve-cells of the ganglia of *Aphrodite aculeata*.

Nerve-fibres.

We may conveniently divide nerve-fibres into the two classes of (1) medullated, (2) non-medullated nerve-fibres; the former are much the more abundant.

1. *Medullated nerve-fibres.* When examined in its yet living condition the medullated nerve-fibre presents the appearance of a perfectly pellucid homogeneous structure which might at first be thought to be a tube with transparent walls, containing a transparent liquid; a careful examination of all facts causes one, however, to reject this view without hesitation.

At death the nerve-fibre undergoes changes in its physical constitution, and it then can be shewn to present (1) a highly transparent membranous envelope, termed the *neurilemma*, in which, or beneath which, are oval flattened nuclei, (2) a central structure, the *axis cylin-*

der, and (3) between the neurilemma and the axis cylinder a white, highly refracting substance, known as the *medullary sheath* or *white substance of Schwann*. At intervals, the white substance is interrupted (nodes of Ranvier).

2. *Non-medullated nerve-fibres*. These differ from the medullated variety in the absence of the white substance of Schwann. They consist of an axis cylinder sheathed in a nucleated neurilemma.

The neurilemma may be absent from both medullated and non-medullated nerve-fibres.

The neurilemma. The very transparent and thin membrane which forms the wall of the nerve-fibre appears to possess characters which closely resemble if they are not identical with those of the sarcolemma. When a medullated nerve-fibre enters a muscular fibre, the neurilemma loses itself upon, and becomes continuous with, the sarcolemma (Kühne). By prolonged boiling both neurilemma and sarcolemma yield gelatin.

The axis cylinder. This structure, which under a high power of the microscope presents the appearance of a cylindrical band, exhibiting marks of fibrillation, is certainly of solid consistence during life, and is composed of a mixture of proteid with complex fat-like bodies. It is partly soluble in a weak aqueous solution of hydrochloric acid (1 to 1000), and in a 10 per cent. solution of common salt. It is not collagenous. It reduces gold solutions very readily in the presence of light; it is stained by ammoniacal solutions of carmine, which leave the white substance of Schwann unstained; this action of carmine is probably dependent upon changes which occur in the axis cylinder at death.

Chromic acid, potassium bichromate, ammonium monochromate and certain other reagents, harden the axis cylinder and render it more easily seen. Perosmic acid, though hardening it, does not stain it black.

The white substance of Schwann. That the medullated nerve-fibre is not homogeneous while it is in a physiological condition, *i.e.* that a distinction between the axis cylinder and medullary sheath exists, may be proved by various considerations, for which the reader is referred to works on histology. The white substance of Schwann appears during life to have a semi-liquid consistence; from optical considerations it would seem to contain suspended solid bodies. At death it undergoes a kind of coagulation. The white substance of Schwann instantly reduces solutions of perosmic acid and becomes black from the presence of metallic osmium. When fresh, the white substance can be squeezed out of the nerve-fibres, and is found to be insoluble in water in which it swells; it is partially soluble in alcohol. The white substance of Schwann is doubtless specially rich in the complex phosphorized fats, in the cerebrin group of bodies, and in the cholesterin, which will be described as the chief constituents of the nervous matter.

Sect. 2. The Proteids found in the Nervous Tissues.

More than one-half of the solids contained in the grey matter, and about one-fourth of the solids of the white matter of the nerve-centres, consist of proteid substances, and yet our knowledge of these is but scanty.

Amongst these proteid bodies are to be mentioned, (1) a proteid substance which is soluble in water and is coagulated at 75° C.; this probably is derived from the grey matter; (2) a globulin-like body which is dissolved by a 10 per cent. solution of sodium chloride and is precipitated from it when the same salt is added to saturation; and (3) an alkaline albuminate, which remains in solution when a 10 per cent. salt solution of brain is boiled; in the solution filtered from coagulated proteids, acetic acid produces an abundant precipitate[1].

Sect. 3. Neurokeratin and Nuclein.

Neurokeratin.

If medullated nerve-fibres are treated with boiling alcohol and ether, so as to extract the fatty matters of the medullary sheath, there is left in its place an irregular framework which is highly refractile, and which is scarcely affected by digestion with trypsin or pepsin. This refractory substance swells when placed in concentrated sulphuric acid and in solution of caustic potash, but only dissolves in these liquids when boiled.

The substance resembles, indeed, the horny matter of epidermis in its power of resisting powerful chemical agents, and has been called by Kühne and Ewald[2], who have studied its properties, Neurokeratin.

The substance is found not only in medullated nerve-fibres, but in the grey matter of the nerve-centres and in the retina; it appears not to be present in non-medullated nerve-fibres.

Mode of separating Neurokeratin.

Ox's brain is washed in water, finely divided, digested for a long time in cold alcohol, again pounded, pressed, treated with alcohol. Then fully extracted with ether; dried in the air, and powdered. The dry powder is shaken through hair sieves and boiled with alcohol, until this liquid dissolves no more cerebrin. The residue is boiled with water, pressed and digested with pepsin and the insoluble residue washed; it is then digested for 24 hours in a weak solution of trypsin containing salicylic acid, and afterwards it is digested at

[1] The most recent observations on the proteids of the brain are contained in a paper by Petrowsky entitled "Zusammensetzung der grauen und der weissen Substanz des Gehirnes." Pflüger's *Archiv*, Vol. VII. p. 367.

[2] A. Ewald und W. Kühne, "Ueber einen neuen Bestandtheil des Nervensystems" (Neurokeratin). *Verhand. d. naturh. med. Vereins zu Heidelberg*, Vol. I. Heft 5.

40° C., for six hours, in a similar trypsin solution which has been rendered alkaline. The residue is washed with cold, and afterwards with hot, solution of sodium carbonate, and then extracted with a ½ p. c. solution of caustic soda. The extracted matter is treated with a little acetic acid, with the object of removing adhering alkali, and is then washed with alcohol and ether. The residue presents the appearance of a yellowish powder which amounts to from 15 to 20 p. c. of the dried residue left after the brain has been fully extracted with alcohol and ether.

Properties. Neurokeratin resembles in its general behaviour the keratin of the horny tissues; it differs from that substance, however, in being less easily soluble in boiling solutions of caustic potash.

When boiled with dilute sulphuric acid for some hours neurokeratin does not, like horn, entirely dissolve. In the solution, both tyrosin and leucin are found; the former being in larger and the latter in smaller quantities than when proteids are similarly treated.

Neurokeratin emits, when ignited, the odour of burning horn; it melts and then burns with a luminous flame. The body contains nitrogen and 2·93 per cent. of sulphur, and leaves 1·6 per cent. of ash.

Nuclein.

When describing the constituents of pus, the propriety of admitting the existence of a definite chemical individual termed *nuclein* was discussed, and the conclusion arrived at, that under that term bodies of the most varied composition had been classed, the common properties of which consisted in resisting the action of the digestive ferments whilst they were soluble in weak solutions of caustic soda. By following processes essentially similar to those by which the alleged nuclein has been separated from pus, v. Jaksch[1] thinks he has discovered nuclein in human brain.

His analyses do not agree with any of the analyses of nuclein obtained from other sources (see p. 242). As v. Jaksch alleges that his body possessed the properties of Miescher's nuclein obtained from the milt of salmon, we quote the ultimate analyses of Miescher's and v. Jaksch's substances.

ANALYSES OF NUCLEIN.

	From spermatozoa of salmon. Miescher.	From human brain. v. Jaksch.	
		(1)	(2)
C.	36·11	50·6	50·5
H.	5·15	7·4	7·8
N.	13·09	13·21	13·15
P.	9·59	1·71	2·08

[1] v. Jaksch, "Ueber das Vorkommen von Nuclein im Menschengehirn." Pflüger's *Archiv*, Vol. XIII. p. 469.

Mr Geoghegan[1] has estimated the amount of the hypothetical nuclein in brain at 1·4 grms. per 1000 grms. of brain-substance.

Sect. 4. The Phosphorized Constituents found in Nervous Tissues.

There is no subject in Physiological Chemistry concerning which it is more difficult to give a statement, which would be accepted as correct by those who have devoted their attention to it, than the chemistry of the complex phosphorized fats which exist in the nervous tissue. In the following pages an attempt will be made to give as impartial an account as possible of the present condition of a subject which is eminently in a transition stage.

Protagon.

Discovery by Liebreich.

In the year 1865, Dr Oscar Liebreich published an important paper[2] upon a new proximate principle which he had separated from the brain. Unlike the numerous bodies, possessed of ill-defined properties, which had, by different writers, received the names of cerebrin, cerebric acid, lecithine, or phosphorized fats, this new body could be extracted by an easy process in a state of purity, and to it, probably as indicating it as the first definitively ascertained specific constituent of brain, Liebreich ascribed the name of Protagon (*πρωταγος*, leading in advance).

Mode of preparation.

The substance was obtained by the following process. An animal was bled to death from the carotid, and a stream of water was passed through the vessels of the head, so as to wash the blood out. The brain, freed from its membranes, was then pounded in a mortar and shaken in a flask with ether and water at 0° C. It was allowed to stand at a temperature of 0°, until the ether had separated, and the treatment with ether again and again repeated.

The brain-matter having been separated by filtration from ether and water, was digested with 85 per cent. alcohol, at a temperature of 45° C. The fluid was filtered hot and allowed to cool at 0° C. A flocculent precipitate then separated, which was collected on a filter and treated with cold ether, until it ceased to dissolve cholesterin. The insoluble mass was dried *in vacuo*, and dissolved in spirit at 45° C. The alcoholic solution was filtered, and allowed to cool *very gradually*, when protagon separated in the form of microscopical needles, differing a little in arrangement and form according to the concentration of the

[1] Edward G. Geoghegan (aus Dublin). "Ueber die anorganischen Gehirnsalze nebst einer Bestimmung des Nucleins im Gehirn." *Zeitschrift f. phys. Chem.* Vol. I. p. 330.

[2] Oscar Liebreich, "Ueber die chemische Beschaffenheit der Gehirnsubstanz." *Annalen der Chemie und Pharmacie*, Bd. CXXXIV. S. 29—44.

solution. The substance thus obtained could be recrystallized repeatedly. As a result of his analyses Liebreich ascribed to protagon the formula $C_{116}H_{241}N_4O_{22}P$.

Properties of Protagon, according to Liebreich.

Protagon is soluble with difficulty in cold, but more easily in warm alcohol and ether. At higher temperatures than 55° C., alcohol appears to decompose protagon. In water protagon swells and presents the appearance of an opaque jelly, ultimately dissolving so as to form an opaque solution. Liebreich found that protagon was soluble in glacial acetic acid, which deposited it again in a crystalline form, when subjected to the action of cold.

When boiled with a solution of barium hydrate protagon is decomposed, yielding glycerin-phosphoric acid, fatty acids of which he isolated stearic acid in a state of purity, and a base to which he gave the name of *neurine*, and to the platinum compound of which he ascribed the formula $C_5H_{14}NCl_3Pt$. This base was afterwards shewn to be identical with the base which Strecker had separated from bile and termed *choline*.

Although the absolute accuracy of a large number of Liebreich's facts has been placed beyond question, the cardinal fact itself—that protagon is a definite phosphorized principle contained in nervous matter—had, until lately, come to be universally denied.

Hypothesis that protagon is a mixture of cerebrin and lecithin.

By Diaconow, Hoppe-Seyler, and Thudichum it is denied that any such definite substance exists, and Liebreich's protagon is held to be a mechanical admixture of a phosphorized body termed *lecithin*, $C_{44}H_{90}NPO_9$, with a nitrogenous, non-phosphorized, body termed *cerebrin*. The presence of phosphorus in protagon is said to be due to contamination with lecithin, and in support of this view it is alleged that by extracting protagon with ether, the substance loses more and more phosphorus. According to Diaconow and Hoppe-Seyler's admission, the phosphorus does adhere most obstinately and cannot be entirely got rid of, though Dr Thudichum thinks he has, by mere extraction with ether, obtained cerebrin (or cerebrines) quite free from phosphorus.

According to Diaconow[1] by repeated extractions with ether, the P contained in protagon may be made to sink to 1 per cent., whereas, according to Liebreich's formula, it should contain 1·5 per cent. Actually, in the three determinations which he made, Liebreich obtained 1·1, 1·1, and 1·5, as the amount of phosphorus in 100 parts, but, unfortunately, he seems to have concluded that the highest number was correct and made it the basis of his calculation.

In the year 1877, the Author, assisted by Mr Leopold Larmuth, Platt Physiological Scholar, commenced in the Physiological Labora-

[1] Diaconow, "Das Lecithin im Gehirn." *Centralblatt für die medicin. Wissenschaften* (8 Februar 1868). Nr. 7, Pag. 97.

tory of Owens College, a series of experiments intended to determine whether Liebreich's protagon existed or not. This preliminary investigation shewed that by Liebreich's process there is always obtained a body having the physical properties of protagon, and containing phosphorus in a proportion sufficiently near to that indicated by him; it was found that the amount of phosphorus in specimens of protagon which had been crystallized from alcohol four or five times, was not smaller than that present in protagon which had only once been crystallized, though a thorough treatment with ether preceded each recrystallization.

These first experiments, so far as they went, were perfectly satisfactory. It appeared, however, quite essential, before forming a definite opinion, to extend them very considerably, and especially to prove the definite nature of protagon by a large number of analyses, indicating not merely the amount of phosphorus, but also that of the other elements present in it.

This investigation was subsequently carried on by the Author in conjunction with Dr E. Blankenhorn, with the result of proving to their entire satisfaction that protagon is a definite chemical body[1].

The process of Blankenhorn and the Author for preparing protagon.

Perfectly fresh ox's brains are freed from blood and from adhering membranes as completely as possible, and are then digested for many hours (18 to 24) in 85 per cent. alcohol in a large incubator kept constantly at 45° C. The fluid is filtered whilst hot, and the insoluble matter is again treated with fresh quantities of spirit, the proceeding being repeated four or five times, as long, indeed, as the filtrate when cooled to 0° deposits a fair quantity of white flocculent precipitate. This precipitate is collected on a filter, and being then transferred to a stoppered bottle is thoroughly and repeatedly agitated with ether, in order to dissolve cholesterin and other bodies soluble in ether[2]. The ether having been removed, first by decantation and then by filtration, the substance left undissolved by it is first of all dried between sheets of filtering paper in air, and afterwards over sulphuric acid or phosphorus pentoxide.

[1] Gamgee und Blankenhorn, "Ueber Protagon," *Zeitschrift f. physiol. Chemie*, Vol. III. (1879) p. 260. "On Protagon," *Journal Physiology*, Vol. II. (1879) p. 113.

[2] At first we commenced by repeating exactly the process of Liebreich in all its details; one of the steps of that process we had found fraught with peculiar difficulty, and we soon ascertained that it could be dispensed with without prejudicially affecting the success of the operations. The step to which we refer consists in agitating the freshly pounded brain repeatedly with water and ether at 0° C., so long as the ether dissolves considerable quantities of substance, then filtering and placing the insoluble matter in 85 per cent. alcohol at 45° C. When pounded brain is so treated with water and ether it swells up and the separation of the ether is most incomplete. The process of filtration is one which is attended with great difficulty, even when carried out in the only way in which we found it possible, viz., in the woven bags sold for household purposes, for straining jellies, &c. It was, however, apparent that however prolonged the ether washing, it never succeeded in freeing the brain from cholesterin and other matters soluble in ether, and that the removal of these bodies from protagon was most readily effected at a later stage of the operations.

The resulting mass, which has a snow-white colour, is reduced to powder, moistened with a little water, and digested for many hours with alcohol heated to 45° C. From the filtered liquid, if this be allowed to cool *very gradually*, the protagon separates in the form of microscopic crystals, mostly arranged in rosettes, the appearance and arrangement of which differ somewhat, as Liebreich very exactly pointed out, according to the degree of concentration of the solution. The once crystallized protagon thus obtained is collected on a filter, washed with ether, and dried first of all in air and ultimately over P_2O_5. It is then recrystallized as many times as required, the process always commencing by pulverizing and thoroughly shaking with cold ether.

Results of ultimate analyses of protagon.

With the object of proving the definite nature of protagon, the Author and Dr Blankenhorn subjected the body to repeated recrystallization, subjecting the product of the successive operations to ultimate analysis. The following are the results of these analyses :—

	Protagon once recrystallized (dog).		Protagon twice recrystallized (ox).		Protagon twice recrystallized (ox).		Thrice recrystallized (ox).	Four times recrystallized (horse).
	No. 5.	No. 6.	No. 1.	No. 2.	No. 3.	No. 4.	No. 8.	No. 7.
C ...	66·3	66·6	66·46	66·58	66·34	66·35	66·30	66·26
H ...	10·52	11·06	10·96	10·72	10·56	10·78	10·467	10·48
N ...	..	..	2·3	2·6	2·40	..	2·29	..
P ...	..	..	1·094	1·107	1·032	1·081	1·027	..
O								

From the above numbers we have deduced for protagon the empirical formula $C_{160}H_{308}N_5PO_{35}$.

	Calculated.		Mean found.
C_{160}	1920	66·45	66·39
H_{308}	308	10·66	10·69
N_5	70	2·42	2·39
P.....	31	1·07	1·068
O_{35}	560	19·40	
	2889	100·00	

From the results of these analyses it appears to the Author that the existence of protagon as a definite chemical individual is well-nigh proved.

Stability of protagon.

It has been alleged by Diaconow and Hoppe-Seyler that by prolonged treatment with ether and alcohol the whole of the phosphorus of protagon may be removed, and these authors regard protagon as a mixture of a nitrogenous body (cerebrin) with a phosphorized substance (lecithin). The most careful investigation of the matter by the Author has led to entirely opposite con-

clusions. Pure protagon is remarkably rebellious to the action of even boiling alcohol, though that action be continued for hours: and the most persistent attempts to separate lecithin from it have failed. At the same time there can be no doubt that protagon when treated with certain reagents which decompose it, especially when digested with alkalies, yields, amongst other bodies, certain of the most characteristic of the products of decomposition of lecithin. But to conclude from the presence of these, the presence of lecithin, is obviously unphilosophical.

There can be no doubt that protagon is accompanied in the brain by large quantities of a body or bodies which may provisionally be conveniently classed under the term of cerebrin, and likewise by smaller quantities of other phosphorized bodies, containing a percentage of phosphorus very close to that found in lecithin, yielding the same products of decomposition, and the separation of which presents extraordinary difficulties. Yet, the Author is convinced that unquestionably the only well characterized phosphorized proximate principle, which can with our present methods be separated with certainty and whose existence will be confirmed by future researches, is Protagon.

Liebreich's researches on the products of decomposition of protagon.

1. *Action of Alkalies.* Liebreich discovered that when protagon is boiled for 24 hours with a saturated solution of barium hydrate, the solution contains the barium salt of glycerin-phosphoric acid, $(C_3H_5)(OH)_2.O.PO(OH)_2$; a base called Neurine, which was afterwards shewn to be identical with a base previously obtained by Strecker from ox bile and termed by him Choline ($C_5H_{15}NO_2$); and barium salts of several fatty acids, especially of stearic acid.

There can be no doubt that there is formed, in addition, a certain quantity of a cerebrin-like body or a mixture of such bodies, *i.e.* a substance is obtained which is not soluble in ether, which dissolves in boiling alcohol and is deposited from it in a nodular form on cooling: which contains nitrogen but no phosphorus. The experiments of the Author have shewn him that the quantity of this body which is formed is very much smaller than would be the case on the hypothesis that protagon is a mixture or even a conjugated compound of lecithin and cerebrin.

2. *Action of acids.* When protagon is boiled with hydrochloric acid in the absence of light, a yellowish liquid is obtained, which deposits flocculi of a body free from phosphorus; and which becomes coloured when exposed to light. In this process there is set free a laevogyrous, non-fermentable sugar.

The chief products of decomposition of protagon will be described at length under the head of lecithin and cerebrin; under the latter the cerebrin body which accompanies protagon will be discussed.

LECITHIN.

Besides the nervous tissues, there are others in which organic phosphorized ingredients occur in considerable proportions; these are found in large quantities in the ovum, in spermatozoa, etc.

The first satisfactory study of the phosphorized organic bodies was made by Gobley[1], who described under the name of *Lecithine* a viscous proximate principle which he had separated from the eggs of the carp. This body was entirely soluble in ether, soluble with difficulty in cold, but readily in hot alcohol, from which it was deposited on cooling. Gobley[2] found that this body, when ignited, yielded an ash possessed of a strong acid reaction, owing to the presence of phosphoric acid. He further shewed that when decomposed with acids or alkalies, his lecithin yielded glycerin-phosphoric acid and fatty acids, amongst which he cited oleic and margaric. Besides lecithin, Gobley separated, by a process which would certainly cause decomposition of any complex proximate principle, a body which he termed Cerebrin, which contained 0·43 p.c. of phosphorus.

In researches on the brain which were anterior to his most mature investigations on lecithin from eggs, Gobley arrived at the conclusion that the phosphorized matter of the brain resembles, if it be not identical with, that obtained from eggs, and this view of Gobley's is the one which has commended itself almost universally to physiological chemists.

After the publication of Liebreich's memoir on Protagon attention was again directed to the phosphorized proximate principles of the body, it being doubtless surmised that the well-defined protagon would be discovered where earlier observers had found less sharply characterized bodies. This surmise was, however, soon disproved.

In a paper published in Hoppe-Seyler's *Untersuchungen*, by one of his own pupils, Parke[3], "On the Chemical Composition of the Yolk of Egg," in which the amount of protagon present was calculated on Liebreich's data from the amount of phosphorus found in the alcoholic extract of the yolk of egg, the observation was made that, by calculation, more protagon was found than corresponded to the whole weight of the alcohol extract.

In a paper immediately succeeding that of Parke's, Hoppe-Seyler[4] clearly expressed his conviction that the yolk of egg contains no protagon but lecithin, this being the name which Gobley had given to the chief phosphorized constituent of the yolk. He further stated, that experiments made in his laboratory by Herr Jüdell had

[1] Gobley, *Journal de Chimie et Pharmacie*, Vol. XVII. (1850) p. 401: Vol. XVIII. (1850) 107.

[2] Gobley, *Journ. de Pharm. et Chimie*, Vol. XI. (1847) p. 409, and XII. (1847) 1.

[3] Parke, "Ueber die chemische Constitution des Eidotters," *Med.-chem. Untersuchungen*, Heft 2, p. 213.

[4] Hoppe-Seyler, "Ueber das Vitellin, Ichthin und ihre Beziehung zu den Eiweissstoffen." *Med.-chem. Untersuchungen*, Heft 2, p. 215.

shewn that the ether extract of red blood-corpuscles, besides cholesterin, contained a body, the amount of phosphorus in which corresponded to 8·25 p.c. of P_2O_5 (that is to say, containing 3·6 p.c. of phosphorus), and which therefore could not be protagon. Hoppe-Seyler had by this time obviously commenced to entertain doubts as to the existence of protagon, though he did not commit himself to a denial of its presence in the brain; indeed, by implication he rather admitted his belief in its existence[1].

Another of Professor Hoppe-Seyler's pupils, Dr Diaconow[2], now continued the investigation.

In a paper on the bodies containing phosphorus which are present in the hen's egg and in the ova of the sturgeon, he came to the following conclusions:

1. Gobley's lecithin and the phosphorized bodies which are obtained from Ichthin and Vitellin yield on boiling the same products of decomposition as protagon.

2. They contain about twice as much phosphorus as protagon, so that they are either altogether distinct from protagon, or they consist of a mixture of protagon with a second phosphorized body.

3. In any case protagon is not the only phosphorized proximate principle of the body.

4. The discovery of phosphoric acid in alcoholic or ethereal extracts of different animal organs, does not entitle us to conclude that protagon is present.

5. The quantity of phosphoric acid found in an ethereal extract, freed from cholesterin and fats, affords no estimate of the quantity of protagon.

A short time after the appearance of the preceding, Diaconow published a second paper[3] in which he described the properties of the phosphorized constituent of yolk of eggs, to which he correctly ascribed the name which Gobley had given to it, giving the results of analyses, and naming the chief products of its decomposition.

Diaconow's description of Lecithin.

According to Diaconow's description of lecithin from eggs this body has the following characters:

Pure lecithin presents the appearance of a yellowish-white, waxy, very hygroscopic solid, which when in thin layers shines with a silky lustre; it is soluble in ether and alcohol, it swells in water, and on shaking it in, or stirring it with, water it forms a starch-like solution which filters with difficulty. When ignited it burns away, leaving as only residue phosphoric anhydride[4]. The chemical formula of the body calculated from its

[1] "Ob aber neben Protagon auch Lecithin sich in der Hirnmasse findet habe ich nich untersucht." Hoppe-Seyler, *Ibid.* p. 220.

[2] Diaconow, "Ueber die phosphorhaltigen Körper der Hühner- und Störeier" (Vorläufige Mittheilung). Hoppe-Seyler, *Med.-chem. Untersuchungen*, Heft 2, p. 221.

[3] Diaconow, "Ueber die chemische Constitution des Lecithin," *Centralblatt für die med. Wissenschaften*, 1868, No. 1, p. 2.

[4] This is a curious mistake which has been repeated by all physiological chemists; the residue is one of metaphosphoric acid.

ultimate analysis (C = 64·27 p.c.; H = 11·4; N = 1·8; P = 3·8) is $C_{44}H_{90}NPO_9 + H_2O$. When decomposed it yields glycerin-phosphoric acid, stearic acid and neurine—the very same products which Liebreich had obtained from protagon.

Compounds of lecithin.

Lecithin forms compounds both with bases and acids, as for example with potassium and hydrochloric acid, and the latter forms a platinum compound (Strecker); in the case of distearyl-lecithin (see 'constitution of lecithin') this compound would have the formula

$$2\ (C_{44}H_{90}NPO_9 . Cl) + PtCl_4.$$

A similar compound with cadmium chloride exists. The platinum compound is soluble in ether, but it may be precipitated from the ethereal solution by an excess of alcohol.

Diaconow's assertion of the presence of lecithin in brain.

Diaconow had in the meantime directed his investigation to the brain, and one month after the publication of his first paper there appeared a second[1] which has exerted a most weighty influence, causing physiological chemists to come to the conclusion that Liebreich's protagon does not exist as a definite proximate principle, but that it consists of a mixture of lecithin with a body free from phosphorus, *cerebrin*.

Diaconow's method of separating lecithin from brain.

Brain freed from its membranes and from blood, is finely divided and repeatedly extracted with ether; the residual mass is digested with absolute alcohol at 40° C., and the alcoholic solution thus obtained is cooled to 0°; the precipitate which separates is filtered off, and washed with a little cold absolute alcohol and afterwards once again with ether. A portion of the substance dissolves in ether, whilst another, protagon, remains as a residue. The latter is repeatedly extracted with ether at ordinary temperatures, and the collected ethereal extracts are subjected to distillation; the residue is dried at 40°, dissolved in a little absolute alcohol, and the alcoholic solution is cooled. There separates a white substance having the composition and properties of lecithin. The substance is amorphous, non-pulverizable, hygroscopic, swells in water and when shaken with it forms an emulsion. When burned it leaves as a residue phosphoric anhydride (?). When decomposed with lime- or baryta-water it yields in addition to neurine, barium stearate and glycerin-phosphate.

Analyses of the body separated in this way by Diaconow furnished the following results.

[1] Diaconow, "Das Lecithin im Gehirn." *Centralblatt für die medicinischen Wissenschaften*, No. 7, 8th Feb. 1868, p. 97.

1. 0·0678 substance gave 0·0083 $Mg_2P_2O_7$ = 7·83 % P_2O_5.
2. 0·0985 „ „ 0·0123 „ = 7·98 % „
3. 0·1833 „ „ 0·024 Pt = 1·85 % N.

The formula of lecithin, $C_{44}H_{90}NPO_9$, demands 8·378 % of P_2O_5 and 1·17 % of N.

Upon these facts, and these alone, so far as the author is aware, all subsequent writers have based their belief in the presence of lecithin in the brain, adopting the views of Diaconow and Hoppe-Seyler that protagon is a mixture of lecithin with cerebrin[1].

The Author's criticism of the observations of Diaconow.

It is unquestionably true that the precipitate which separates from an alcoholic solution of brain, contains, besides protagon, cholesterin, and a body for which we may retain the name of cerebrin, small quantities of bodies soluble in ether which have a much higher percentage of phosphorus than protagon, and which possess the general smeary characters of lecithin. But these bodies are present in very small quantities, and are readily removed by ether washing. Protagon which has been several times recrystallized yields no such body as lecithin to ether, in which liquid it is at ordinary temperatures practically insoluble.

From his own observations then he would conclude that whilst it is true that the brain yields to alcohol other phosphorized bodies than protagon, the latter is much the most abundant of the phosphorized products, and by no action of ether can it be split up into lecithin and a non-phosphorized cerebrin, it is possible and indeed probable that amongst the phosphorized principles, lecithin is to be reckoned. No sufficient proof of its identity has however yet been furnished. It is indeed apparent to the author from his own work, no less than from a careful study of the researches of Thudichum, that the phosphorized ingredients are numerous.

Description of some of the products of decomposition of lecithin and protagon.

Whichever the view adopted, it appears that certain of the products of decomposition of lecithin and protagon are the same. The chief of these are glycerin-phosphoric acid, neurine or choline, and fatty acids; the two former of these bodies will now be described.

Glycerin-phosphoric acid ($C_3H_9PO_6$).

When distearyl-lecithin is decomposed by boiling with alkaline solutions, it combines with the elements of water, with the formation

[1] The author has attempted to separate lecithin from brains by Diaconow's method and has failed entirely.

of stearic acid, glycerin-phosphoric acid and choline, as shewn by the following equation :—

$$\underbrace{C_{44}H_{90}NPO_9}_{\text{Lecithin.}} + \underbrace{3\,H_2O}_{\text{Water.}} = \underbrace{2\,(C_{18}H_{36}O_2)}_{\text{Stearic acid.}} + \underbrace{C_3H_9PO_6}_{\text{Glycerin-phosphoric acid.}} + \underbrace{C_5H_{15}NO_2}_{\text{Neurine.}}.$$

Preparation of glycerin-phosphoric acid.

This acid may be prepared by the decomposition of lecithin by means of caustic baryta, or synthetically in the following manner :—

Phosphoric anhydride is added in equivalent proportions to glycerin which is kept cool by a freezing mixture. Much heat is evolved and the new acid is formed. According to the author's experiments the yield of acid is extremely small. The solution is diluted with water, neutralized with barium carbonate, filtered from the large quantity of barium phosphate which is formed, and the filtrate is exactly neutralized with dilute sulphuric acid. The filtrate from deposited barium sulphate is concentrated *in vacuo* at a low temperature; in this manner a watery solution of the acid is obtained. This solution cannot be concentrated beyond a certain point, as it decomposes.

Properties and compounds.

Glycerin-phosphoric acid is a syrupy liquid possessed of both an acid and sweet taste. It forms salts which are for the most part soluble in water, but insoluble in alcohol; the lead compound is an exception to the former statement.

The barium compound has the composition $C_3H_7BaPO_6$. Thudichum and Kingzett[1] describe a hydrate having the composition $C_3H_7BaPO_6H_2O$.

The normal calcium salt has the composition $C_3H_7CaPO_6H_2O$; it is less soluble in hot than in cold water, so that a solution appears to coagulate when boiled, like a solution of albumin. An acid salt has been described having the formula C_3H_7CaPO , $C_3H_9PO_6$.

A soluble zinc salt ($C_3H_7ZnO_6$) and an insoluble lead salt ($C_3H_7PbO_6$) also exist; the latter is prepared by adding a solution of acetate of lead to the barium compound.

Constitution.

By studying the appended graphic formulae of glycerin, phosphoric acid, and glycerin-phosphoric acid the reader will apprehend the view which is held of the constitution of the last-named body.

(1) $C_3H_5\begin{cases}OH\\OH\\OH\end{cases}$ $\quad C_3H_5(OH)_3$ Glycerin.

(2) $PO\begin{cases}OH\\OH\\OH\end{cases}$ $\quad PO(OH)_3$ Orthophosphoric acid.

[1] Thudichum and Kingzett, "On glycero-phosphoric oxide and its salts, as obtained from the phosphorized constituents of the brain." *Journ. Chem. Soc.*, July, 1876.

(3) $C_3H_5\begin{cases}OH\\OH\\O-PO\begin{cases}OH\\OH\end{cases}\end{cases}$

$C_3H_5(OH)_2 . O . PO(OH)_2$

Glycerin-phosphoric acid.

Neurine[1] *(Choline).* $C_5H_{15}NO_2$.

Preparation. This body, which was first obtained by Strecker[2] from bile (and termed choline), and afterwards independently discovered as a product of decomposition of protagon by Liebreich, and of lecithin by Diaconow, may be obtained by decomposing either of the latter bodies by boiling them for at least an hour with baryta water. The liquid is then filtered, treated with CO_2, then boiled, filtered and concentrated at a gentle heat. A syrupy residue is obtained which is extracted with absolute alcohol; the solution is filtered, hydrochloric acid is added, so as to induce a slight acid reaction, and then a solution of platinum tetrachloride is added; a yellow precipitate (composed of a double salt of neurine and platinum tetrachloride) falls, which is washed in alcohol, dissolved in water and decomposed by H_2S. The filtrate from the precipitate of platinum sulphide is concentrated in the water bath. In this way hydrochlorate of neurine is obtained and from this the base is set free by treatment with silver oxide.

Properties. Neurine is a syrupy liquid, soluble in alcohol and ether and possessing a marked alkaline reaction. It does not coagulate albumin. Its solutions dissolve fibrin.

Compounds. Besides the compounds with hydrochloric acid, previously referred to, neurine forms compounds with carbonic and sulphuric acids; its hydrochlorate forms double salts with platinum tetrachloride and with gold chloride. The following are the rational formulae of the hydrochlorate and of the platinum and gold compounds:

(1) $N(CH_3)_3(C_2H_4 \cdot OH)Cl$.

(2) $[N(CH_3)_3(C_2H_4 \cdot OH)Cl]_2PtCl_4$.

(3) $N(CH_3)_3(C_2H_4 \cdot OH)Cl + AuCl_3$.

Products of decomposition. Amongst numerous and highly interesting decompositions it may be mentioned that, when heated, neurine splits up into glycol and trimethylamine:—

$$\underbrace{C_2H_4\begin{cases}OH\\N(CH_3)_3\end{cases}OH}_{\text{Neurine.}} = \underbrace{C_2H_4\begin{cases}OH\\OH\end{cases}}_{\text{Glycol.}} + \underbrace{N(CH_3)_3}_{\text{Trimethylamine.}}$$

[1] In writing this description of Neurine the author has availed himself very freely of the excellent account in Hoffmann's *Lehrbuch der Zoochemie*, page 114 et seq.

[2] Strecker, "Ueber das Lecithin." *Ann. d. Chem. u. Pharm.*, Vol. LXXII. p. 77.

Synthesis. Neurine has been produced synthetically in two ways:
1st. By bringing together a concentrated solution of trimethylamine with ethene oxide, thus:—

$$\underbrace{\begin{matrix}CH_2\\ \\ CH_2\end{matrix}\!\!>\!O}_{\text{Ethene oxide.}} + \underbrace{N(CH_3)_3}_{\text{Trimethylamine.}} + \underbrace{HOH}_{\text{Water.}} = \underbrace{\begin{matrix}CH_2.OH\\ \\ CH_2-N(CH_3)_3OH\end{matrix}}_{\text{Neurine.}}$$

2nd. By heating in a sealed tube a mixture of ethene-chlorhydrin and trimethylamine, thus:—

$$\underbrace{\begin{matrix}CH_2.OH\\ \\ CH_2Cl\end{matrix}}_{\text{Ethene-Chlorhydrin.}} + \underbrace{N(CH_3)_3}_{\text{Trimethylamine.}} = \underbrace{\begin{matrix}CH_2.OH\\ \\ CH_2-N(CH_3)_3Cl\end{matrix}}_{\text{Neurine Hydrochlorate.}}$$

Constitution of Lecithin.

Having now described the properties of lecithin as observed by Diaconow and Strecker, we have to approach the question of the constitution of that body.

The lecithin which Diaconow believed to exist in the brain was supposed by him to yield as a product of decomposition, and as the only fatty acid, stearic acid, and it may be therefore termed, for reasons which will be apparent immediately, distearyl-lecithin. This body and indeed all lecithins, for there are probably many lecithins, are derived from glycerin-phosphoric acid.

If in glycerin-phosphoric acid two of the atoms of hydroxyl-hydrogen in the glycerin be replaced by two molecules of stearyl, we shall obtain an acid to which the name of distearyl-glycerin-phosphoric acid was given by Diaconow. This acid would have the constitution $C_3H_5(C_{18}H_{35}O_2)_2O.PO(OH)_2$: it was actually obtained by Diaconow on shaking an ethereal solution of lecithin with sulphuric acid, the products of the reaction being this body and neurine-sulphate.

Now distearyl-glycerin-phosphoric acid may unite itself with neurine to form lecithin.

The mode of attachment of neurine to distearyl-glycerin-phosphoric acid cannot be represented with certainty by any formula. The three following formulae for distearyl-lecithin have been suggested and represent the different views which have been held:—

(A) Diaconow:[1]

$$C_3H_5\begin{cases}(C_{18}H_{35}O_2)_2\\ O-PO\begin{cases}OH\\ O-N(CH_3)_3-C_2H_4OH\end{cases}\end{cases}$$

[1] *Centralblatt f. d. med. Wiss.*, 1868, Nr. 1, s. 3.

(B) Strecker:[1]

$$C_3H_5\begin{cases}(C_{18}H_{35}O_2)_2\\O-PO\begin{cases}OH\\O-C_2H_4-N(CH_3)_3OH\end{cases}\end{cases}$$

(C) Hoppe-Seyler:[2]

$$C_3H_5\begin{cases}(C_{18}H_{35}O_2)_2\\O-PO\begin{cases}OHN(CH_3)_3\\O-C_2H_4-OH\end{cases}\end{cases}$$

Distearyl-lecithin may be looked upon as the type of the lecithins; but we can easily conceive of lecithins which only differ from this one by the radicals of oleic acid or of palmitic acid having taken the place of stearyl. We should thus have dipalmityl-lecithin, dioleyl-lecithin or oleyl-palmityl-lecithin formed; such bodies probably exist.

Phosphorized Principles other than Protagon and Lecithin.

In a very elaborate research on the phosphorized constituents of the brain, Thudichum[3] has come to the conclusion that he has separated at least three well characterized bodies or groups of bodies.

The bodies belonging to these groups are distinguished as (1) the Kephalines, (2) the Myelines, (3) the Lecithins.

Kephaline. Belonging to the first group is a body *Kephaline*, which is said to be exceedingly soluble in ether; this body is soluble in hot alcohol, but less so than either lecithin or myeline. To kephaline is ascribed the formula $C_{42}H_{79}NPO_{13}$. It does *not form* definite compounds with platinum or cadmium.

Myelines. The myelines are far less soluble in ether than kephaline or the kephalines, and less soluble in alcohol than the lecithins.

Various myelines have been described by Thudichum including bodies having the following formulae:—$C_{40}H_{85}NPO_8$; $C_{40}H_{75}NPO_{10}$; $C_{49}H_{82}N_2PO_{10}$; $C_{52}H_{103}N_2PO_9$; $C_{39}H_{81}NPO_8$; $C_{39}H_{82}N_2PO_8$.

Thudichum's summary of his researches on the phosphorized principles. The following are the main characters of the phosphorized principles of the brain as summarized by Dr Thudichum. In quoting them the author in no respect expresses his agreement with Dr Thudichum's conclusions.

"The group of the phosphorized bodies contains the phosphorus in the form of phosphoric acid, combined proximately with glycerine, so that by chemolysis they all yield glycero-phosphoric acid, but they differ in the manner in which they contain the nitrogen, and the acid radicles which constitute the great bulk

[1] *Ann. Chem. Pharm.*, 1868, Bd. CXLVIII. s. 77.
[2] *Physiologische Chem.*, 1877, 1 Theil. s. 80.
[3] Thudichum, "Researches on the Chemical Constitution of the Brain." *Reports of Medical Officer of the Privy Council and Local Government Board*, 1874, p. 113 et seq.

of their substance, and according to these differences must be divided into sub-groups. We thus obtain the sub-groups of the *kephalines*, *myelines*, and *lecithines*.

"Of these the *kephaline* sub-group, itself hitherto unknown, includes members which contain the nitrogen in either one or two forms, one being either choline or neurine, another hitherto unknown; and they contain the fatty acid radicles also in forms with which chemistry is at present unacquainted, and the members of this sub-group further vary in the amount of oxygen which they contain in a manner so as to be sharply characterised thereby. This variability of the constituent oxygen may be transitional, but must not be confounded with that remarkable reaction of the bodies of this group which I describe as their oxydisability.

"The *myeline* sub-group, also new, contains the nitrogen in two forms, of which one is choline, the other amide in a fatty acid radicle. The fatty acid radicles vary, and are mostly new forms, some known forms. The members of this group consequently vary in carbon, hydrogen and oxygen; little in nitrogen, never in phosphorus. They are not oxydisable after the manner of kephaline, though there is an oxy-myeline after the manner of oxy-kephaline. They are the least soluble of the entire group, the least decomposable, and stand the highest temperatures, being unchanged by fusion at a heat above the boiling point of water.

"The *lecithine* sub-group, well known from the chemistry of eggs, is only with difficulty evolved from the brain, on account not only of the many stages of the processes necessary for their isolation, but also on account of a prominent feature of its members, namely, their readiness to decompose when in the anhydrous state. This tendency to apparently spontaneous lysis into proximate nuclei prevents the inquirer fixing properties and varieties with the same precision as in the previous groups; but it furnishes a valuable key to the explanation of many changes in the sick body, which may arise, or have been proved to arise, from their decomposition.

"The chemical characteristics of these sub-groups may be summarized thus: the kephalines possess the tendency to be oxydised, oxydisability; the myelines are not easily changed by any agent or influence, and possess therefore stability; the lecithines easily fall to pieces, they are afflicted with lability.

"In language more technically chemical: the *kephalines* have on the outside of their molecules free affinities for oxygen; this gas they bind in several ways; when the oxygen combined with a molecule has attained a certain quantity, the avidity (intensity of affinity) of the molecule increases to this extent, that it monopolises all available oxygen to itself until the limits of its oxydisability (at present unknown) are attained; until its free affinities are satisfied. Until then the rest of the molecules, if the supply of oxygen be insufficient to oxydise all to the same point, are not oxydised. The kephalines, however, are not in a state of atomic tension, and therefore do not fall to pieces so easily as the lecithines, but require for lysis the influence continued for some time, of powerful extraneous affinities in the presence of water and heat.

"The *myelines* have no apparent free affinities for oxygen; they are not affected by heat to and above boiling water except to the extent of

fusion; their atoms are not in a state of chemical tension, but require for vibration into permanent decomposing distances the influence of strong external affinities, water and heat.

"The *lecithines*, however, are in a state of great atomic tension, and therefore slight external affinities or dissociating impulses suffice to effect their decomposition. Such a slight impulse is the attraction of absolute alcohol for their fatty acid radicles in the absence of external water. The water given out by the alcohol in becoming ethylic ether serves to enable the radicle of glycero-phosphoryle to become glycero-phosphoric acid, and to remain in combination with the choline evolved[1]."

Sect. 5. Non-phosphorized nitrogenous bodies of unknown constitution.

Cerebrin (?) *or Cerebrins* (?).

Müller's cerebrin.

In the year 1858 Müller published an account of a non-phosphorized body which he had obtained from brain by the following process. The brain was pounded up with baryta water to the consistence of a thin milk and then boiled; the precipitate which formed under these circumstances was extracted with boiling alcohol: on cooling, the alcoholic solution deposited an abundant precipitate. This was treated with ether to separate cholesterin and fats, and then recrystallized from boiling alcohol. The purified body thus obtained, which was termed cerebrin by Müller, possessed the following characters:—it was a loose, white, very light, powder, destitute of smell and taste, soluble in boiling alcohol and ether, but insoluble in water, cold alcohol and ether. Under the microscope the body presented the appearance of small round balls[2]. Müller published analyses of this body (only two carbon determinations being given) and to it he ascribed the formula $C_{24}H_{33}NO_6$.

The following are the results of the analyses of Müller's cerebrin:

	Calculated.	Found. (1)	Found. (2)
C.	68·23	68·35	68·56
H.	11·04	11·30	11·25
N.	4·68	4·69	4·53
O.	16·05	15·66	15·66

That a body produced by the prolonged action of a solution of boiling barium hydrate on so complex an organic mixture as brain should be a definite proximate principle of the unaltered organ would appear in the highest degree unlikely; even more unlikely than that it should be a definite principle at all. Yet, without any sufficient proofs, the existence of Müller's cerebrin has found favour with all those who have doubted the existence of protagon, and, since the time

[1] Thudichum, *Op. cit.* p. 198.

[2] Müller, "Ueber die chemischen Bestandtheile des Gehirns." *Ann. d. Chem. u. Pharm.*, Vol. cv. p. 361, 2te Abth.

when Diaconow and Hoppe-Seyler first promulgated this idea, the latter body has been generally considered to be a mixture of cerebrin with lecithin.

The author, in his criticism of the position which had been taken up by Diaconow and Hoppe-Seyler, tried to shew that those who had manifested great scepticism in Liebreich's protagon had taken for granted the existence of a body whose investigation had been infinitely more incomplete[1]. The justness of the criticism has been thoroughly confirmed by the subsequently published researches of Geoghegan[2].

Geoghegan's mode of preparing cerebrin.

Instead of boiling pounded brain with caustic baryta, as Müller had done, Geoghegan extracted pounded brain with cold alcohol and ether, then boiled it in alcohol. The white body which separated on cooling, and which according to Geoghegan is a mixture of cerebrin, cholesterin and lecithin, was treated with ether so as to separate cholesterin, and then boiled with baryta water. The insoluble residue was dissolved in alcohol and crystallized.

It was analysed with the result of finding that it contained only one-third of the amount of nitrogen which had been found by Müller; to it the empirical formula $C_{57}H_{110}N_2O_{25}$ is ascribed.

	Mean of Geoghegan's analyses of cerebrin.	Mean of Müller's analyses.
C.	68·74	68·45
H.	10·91	11·20
N.	1·44	4·50

The author's researches on cerebrin.

The author's researches on cerebrin, though far from complete, were made immediately prior to the publication of Geoghegan's paper, though they have been hitherto unpublished. They have led him to the following conclusions:

(1) By the action of ether, however prolonged, or of alcohol, a phosphorus-free cerebrin cannot be obtained from protagon; though by boiling with alcohol for many hours protagon appears to be decomposed, so that by separating the substance which falls first on cooling and subjecting it again to prolonged treatment with boiling alcohol, a body is obtained which differs somewhat in physical characters from protagon; if this body be many times subjected to the action of boiling alcohol and to the above referred-to process of separation, a substance is obtained containing less phosphorus than protagon and having a different composition. This body, which was certainly not absolutely pure, was analysed with the following results:—

		(1)	(2)
C.	in 100 parts	64·44	64·23
H.	„ „	10·46	10·54
N.	„ „	3·12	

(2) By the action of caustic baryta on protagon there is obtained

[1] Gamgee and Blankenhorn, *Op. cit. Journ. of Phys.*, p. 121.

[2] Geoghegan, "Ueber die Constitution des Cerebrins." *Zeitschrift f. phys. Chemie*, Vol. III. (1879), p. 332.

a cerebrin-like body, which agrees fairly in so far as the C and H with Geoghegan's body. The nitrogen has not yet been determined.

C. in 100 parts 68·95
H. „ „ 11·32

(3) In addition to protagon, and other phosphorized matters, there is always extracted from brain by alcohol at 45°, a very considerable quantity of a body, which, in order to distinguish it, the author provisionally termed *pseudo-cerebrin*. This body is less soluble in 80 p. c. alcohol at 45° than protagon, so that on subjecting impure protagon to repeated crystallization from 80 p.c. alcohol there accumulated residues consisting of the cerebrin-like body. The latter is a white, pulverulent body, very unlike protagon to the naked eye and separating under the microscope in the form of very large nodular masses. After repeated recrystallization from alcohol it was found to be practically free from phosphorus (containing only 0·08 p. c.).

On analysis this body has given results which are not widely different from those of Geoghegan, though they are sufficiently discrepant to render it certain that the substances analysed by that observer and himself were not identical. Whilst the author would refrain from speaking with confidence of the absolute purity of '*pseudo-cerebrin*,' he has, however, no hesitation in asserting that Geoghegan's substance, from the method of preparation, is necessarily a mixture of the so-called pseudo-cerebrin just referred to with a 'cerebrin' obtained by the action of barium hydrate on protagon—and therefore much more impure than the body now provisionally described by the term of pseudo-cerebrin.

Analyses of '*pseudo-cerebrin*' found by the Author to accompany Protagon.

	(1)	(2)	(3)	(4)	Mean.
C.	68·97	68·95	69·01	68·67	68·89
H.	11·7	11·17	11·60	12·10	11·87
N.	1·76	1·95	1·64	2·01	1·83
O.					17·41
					100·00

From the above analyses the author deduces for '*pseudo-cerebrin*' the empirical formula $C_{44}H_{92}NO_8$.

It would therefore appear to the author that whilst protagon cannot be separated by the action of solvents into a non-phosphorized cerebrin and a phosphorized body, yet such non-phosphorized bodies exist by its side in the brain, and can be obtained from protagon by the action of caustic baryta.

Geoghegan's researches in the decomposition of cerebrin. Cetylid.

By the action of concentrated sulphuric acid on cerebrin, this author has obtained a body to which he ascribes the formula $C_{22}H_{42}O_5$ and believes to be a derivative of cetyl-alcohol, and which he terms Cetylid. On fusion with caustic potash this body evolves a mixture of CH_4, H, and N, whilst palmitic acid is formed; a portion of the N is left in the form of an ammoniacal salt.

Thudichum's researches on the cerebrins.

Under the name of cerebrins, Thudichum describes a class of nitrogenous bodies free from phosphorus, which he believes to exist in the brain. Certain of these bodies he obtained by following substantially Müller's process; others by extracting brains with alcohol at 45° C., and purifying the substance obtained by various solvents. He believes Müller's cerebrin to be the lowest representative of a group of nitrogenous prinicples of the brain which are free from phosphorus, contain nitrogen, and vary in the number of carbon atoms which they contain, for each nitrogen atom, between 17 and 48. "Whatever may be the ultimate explanation of these differences of composition must be left for future inquiry. Meanwhile it is certain that these differences do but slightly affect the external appearance and bearing towards solvents of these bodies, so that by describing the general properties of one we describe the general properties of all members of the group, while differentiating characters and means are most difficult of discovery and application."

"The cerebrins are all soluble in hot alcohol, particularly in absolute alcohol, and deposited on cooling; they are very little soluble in cold absolute alcohol, much less soluble indeed than myeline, which can thus be separated from the cerebrins. The mixture is dissolved in hot alcohol and allowed to cool; nearly all cerebrin falls down, much myeline remains in solution. The deposit is separated from the liquid, and subjected to this treatment until it is free from phosphorus."

The following are the three chief bodies which Thudichum classes amongst the cerebrins.

Cerebrin $C_{34}H_{66}N_2O_8$ (Müller).
Phrenosine $C_{34}H_{67}NO_8$.
Kerasine $C_{46}H_{91}NO_9$.

Sect. 6. Cholesterin ($C_{26}H_{44}O + H_2O$).

Amongst the most abundant of the constituents of the nervous tissues, and especially of the white matter, is the beautiful, crystalline, non-nitrogenous, body cholesterin. This body, which is very freely soluble in ether, cold or hot, is also freely dissolved by warm alcohol, which in great part deposits it on cooling; in consequence of its solubility in these two fluids, cholesterin finds its way into both the ethereal and alcoholic extracts of the nervous tissues[2].

Preparation of cholesterin from brain or spinal cord.

The tissue from which cholesterin is to be extracted may be placed in cold alcohol for some days, so as to deprive it of the greater part of its water. The hardened substance is then finely divided and digested in boiling alcohol. The alcoholic solution is filtered

[1] Thudichum, "Researches on the Chemical Constitution of the Brain." *Reports of the Medical Officer of the Privy Council and Local Government Board*, London, 1874, pp. 113—247.

[2] The author some years ago performed a number of experiments with the object of determining whether cholesterin preexists in the nervous tissues or is merely one of the products of the decomposition of more complex bodies. These experiments led him to the conclusion that cholesterin exists preformed in the brain.

through a heated funnel and the filtrate is cooled. The deposit, which consists of cerebrin, protagon, other complex phosphorized bodies, and cholesterin, is collected on a filter, washed with cold alcohol, and then, after being pressed between filter paper, is shaken in a stoppered bottle with ether; the ethereal solution is filtered, the ether is distilled off, and the residue, consisting of impure cholesterin mixed with some lecithin, is heated in a water bath for an hour with an alcoholic solution of caustic potash. The contents of the flask are then evaporated to dryness on the water bath, and the dry residue is washed with water and dissolved in a mixture of ether and alcohol, from which it is allowed to crystallize by the spontaneous evaporations of the solvents.

Properties. Pure cholesterin separates from its solutions in anhydrous ether or chloroform in the form of needles containing no water of crystallization; but from alcohol it separates in the form of rhombic tables.

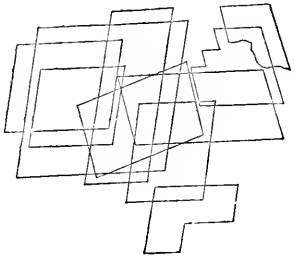


FIG. 60. CRYSTALS OF CHOLESTERIN AS IT SEPARATES FROM ALCOHOL OR ETHER CONTAINING WATER. (Frey.)

Cholesterin is insoluble in water, alkalies and dilute acids; it is very slightly soluble in cold, but soluble in 9 parts of boiling, alcohol. It is highly soluble in ether cold and boiling, in chloroform, benzol, and in solutions of salts of the bile acids.

Dry cholesterin melts at 145°, and distils *in vacuo* at 360°. Its solutions exert a left-handed rotation on the plane of polarization. The specific rotatory power of solutions of cholesterin $(a)_D = -31°{\cdot}6$. Amongst the most useful reactions for detecting cholesterin are the following :—

1. When treated with concentrated sulphuric acid, and afterwards a little iodine, a play of colours, of which blue, green, and red are the most prominent, is produced. This reaction may be employed as a more stringent proof than that offered by the microscopic characters of the crystals, and it may be well observed under the microscope.

2. When cholesterin is gently heated with five volumes of sulphuric acid and one volume of water, the edges of the crystals are seen to become of a carmine colour; this reaction admits of

being performed on a microscopic slide, and the results may be watched under the microscope.

3. When cholesterin is dissolved in chloroform, and the chloroformic solution is shaken with an equal volume of strong sulphuric acid, the chloroform becomes successively blue, red, cherry-red, and ultimately purple, whilst the subjacent sulphuric acid acquires a marked green fluorescence.

4. When heated gently with a mixture of one volume of solution of ferric chloride and two volumes of hydrochloric acid, cholesterin assumes a violet or blue colour.

Compounds and derivatives of cholesterin.

Cholesterin is a monad alcohol, and it readily forms compounds with certain acids as with the volatile fatty acids. By the action of bromine upon cholesterin, both bodies being dissolved in carbon disulphide, *Cholesterin dibromide* ($C_{26}H_{41}OBr_2$) is formed.

By the action of phosphorus pentachloride on dry cholesterin, cholesteryl chloride $C_{26}H_{43}Cl$ is obtained. By the action of an alcoholic solution of ammonia upon the chloride, cholesterylamine $C_{26}H_{43}NH_2$ is obtained. By treating a boiling alcoholic solution of cholesteryl chloride with sodium amalgam, a crystalline carbo-hydrate having the composition C H and a melting point of 90°, is obtained.

By the action of boiling nitric acid on cholesterin, cholesteric acid is obtained, $C_8H_{10}O_5$. This body is one of the substances obtained when cholic acid is oxidized in a similar manner.

When oxidized by means of chromic acid, cholesterin yields oxycholic acid, $C_{24}H_{40}O_6$. The two last compounds establish a close relationship between cholesterin and the bile acids.

SECT. 7. EXTRACTIVE MATTERS OCCURRING IN THE NERVOUS TISSUES WHICH ARE COMMON TO THESE AND TO OTHER TISSUES, ESPECIALLY THE CONTRACTILE.

It is a fact worthy of notice that the brain contains considerable quantities of the same bodies which are found in muscle, viz. creatine, xanthine, hypoxanthine, inosit, and lactic acids; in addition it contains leucine, uric acid and probably urea.

According to W. Müller[1] the quantity of inosit in ox brain amounts to 0·8 parts per 1000. The same author separated 0·6 grms. of uric acid from 50 pounds of ox brains. Müller found creatine in the brain of man, but not in that of the ox.

The quantity of lactic acid separated from ox brain is said to amount to 0·5 per 1000, and, strangely, to be identical with the lactic acid of fermentation. As Kühne has remarked, this lactic acid may take its origin from the inosit of the brain.

[1] Müller, *Annal. der Chemie u. Pharm.*, Vol. CIII. (1857), p. 131.

Sect. 8. The inorganic constituents of the nervous tissues.

Brain is extremely poor in inorganic matters, though it is difficult from the discrepant results of various writers to give reliable facts as to the exact amount of these; the estimates vary between 0·1 and 1 per cent. of the fresh brain.

Not only are statements discrepant as to the total quantity of brain ash, but also as to the relative amounts found in the white and grey matters. It appears to be true that the ash of the grey matter has an alkaline, whilst that of the white has an acid reaction.

The following table exhibits the results of the analyses of the mineral matters of brain made in Hoppe-Seyler's laboratory by Geoghegan[1].

INORGANIC MATTERS CONTAINED IN 1000 PARTS OF BRAIN.

	(1)	(2)	(3)	(4)
Cl	1·20	0·430	1·320	1·064
PO_4	1·40	0·956	2·016	1·392
CO_3	0·796	0·244	0·548	0·330
SO_4	0·220	0·102	0·136	0·132
$Fe(PO_4)_2$	0·010	0·096	0·098	0·032
Ca	0·005	0·020	0·014	0·022
Mg	0·016	0·068	0·060	0·072
K	1·630	0·580	1·778	1·520
Na	1·000	0·450	1·114	0·780
Total Ash	6·277	2·946	7·084	5·344

Sect. 9. General summary shewing the results of quantitative analyses of brain, spinal cord and nerves.

1. *Proportion of Water.*

The amount of water is much larger in grey than in white matter, in early than adult life. The following are observations made by Weisbach on the brains of men.

PROPORTION OF WATER IN 100 PARTS.

	Age 20 to 30	Age 30 to 50	Age 70 to 94
White substance of brain	69·56	68·31	72·61
Grey „ „ „	83·36	83·60	84·78
Cerebellum	78·83	77·87	80·34
Pons Varolii	73·46	72·55	72·74
Medulla oblongata	74·43	73·25	73·62

In the foetus the brain contains between 87·9 and 92·6 per cent. of water.

[1] Geoghegan, "Ueber die anorganischen Gehirnsalze." *Zeitschr. f. phys. Chem.*, Vol. I. p. 330.

The proportion of water in the spinal cord is less than in the brain. Thus Bernhardt obtained the following results :—

PROPORTION OF WATER IN THE SPINAL CORD AND MEDULLA OBLONGATA.

Cervical portion of cord	73·05 p. c.
Lumbar „ „	76·04 „
Medulla oblongata	73·90 „
Cortex of brain	85·86 „
White matter of brain	70·08 „
Sympathetic cord	64·30 „

2. *Proportion of the chief organic constituents of ox brain* (Petrowski[1]).

	Grey matter.	White matter.
Solids	18·40 p. c.	31·65 p. c.
Water	81·60 „	68·35 „
Albumin and Gelatin	55·37	24·72
Cerebrin	0·53	9·55
Lecithin	17·24	9·90
Cholesterin	18·68	51·91
Substances insoluble in anhydrous ether	6·71	3·34
Salts	1·45	0·57

The above analyses, though interesting as shewing the varying proportions of certain of the brain constituents, such as water, proteids, and cholesterin, must not be considered as throwing any light upon the nature or distribution of the phosphorized constituents. The phosphorus present in the mixed alcoholic and ethereal extracts having been determined, a calculation was made upon the unwarrantable assumption that all the phosphorus was derived from lecithin. The reader who has perused the preceding pages will understand the unfounded nature of this surmise.

Sect. 10. The chemical processes connected with the activity and death of the nervous tissues.

We are acquainted with singularly few facts which throw a light upon the chemical processes which have their seat in the organs of the nervous system.

The great vascularity of the central organs as compared with the nerves, and especially of the grey matter of the central organs, establishes a presumption that processes have their seat in the nerve cells of the grey matter which demand an abundance of oxygen.

[1] Petrowsky, "Zusammensetzung der grauen und der weissen Substanz des Gehirns." Pflüger's *Archiv*, Vol. VII. p. 367.

Observation of the living organism also teaches us that the proportion of oxygen which is supplied to certain of the central organs influences their activity in a remarkable manner; thus the activity of the respiratory centre in the medulla is affected chiefly by the amount of oxygen of the blood which traverses it. Again, an adequate supply of oxygen to the brain appears to be a condition essential to the proper exercise of the mental functions, and it is probably in consequence of deprivation of oxygen that the moment blood is cut off from the brain, as by ligaturing or compressing some of the large arteries supplying it, all mental acts cease. When, however, we direct our inquiries to the nature of the processes which have their seat in the nerve cells we are obliged to conclude that we are yet altogether in the dark.

The nerve fibre is much less directly influenced by a supply or absence of oxygen than the central organs, and it is probably for this reason, amongst others, that it survives, even in warm-blooded animals, after the brain and spinal cord have ceased to manifest any signs of vitality.

The only change of a chemical nature which has been proved to occur in nerves as a result of long continued activity, or at death, is a change in the reaction of the axis cylinder, which from an alkaline changes to an acid reaction. The grey matter of the brain having an acid reaction even during life [1], no change can be observed to occur at death.

When nerve fibres are cut off from their connection with certain nerve cells, whilst the life of the animal is preserved, they gradually undergo a fatty degeneration which affects the axis cylinders and ultimately leads to an abolition of their power to act as conducting organs.

[1] Gscheidlen, "Ueber die Reaction der nervösen Centralorgane." Pflüger's *Archiv*, Vol. VIII. p. 171.

CHAPTER XI.

CHEMICAL HISTORY OF CERTAIN OF THE PERIPHERAL TERMINATIONS OF THE NERVOUS SYSTEM AND OF THE ACCESSORY STRUCTURES CONNECTED WITH THEM,—THE TISSUES AND MEDIA OF THE EAR, THE TISSUES AND MEDIA OF THE EYE.

Introductory.

DIRECTLY or indirectly all the nerve fibres of the organism are connected *centrally* with nerve centres, of which we have examined the chemical history, so far as it is at present known to us. *Peripherally* nerve fibres either commence in certain special *end-organs* capable of being influenced by movements in the external medium and of transmitting the influence through the nerves to the nerve centres (*afferent nerve fibres*), or they terminate in structures of which the immense majority are concerned in bringing about changes in the position of different organs of the body, and changes in the relation of the organism to the medium which it inhabits (*efferent nerve fibres*). Fibres of the latter class terminate by peculiar end-organs in the contractile tissues which have formed the subject matter of Chapter IX.

In the present chapter there remains to be discussed the chemical history, so far as it is known, of the peripheral nervous end-organs which are connected with afferent nerves, though unfortunately it is only in connection with the eye that any detailed information is available. For reasons of expediency we shall consider not merely the chemical facts relating to the actual nervous structures, but also those relating to the accessory apparatus with which they are connected.

SECT. 1. THE TISSUES AND MEDIA OF THE EAR.

The organ of hearing of vertebrates, reduced to its simplest form, consists of a membranous sac of greater or less complexity, termed the membranous labyrinth, on the inner surface of which are situated

epithelial structures which are directly continuous with fibres of the auditory nerve. The sac contains a liquid termed *endolymph* and is usually separated from the bony or cartilaginous structures wherein it lies, by a liquid termed *perilymph*, through which sound-waves have to be transmitted before they can affect the structures contained in the membranous labyrinth. On the inner surface of the membranous labyrinth, in certain situations, are crystalline bodies usually termed *otoliths* or *otoconia*.

Perilymph and Endolymph.

Perilymph. According to Dähnhardt[1] the perilymph of the haddock is a somewhat tough gelatinous liquid, rich in mucin, and containing a proteid matter precipitable by acids but not coagulated by heat. It contains from 2·1 to 2·2 per cent. of solid constituents. Its chief saline constituent is common salt.

Endolymph. According to the same observer the endolymph of the haddock is a clear liquid, containing 1·5 per cent. of solid matter; the quantity of mucin is small, and albumin is absent.

Otoliths, Lapilli, or Otoconia.

In the vestibular sacs of the membranous labyrinth of most (though not of all) fishes, lying free upon the surface of the epithelium and bathed by endolymph, lie small concretions, termed *otoliths, lapilli,* or *otoconia,* which are either pulverulent, as in the plagiostomatous fishes, or hard and stony, as in the osseous fishes. In these cases the otoliths rest freely on the surface of the long projections of the hair-cells which line the otolith sacs.

Although by no means universally distributed, similar concretions are met with in the vestibular sacs and in the ampullar commencements of the semicircular canals throughout the various groups of vertebrate animals, though as a general rule they do not present themselves as individual *lapilli,* lying free, but rather as pulverulent crystalline concretions lying imbedded in the epithelial lining of the sacs. In the latter case the individual crystals are surrounded and held together by a slimy organic matter. Otoliths also occur in many invertebrate groups.

According to Johannes Müller, the otoliths of the osseous fishes have a structure similar to that of the enamel of the teeth, though the statement is one which cannot, on morphological grounds, be comprehended and invites further examination[2].

[1] Dähnhardt, "Endolymphe u. Perilymphe." *Arbeit. d. Kieler physiol. Instituts,* p. 103.

[2] For much interesting information on otoliths consult Müller's *Elements of Physiology,* translated by Baly. Vol. II. p. 1129 et seq.

Crystalline appearance of pulverulent otoliths.

The fine pulverulent otoliths which occur in most animals present the appearance of microscopic crystals, presenting remarkable variations in size. Their form is shewn in the accompanying illustration.

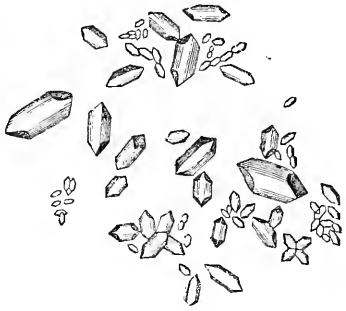


FIG. 61. OTOLITHS, COMPOSED OF CALCIUM CARBONATE. (From Frey, after Funke.)

According to Dähnhardt[1] otoliths contain from about 74·5 to 77·5 per cent. of mineral matter, composed chiefly of calcium carbonate in the form of crystals. The organic matter resembles mucus.

The membranous Labyrinth.

As yet no information whatever is possessed in reference to the composition of the walls of the labyrinth. Mainly these are composed of connective tissue, which is said to resemble the cornea in structure[2]. Of the chemical characters of the epithelium of the labyrinth nothing whatever is known.

SECT. 2. THE TISSUES AND MEDIA OF THE EYE.

The Cornea.

The ground-substance of the cornea presents, as has already been pointed out (p. 271), very great similarity in chemical composition to that of hyaline cartilage, and until lately it was asserted without contradiction that both tissues, when subjected to prolonged boiling, yield *chondrin*, although that substance, as obtained from the cornea, was said to possess somewhat special reactions. As we have already pointed out, Morochowitz[3] denies the existence of chondrin and looks

[1] Dähnhardt, "Endolymphe u. Perilymphe." *Arbeit. d. Kieler physiol. Instituts*, p. 106.

[2] Hensen, *Op. cit.* p. 68.

[3] Morochowitz, "Zur Histochemie des Bindegewebes." *Separat-Abdruck aus den Verhandlungen des naturhist.-med. Vereins zu Heidelberg.* Vol. I. Part 5.

upon the ground-substance of the cornea, like that of hyaline cartilage, as composed of collagenous and mucin-yielding bodies.

When digested in sulphuric acid the cornea may be split up into lamellae, whilst potassium permanganate separates these into fibrils which are broader than those of the fibrillar connective tissue (Kühne).

Acetic acid first renders the cornea transparent and afterwards causes it to swell, though the ground-substance does not dissolve. After digestion in dilute mineral acids, the ground-substance of the cornea becomes readily soluble in boiling water.

When the cornea is heated to 55° C. it becomes opaque, in consequence partly of changes in the corpuscles: in part, however, because of the coagulation of proteids previously existing in solution in the parenchymatous fluid bathing the tissue (Kühne)[1]. By treating the cornea with water, this liquid dissolves alkaline albuminates and a globulin which, according to Schmidt, possesses fibrinoplastic activity.

Presence of Myosin in the protoplasm of the cornea corpuscles.

In the course of his beautiful studies on the histological structure of the yet living cornea corpuscles Kühne[1] was led, from the eminently contractile character of their bodies, from their behaviour to stimuli, and from the changes which they undergo at death, to surmise the close relationship of their protoplasm to the substance of muscle. This relationship, according to Bruns[2], is further evidenced by the fact that the cornea contains myosin, doubtless derived from its corpuscles.

To obtain myosin, Bruns separated the cornea from the sclerotic, and placed the finely divided structure in saturated solution of NaCl for 24 hours. The solution on being treated with large quantities of distilled water deposited a precipitate, soluble in weak solutions of NaCl (containing less than 10 per cent.) and in water containing 1 part in 1000 of hydrochloric acid.

Results of analyses of cornea.

The following is an analysis of the cornea by His.

Water in 1000 parts	758·3
Collagen	203·8
Organic matters insoluble in water .	28·4
Soluble salts	8·4
Insoluble „	1·1
	1000·0

Sclerotic.

No special information is possessed in reference to the sclerotic, which, however, consists of collagenous connective tissue.

[1] Kühne, *Untersuchungen über das Protoplasma.* See section entitled "Das Protoplasma der Zellen in der Cornea" (p. 123—131).

[2] Bruns, "Chemische Untersuchungen über die Hornhaut des Auges." Hoppe-Seyler's *Untersuchungen*, p. 260.

Aqueous Humor.

This liquid which fills the anterior chamber of the eye is free from all formed elements. Although the anterior chamber must be looked upon as essentially a lymph space, and the secretion of the aqueous humor like that of lymph is essentially dependent upon the arterial pressure[1], yet it possesses a very different chemical composition.

Physical properties. Aqueous humor is a perfect transparent liquid of specific gravity 1003—1009, possessed of alkaline reaction.

Chemical constituents. Aqueous humor contains a trace of a proteid matter which is stated by Kühne to be fibrinoplastic, it also contains about 4 parts per 1000 of extractive matters, amongst which is urea, and from 7 to 8 parts per 1000 of mineral matters.

The following are the results of an analysis by Lohmeyer[2] of the aqueous humor of a calf.

Water per 1000	986·87
Proteids	1·22
Extractive matters	4·21
Sodium chloride	6·89
Other mineral matters . . .	0·81
	1000·00

Crystalline Lens.

The crystalline lens is composed of concentric layers of fibres, which are essentially elongated cells, and which usually present more or less marked serrated edges. The structure is bounded externally by a *capsule* composed of a structureless membrane which appears to possess physical and chemical characters similar to those of the sarcolemma of muscle.

The crystalline lens is not homogeneous, as its refractive index increases as we pass from the more external to the more internal layers —an optical property which probably bears a relation to the fact that the specific gravity of the central portion of the lens is, according to Chevenix[3], greater than that of the superficial layers, in the proportion of 1194 to 1076.

Chemical constituents of the Lens. The lens contains about two-thirds of its weight of water; its solid matters consist chiefly of a globulin (about 24·6 p.c.) besides some serum albumin; in addition they contain small quantities of fat, traces of cholesterin and salts.

[1] Chavvas, "Secretion des Humor aqueus im Bezug auf die Frage nach den Ursachen der Lymphbildung." Pflüger's *Archiv*, Vol. xvi. p. 143.

[2] See Gorup-Besanez, *Lehrbuch d. phys. Chemie*, 4to. ed. (1878), p. 401.

[3] Chevenix, quoted by Kühne, *Lehrbuch*, p. 404.

The globulin contained in the lens.

Under the name of Crystallin, Berzelius described the proteid belonging to the group of *globulins* which forms the chief solid constituent of the crystalline lens. This substance is soluble in water holding oxygen in solution, forming an opalescent liquid, which is precipitated by CO_2. According to Laptschinsky[1], acetic acid does not precipitate this body, which, however, separates in a flocculent form when its solution is heated to 70° C. According to this author the lens behaves fibrinoplastically: according to Kühne it does not possess that property.

The cornea becomes opaque after death; it is believed (Kühne) that this is not due to any coagulation of a soluble proteid, but to diffusion phenomena, leading to the formation of vacuoles in the lens-fibres, which necessarily impair the passage of light.

Results of quantitative analyses of the lens.

The following are the results of four analyses of the crystalline lens of the ox, made by Laptschinsky.

Proteids in 100 parts	34·93
Lecithin	0·23
Cholesterin	0·22
Fats	0·29
Soluble salts	0·53
Insoluble salts	0·29

The following are the results of other analyses of the lens of the ox made by Hoppe-Seyler and Laptschinsky.

	Hoppe-Seyler	Laptschinsky
Proteids in 100 parts	33·03	34·72
Aqueous extract	0·94	0·95
Alcohol extract	0·52	0·37
Insoluble salts	0·12	0·17
Soluble salts	0·61	0·50
Ash obtained on incinerating the aqueous extract	0·52	0·39
Ash obtained on incinerating the alcoholic extract	0·08	0·11
Ethereal extract	—	0·45

Changes of the lens in cataract.

By introducing solutions of salts or of sugar under the skin of frogs a form of cataract is induced in which the structure presents vacuoles; these have been produced apparently by the abstraction of water from the lens; the cataract which occurs in the course of some cases of diabetes is probably induced in this way.

In genuine cataract the more common change consists, however, in a fatty degeneration of the lens in which cholesterin is abundantly deposited; occasionally it is said that the lens is the seat of a deposition of calcareous salts.

[1] Laptschinsky, *Ein Beitrag zur Chemie des Linsengewebes.*

The Vitreous Body.

The vitreous body or humor consists essentially of mucous connective tissue. In the very loose and large meshes of the tissue is contained a large quantity of watery fluid, containing a small quantity of proteids and said to be specially rich in urea (according to Picard containing 0·5 per cent. of that body).

The following is an analysis of the vitreous body by Lohmeyer[1].

Water in 1000 parts	986·400
Membranes	0·210
Proteids and mucin (chiefly the latter)	1·360
Fats	0·016
Extractive matters (urea, &c.) . .	3·206
Sodium chloride	7·757
Other mineral matters . . .	1·051
	1000·000

The Choroid.

The middle coat of the eye or *Choroid* is eminently vascular and contains, imbedded within its substance, branched pigment-cells very similar to those of the frog's skin. Until the researches of Max Schultze had shewn that they properly belonged to the retina, the layer of hexagonal pigment-cells (retinal epithelium) which we shall describe in the sequel were described as an integral part of the choroid.

All that need now be said in reference to the chemistry of the choroid is that its branched pigment-cells contain a pigment which appears to be similar to, if not absolutely identical with, that which under the term of *Fuscin* we shall describe as the pigment of the *retinal* epithelium.

THE RETINA.

Introductory.

The retina is the most internal of the tunics of the eye, and contains the complex terminations of the optic nerve.

This membrane which by its internal surface lies in contact with, or applied to, the external surface of the vitreous body and which is covered externally by the vascular choroid, possesses during life an exquisite transparency and doubtless throughout its greater part absolute optical homogeneity, so that undulations of light which have traversed the transparent media of the eye and impinge upon the inner surface of the retina, may penetrate to the very peripheral structures which they are destined to throw into action.

The retina possesses a connective-tissue framework, wherein lie imbedded the greater part of its nervous elements, but which does

[1] Quoted by Gorup-Besanez.

not extend so far as to afford support to those structures (the *rods* and *cones*) which are eminently the end-organs of the optic nerve or to those pigmented epithelium cells which afford a close investment to the outer limbs of the rods.

Description of the ten layers of the Retina.

In the accompanying engraving the structure of the retina is semi-diagrammatically represented, so as to shew with clearness the position, and the mutual relations, of the ten layers which, since Max Schultze's description, histologists have agreed to distinguish.

The first layer (1, Fig. 62) is composed of the so-called *membrana limitans interna*, which is a fibrillated membrane belonging to the connective-tissue framework.

The second layer (2, Fig. 62) is the *nerve-fibre layer* and is composed of naked axis-cylinders continuous with the optic nerve fibres which having pierced the sclerotic and cornea enter the retina at the '*colliculus nervi optici*.'

The third layer (3, Fig. 62) the *nerve-cell or ganglionic layer*, is com-

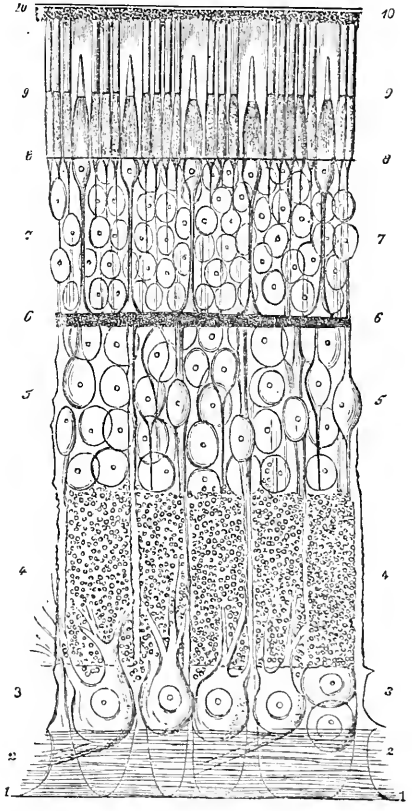


FIG. 62. DIAGRAMMATIC SECTION OF THE RETINA. (Max Schultze.)

posed of multipolar nerve-cells which unquestionably communicate by certain of their processes with the fibres of the second layer, and by other more delicate processes with the delicate reticulum which constitutes a great part of the fourth layer.

The fourth layer (4, Fig. 62), termed the *inner molecular layer*, is composed in part of fibres belonging to the connective-tissue framework, which afford support to a delicate reticulum which doubtless is the medium of communication between the layers which lie internal and external to it.

The fifth layer (5, Fig. 62), or *internal granular layer* (also *inner nuclear layer*), is conspicuous for the presence of the so-called *granules*, viz. small transparent nucleated spherical bodies with two poles, of which one points towards the inner the other towards the outer molecular layer, and which are doubtless connected with the networks of those layers. These granules are considered by all to belong to the nervous elements of the retina.

In addition we observe, however, in the fifth layer certain *granules* which are probably connective-tissue cells, and radiating fibres (fibres of Müller) which belong to the connective-tissue framework, which is specially well developed in the granular layers.

The sixth layer (6, Fig. 62), or outer molecular layer, possesses a structure similar to that of the fourth or inner molecular layer, consisting of fibres of which some doubtless belong to the connective-tissue framework and merely afford support for a truly nervous reticulum; this outer molecular layer is much less deep than the inner molecular layer.

The seventh layer (7, Fig. 62), or *external granular layer* (also *outer nuclear layer*), presents many strata of bodies resembling in the main those characteristic of the inner granular layer, and like them presenting nuclei and two poles of which the inner pass to the reticulum of the outer molecular layer; the *granules* offer however peculiarities:—1stly their external poles very clearly are connected with either the rods or cones of the ninth layer: 2ndly the granules which are connected with the cones (*cone-granules*) are larger, are situated in the more external strata of the layer, and are directly joined to the cones without the intermediation of fibres, whilst the *rod-granules* are smaller, are joined to the rods by fibres and present two transverse stripes.

The eighth layer (8, Fig. 62) is composed of the external limiting membrane and, like the first layer, is composed of a fibrillated membrane which forms the external boundary of the connective-tissue framework of the retina; within this boundary the retina is vascular, outside it is absolutely free from blood-vessels. The external limiting membrane is perforated by the communications between the rods and cones and the outer granular layer.

The ninth layer (9, Fig. 62), *bacillary layer* or *layer of rods and cones*, is composed of the bodies which are by common consent and for undeniable reasons considered to be the end-organs which are directly excited by luminous undulations, which initiate the state of activity,

which travels through the more internal layers of the retina and ultimately stimulates the optic nerve fibres.

Particular description of the Visual Epithelium.

The rods and cones are included by Kühne in the term of *visual cells* (Sehzellen) or *visual epithelium cells*, and must be distinguished from the *retinal epithelium cells*, viz. the pigmented epithelium cells of the 10th layer. Both rods and cones are distinctly nervous elements in so far that they are doubtless in unbroken connection with the layer from which nerve fibres ultimately spring. The rods and cones possess some points in common and some which are distinguishing. "Each consists of two distinct segments—an inner and an outer; the division between the two occurring, in the case of the rods, about the middle of their length (in man); in the cones at the junction of the finer tapering end-piece with the basal part; consequently, the outer and inner segments of the rods are nearly similar in size and shape, the inner being, however, slightly bulged as a rule, whereas the inner segment of the cone far exceeds the outer one in size, the latter appearing merely as an appendage of the inner segment (fig. 63).

"The two segments both of the rods and cones exhibit well-marked differences, both in their chemical and optical characters, as well as in the structural appearances which may be observed in them. Thus while in both the outer segment is doubly refracting in its action upon light, the inner is, on the contrary, singly refracting: the inner is stained by carmine, iodine, and other colouring fluids, whilst the outer remains colourless. The outer segment in both shews a tendency to break up into a number of minute superimposed disks, whereas the inner segment is itself again distinguishable into two parts—an outer part, apparently composed of fine fibrils, and an inner part, homogeneous, or finely granular[1]," by which they communicate directly, in the case of the rods with a rod-fibre, in the case of the cones with a cone-granule. The inner limbs of the rods are longer than those of the cones; on the other hand the outer limbs of the cones are much shorter than those of the rods so that the latter project above the former.

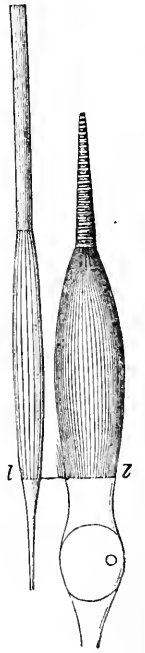


FIG. 63. A ROD AND CONE FROM THE HUMAN RETINA. (Schultze.)

Retinal Epithelium.

The tenth layer (10, Fig. 62), or *pigmentary layer*, is composed of a single layer of hexagonal pigmented epithelium cells, forming a mosaic which covers the outer limbs of the rods; these cells are characterized by possessing long processes which extend from their anterior faces in a beard-like fashion, and lie in the crevices between the rods and cones.

[1] Quain, *Elements of Anatomy*, 8th ed. (edited by Schäfer), Vol. II. p. 613.

Kühne has made the discovery that the protoplasm of these cells is the seat of remarkable movements, as proved by the different distribution of the pigment in them, dependent upon the degree of illumination to which the eye has been subjected. In the pigment cells of a frog which has been kept for several hours in the dark, the pigment is found to be confined to the cell bodies and the roots of the processes coming from these. But if microscopical sections be made of the eyes of frogs which have remained for some time in the sunlight, the pigment will be found to have extended itself much further forward in the cell processes towards the *membrana limitans externa*, a proportionally smaller quantity remaining in the cell bodies. In the eyes of frogs which have been exposed to light, the retina, when removed,

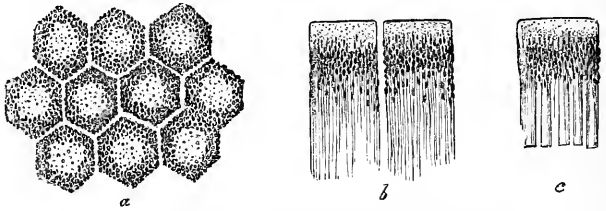


FIG. 64. RETINAL EPITHELIUM CELLS. (Max Schultze.)
(*a*) Cells seen from external surface; (*b*) and (*c*) Cells seen in profile, with processes projecting inwards.

has much epithelium attached to it. Conversely, in the eyes of frogs which have not been exposed to light, the retina can be removed without its epithelial covering. These facts will be again referred to in discussing the functions of the retinal epithelium in regenerating the visual purple.

Tapetum.

In animals possessing a *tapetum*, the epithelial layer of the retina is unpigmented in the tapetal area, and the choroid is composed anteriorly of a dense, strongly light-reflecting tissue. In some animals, as the sheep or ox, the tapetum is composed of fine fibrous tissue. In others, as the dog and cat, it is made up of several layers of unpigmented cells which are filled with exceedingly fine crystals (Max Schultze). Some fish, as the bream (*Abramis Brama*) possess a so-called *pseudo-tapetum* (Brücke, Kühne and Sewall[1]); in the bream the retinal epithelium contains, in certain areas, both dark pigment and amorphous strongly light-reflecting *Guanin*, so that the epithelium seen from before presents a bright or a dark surface according as the pigment, under the influence of darkness or light, is found in the bases or processes of the cells.

Variations in the structure of the retina in different regions.

The preceding description of the structure of the retina does not apply to every part of its surface, though we must refer the reader who requires detailed information on this subject to treatises on histology. Suffice it to say that at the entrance of the optic nerve (*Colliculus nervi optici*) the nerve fibre layer is immensely

[1] Kühne und Sewall, "Zur Physiologie des Sehepithels." *Verhandl. d. naturhist. Vereins zu Heidelberg*, N.S. Vol. II. Heft v. (June, 1880).

developed, whilst the other nervous layers are absent. At the so-called *macula lutea*, and especially at its central depression, the *fovea centralis*, cones are found to the exclusion of rods; at the periphery of the *macula lutea* cones are found, each surrounded by a circle of rods, whilst over the rest of the retina the cones are found sparsely distributed amongst the rods.

Variations in the distribution of rods and cones in the retinae of different classes of animals.

The bacillary layer of the retina does not always possess both rods and cones; in some animals we find rods and in others cones, or where both are present their relative number varies. Thus the following animals have no cones:—the ray, the shark, the sturgeon, the bat, the hedgehog, the mole. The following animals have no rods:—lizards, serpents, tortoises, and perhaps all reptiles. All mammals have more rods than cones; nearly all birds have more cones than rods, though in the owl, which is a nocturnal bird, the cones are very few.

Chemical composition of the Retina as a whole.

In consequence of the scanty material at the chemist's disposal little is known as to the general composition of the retina. The reaction of the retina is said to be acid. According to C. Schmidt[1] the retina, besides containing albumin, yields, on boiling, gelatin and mucin. Its alcoholic extract yields a body which gives a crystallizable compound with platinum chloride and which smells of trimethylamine, doubtless due to the decomposition of neurine. As Kühne remarks, we may on general grounds surmise that the retina contains the same bodies as the central nervous systems.

Whilst the living retina is perfectly transparent, at death it becomes opaque, doubtless in consequence of the coagulation of some proteid constituent.

General Chemical facts relating to Rods and Cones.

Chemical structure of the inner limbs of the rods and cones.

The inner segments of both rods and cones are composed of protoplasm which during life is possessed of marvellous transparency; after death this becomes opaque and presents granular deposits, nuclei, and in some cases spherical, lenticular, or paraboloid, highly refractile bodies.

Chemical structure of the outer limbs of the rods.

The outer limbs of the rods are composed of an external envelope, which agrees, in physical characters and in its power of resisting various agents, with *neuro-keratin*. This external envelope encloses contents which morphologically appear as little disks which are separated by an intermediate substance; it is impossible to distinguish between the chemical characters of these two kinds of substances. Kühne has pointed out that the contents of the envelopes consist of a mixture of proteid bodies and of substances soluble in alcohol and

[1] See Kühne, "Chemie der Netzhaut." Hermann's *Handbuch*, Vol. III. Part 1, p. 239.

ether and doubtless similar to, if not identical with, those extracted by these solvents from the nervous tissues. Kühne has found, indeed, that the contents of the outer limbs behave to osmic acid in almost the same manner as the medullary sheath of nerves; to the substance in the contents which exhibits this reaction Kühne ascribes the name of rod-myeloid (Stäbchen-myeloïd), though he does not wish thereby to indicate that it is a definite proximate principle.

Solubility of the outer limbs of both rods and cones in bile.

Max Schultze first pointed out, and his observations are confirmed by Kühne, that the contents of the outer limbs of the rods and cones are dissolved with extraordinary rapidity and ease by bile, the envelope alone remaining.

Colouring matters associated with cones. (Chromophanes.)

The outer limbs of the cones differ from those of the rods in being invariably free from colouring matters. In birds, reptiles and fishes, however, the inner segment of each cone presents a minute globular body, apparently of a fatty nature, and possessed of brilliant and varied colours, violet, blue, green, yellow, and red, though red and yellow are most frequently met with.

The fact that the pigments are held in solution by fats is proved by the intensely brown colour which the coloured globules acquire when treated with perosmic acid and by the fact that they are dissolved by such solvents of fatty bodies as a mixture of alcohol and ether, carbon disulphide, and benzol.

Whilst the colouring matters of the cones are grouped together under the name of *Chromophanes*, Kühne[1] has succeeded in separating, and examining the physical properties of, three distinct colouring matters, a green, a yellow, and a red, which he distinguishes by the names of *chlorophane*, *xanthophane* and *rhodophane* respectively.

Method of separating the Chromophanes.

A large number of eyes (50 to 300) of doves or hens are bisected so as to cut off the anterior segments; the vitreous humor being removed, the posterior segments of the eyes are placed at once in absolute alcohol; as soon as possible the alcohol is poured away and the eyes are thoroughly exhausted with ether. On evaporating the ether, a fiery-red fat is obtained which is dissolved in hot alcohol and saponified by the action of caustic soda, water being used to replace the alcohol as it evaporates. The hard soap which separates from the mother liquor is well dried and then treated successively with petroleum ether, then with ether, lastly with benzol, which dissolve in order *Chlorophane*, *Xanthophane*, and *Rhodophane;* for the methods of purification the reader is referred to the original paper.

General characters of the chromophanes.

All the chromophanes when treated with solution of iodine assume, as Schwalbe pointed out, a blue colour which differs in intensity and shade according to the shade of the particular chromophane. The chromo-

[1] Kühne und Ayres, *Ueber lichtbeständige Farben der Netzhaut.*

phanes slowly become decolourized even in the dark. They are much more rapidly bleached in the light, though very much less rapidly than the visual purple to be afterwards described. Under the most favourable circumstances a solution of chlorophane exposed to the direct rays of the sun will be bleached in a few hours; a solution of xanthophane under similar circumstances will resist for a period three times longer, and a solution of rhodophane for a period twenty times longer. The process of decolourization is stated by Kühne to be dependent upon the presence of oxygen and to be therefore probably due to oxidation changes.

Special characters of Chlorophane. Chlorophane is of a greenish yellow colour; its alcoholic and ethereal solutions possess this tint. They present two absorption bands; these (in the case of a petroleum ether solution) are situated between F and G.

Special characters of Xanthophane. Xanthophane, unlike Chlorophane, is but slightly soluble in petroleum ether, but readily soluble in alcohol, ether and carbon disulphide. The solutions exhibit a strong absorption of the violet end of the spectrum and a single absorption band, which in the case of the ethereal solution is situated near F, and on its violet side. In the case of solutions in bisulphide of carbon the absorption band is situated between *b* and F.

Special characters of Rhodophane. This colouring matter is not at all soluble in petroleum ether or carbon disulphide. It is most readily soluble in oil of turpentine and in alcohol which has been acidified with acetic acid; these solutions become decolourized, after some hours, even in the dark. Solutions in benzol may be kept indefinitely. These solutions exhibit marked absorption of the violet end of the spectrum and a single absorption band between *b* and F.

Colouring matters associated with the rods.
(*Visual Purple or Rhodopsin.*)

Historical Notes. In the year 1851 Heinrich Müller[1] pointed out that the rods of the retina of the frog when seen *en masse* often present a reddish colouration. In 1857 Leidig[2] referred to the satiny-red colour possessed by the retina of the frog. Later Max Schultze drew attention to the satiny-red colour of the rods of the retina of the rat and owl.

These observations did not however attract marked attention and were lost sight of until the publication of a remarkable paper by Boll, presented to the Berlin Academy towards the close of the year 1876[3], in which that observer announced the startling fact that the bacillary layer of the retina of all animals is during life not colourless, but of a purple red colour.

[1] H. Mueller, *Zeitschr. f. wiss. Zoologie*, Vol. III. p. 234.
[2] Leidig, *Lehrbuch d. Histologie*, p. 238.
[3] Boll, "Zur Anatomie u. Physiologie der Retina." *Monatsber. d. Berl. Acad.*, 12 Nov. 1876.

During life, according to Boll, the peculiar colour of the retina is perpetually being destroyed by the light which penetrates the eye; darkness, however, restores the colour, which vanishes for ever almost immediately after death.

The wonderfully suggestive nature of Boll's discovery led Kühne to repeat his observations[1]. Whilst generally confirming the fundamental statement of Boll, Kühne at once was able to correct and amplify Boll's account. In the first place, relying implicitly upon the statements of Boll, he examined, as soon as possible after death, the retinae of animals (frogs and rabbits) which had been kept for some time in darkness. He soon found that the beautiful purple colour persists after death if the retina be not exposed to light; that the bleaching takes place so slowly in gas-light that by its aid the retina can be prepared and the changes in its tint deliberately watched; that when illuminated with monochromatic sodium light the purple colour does not disappear in from twenty-four to twenty-eight hours even though decomposition has set in. These first observations of Kühne on the vision-purple (Sehpurpur), as he termed it, whilst they shewed that the disappearance of the colour is not, as Boll had asserted, a necessary concomitant of death, removed many of the difficulties which stood in the way of a careful investigation. Carrying out his preparations in a dark chamber illuminated by a sodium flame, Kühne was able almost at once to discover the conditions necessary to the destruction of the vision-purple, as well as the most important facts relating to its restoration or removal. Since then the investigation of the retinal pigments and of photo-chemical processes in the eye have formed the subject of continuous and successful studies on the part of Kühne and his pupils, and it is to them that we owe all the important facts relating to this fascinating subject[2].

[1] Kühne, "Zur Photochemie der Netzhaut." Gelesen in der Sitzung des Naturhistorisch-medicinischen Vereins zu Heidelberg, den 5 Jan. 1877.

[2] The following is a list (in the order of publication) of the researches of Kühne and his pupils on the retinal pigments and photochemical processes in the retina which have appeared in the *Untersuchungen aus dem physiologischen Institute der Universität Heidelberg.*

(1) Kühne, "Zur Photochemie der Netzhaut." (2 Abdruck.) *Untersuchung.* Vol. i. Part i.

(2) Kühne, "Ueber den Sehpurpur." *Ibid.*

(3) Kühne, "Ueber die Verbreitung des Sehpurpurs im menschlichen Auge." *Untersuchung.* Vol. i. Part ii. p. 105.

(4) Kühne, "Weitere Beobachtungen über den Sehpurpur des Menschen." *Ibid.* p. 109.

(5) Kühne, "Das Sehen ohne Sehpurpur." *Ibid.* p. 119.

(6) Ewald u. Kühne, "Untersuchungen über den Sehpurpur." *Ibid.* p. 139.

(7) Kühne, "Ueber die Darstellung von Optogrammen im Froschauge." *Ibid.* Vol. i. Part iii. p. 225.

(8) Kühne, "Eine Beobachtung über das Leuchten der Insectenaugen." *Ibid.* p. 242.

(9) Ewald u. Kühne, "Untersuchungen über den Sehpurpur." (Fortsetzung.) *Ibid.* p. 248.

(10) Kühne, "Ueber lichtbeständige Farben der Netzhaut." *Ibid.* Vol. i. Part iv. p. 341.

Having given this brief account of the progress of discoveries on the visual purple, a short abstract of all the more important facts which have been brought to light may be given.

Distribution of the Visual Purple in the Retina.

If the retina of a rabbit or a frog—preferably of one which has been kept for some time in the dark—be quickly removed from the perfectly recent eye, in a room lighted with the help of a monochromatic yellow light, and be taken into the daylight, it will be observed to be of a purple-red colour, which quickly bleaches on exposure. On a closer inspection it will be found that in a horizontal plane cutting the retina the purple colour is more intense, forming a distinct purple band, whilst the *macula lutea* and a rim 3—4 millimetres broad, at the *ora serrata*, are devoid of colour. If the retina be examined under the microscope the purple colour will be found to be limited to the rods, *and to the outer segments of these*, all other parts of the retina looking greenish by contrast. Thus the purple colour varies in fulness directly with the richness of the retina in rods. The more cones, the less visual purple: and *vice versa*. Hence the absence of purple from the *fovea centralis* which contains cones only, and its entire deficiency in the rod-less retinae of reptiles. But, although the colour is confined to the outer limbs of the rods, it must not be supposed that every rod is purple. The rods in the neighbourhood of the *fovea centralis* (viz. in the *macula lutea*) lack colour, as also do the rods in the colourless margin near the *ora serrata*. The cause of the greater depth of purple in the horizontal zone previously referred to has not been discovered, as, for instance, whether it is due to a more intense colouration of each rod segment, or to a greater length of the rod segments.

(11) Ewald u. Kühne, "Untersuchungen über den Sehpurpur." (Schluss.) *Ibid.* Vol. I. part iv. p. 370.
(12) Kühne, "Beobachtungen über Druckblindheit." *Ibid.* Vol. II. Part i. p. 46.
(13) C. Fr. W. Krukenberg, "Ueber die Stäbchenfarbe der Cephalopoden." *Ibid.* p. 58.
(14) Kühne, "Beobachtungen an der frischen Netzhaut des Menschen." *Ibid.* p. 59.
(15) Kühne, "Fortgesetzte Untersuchungen über die Retina und die Pigmente des Auges." *Ibid.* p. 89.
(16) Ayres u. Kühne, "Ueber Regeneration des Sehpurpurs beim Säugethiere." *Ibid.* p. 215.
(17) Ewald, "Ueber die entoptische Wahrnehmung der Macula Lutea und des Sehpurpurs." *Ibid.* p. 241.
(18) Kühne, "Zur Abwehr einiger Irrthümer über das Verhalten des Sehpurpurs." *Ibid.* p. 254.
(19) Kühne, "Notiz über die Netzhaut der Eule." *Ibid.* p. 257.
(20) Mays, "Ueber das braune Pigment des Auges." *Ibid.* Heft III. p. 324.
(21) Kühne, "Notizen zur Anatomie und Physiologie der Netzhaut." *Ibid.* p. 378.

The first two papers in the above list were translated from the German by Mrs Foster, edited with notes by Dr Michael Foster and published under the title "On the Photochemistry of the Retina and on the Visual Purple." London, Macmillan and Co., 1878.

Kühne has recently given a systematic account of his researches under the title of "Chemische Vorgänge in der Netzhaut" in Hermann's *Handbuch der Physiologie*, Vol. I. Part i. (1879) p. 235—337.

With regard to the distribution of the visual purple in the animal kingdom, it is to be remarked that whilst the rod-bearing retinae of vertebrates generally possess it, in a few isolated animals it is inexplicably absent. Thus a species of bat (*Rhinolophus hipposideros*) has no purple, and hens and pigeons want it, though bats have none but rods in their retinae, while the birds mentioned, with a preponderance of cones, yet possess rods also. With these exceptions, all vertebrates with rod-bearing retinae possess the visual purple, and all invertebrates hitherto examined lack it. It is found in day-loving and night-loving animals,—in the sunward-flying eagle and the nocturnal owl, in fishes which inhabit the sombre depths of the ocean, and in the embryo into whose eye light has never fallen.

Method of separation of Visual Purple or Rhodopsin.

Kühne's study of the *visual purple* and of the changes which it undergoes by the action of light were much aided by the discovery of the fact that the colouring matter is soluble in aqueous solutions containing from 2—5 p. c. of crystallized bile.

Colourless crystallized bile is obtained by evaporating ox bile to dryness on the water-bath after mixing it thoroughly with much animal charcoal. The perfectly dry residue is heated with absolute alcohol and a large excess of ether is added to the filtered solution; by this means the salts of the bile acids are precipitated and ultimately acquire a crystalline structure. The precipitate which consists of sodium glycocholate and taurocholate is termed 'crystallized bile.'

The perfect isolation of rhodopsin by this solvent is beset with difficulties, the greatest of which is to avoid contamination with blood-colouring matter. The retinae of certain animals disappoint all attempts to free them from haemoglobin and are therefore unfit for the extraction of visual purple. Fortunately the frog is not among these. Twenty to thirty frog retinae separated in the chamber by the aid of sodium light, are moistened with about 1 c.c. of a 2 p. c. solution of bile salts and shaken, but without violence, for an hour. The mixture is allowed to stand so as to allow of the subsidence of the grosser particles, and the supernatant fluid afterwards poured on to a filter. The solution thus obtained is of a red-purple colour, bleaching to a water-like fluid on exposure to light. The solution is perfectly clear and transparent and does not fluoresce or seem opalescent, if absolutely free from fuscin. It may be concentrated rapidly *in vacuo*, yielding solutions of progressively deeper tints of purple and finally a dark residue resembling ammoniacal carmine, containing dark violet or black amorphous particles. This mass reacts to light after the manner of solutions. It is hygroscopic and its amorphous particles redissolve. If the bile solution of rhodopsin is thrown upon a dialyser the bile escapes, leaving a violet magma capable of being bleached in the sunlight.

Optical characters of Rhodopsin.

When solutions of rhodopsin are exposed to light, the colour changes from a purple tint, through red and orange, to yellow before becoming colourless. According to the rapidity of our observation, therefore, will be our notion of the pristine tint, when we bring the solution into the light to examine it. If our eye fixes it in the red stage first, and then we begin to note the fading, we shall be led to conclude that the original tint was a deep red rather than a violet; and in fact, many observers, as Boll who proposed for the colouring matter the name '*Seh-Roth*', have fallen into this error of description. To obviate this self-deception we must prepare, in the dark, solutions of the visual purple, of strengths becoming progressively weaker, and bring them (one by one in the order of their concentration) into the light. It will be observed under these conditions that the tints of the different strengths run from purple-violet (in the strongest solution) through purple-red, carmine-red and rose-colour, to lilac in the weakest. In other words, the fading of the colour on exposure to light is different from the fading of the colour on progressive dilution. In the former case appears a yellow admixture which is absent from the original colour. Indeed it is to some extent a misnomer to speak mostly of a '*fading*' of the visual purple, for besides itself *fading*, the *visual purple* is converted into a *visual yellow*, which in its turn fades. The hypothesis that visual purple becomes visual yellow in the sunlight, while visual yellow fades in the same circumstances, suffices to explain all the diversities of tint presented by the retina. According to the rapidity of the conversion of purple to yellow, and according to the rate at which the yellow is dissipated altogether, will be the particular tint of an exposed retina. It will be shewn that different regions of the spectrum have different powers of converting and bleaching rhodopsin.

Spectrum of Visual Purple and Visual Yellow.

When light is passed through visual purple and afterwards through a prism, there is obtained a spectrum offering no defined absorption bands, but presenting a general absorption of rays in the centre of the spectrum, from a little to the red side of D to the violet side of G. Visual yellow blocks the rays from the red side of F to the blue end of the spectrum. The most complete absorption by the visual purple is in the region of E: that by the yellow is at G.

Effect of light of different wave-lengths on the Visual Purple.

The characteristic transformation of visual purple in the presence of sunlight opens up a number of interesting questions. Is it to the highly refrangible invisible rays or to the coloured rays that the change is due? Are rays of all degrees of refrangibility in the visible spectrum equally concerned in the action? What is the nature of the conversion of purple into yellow; is it physical or chemical, a synthesis or a splitting asunder of complex into simpler groups? Several of these questions have already received a satisfactory solution, as we shall now attempt to shew. The

entire beam of white light is by far the best transformer of the visual purple—superior to light of any particular wave-length. The less refrangible dark rays at the red end of the spectrum do not bleach the visual purple. Whether the actinic rays at the violet end are capable of *slowly* bleaching is not yet ascertained, though it is certain that if possessed of activity it is almost immeasurably weaker than that exerted by the coloured rays. Of the visible rays of the spectrum those bleach the visual purple most freely and quickly which the visual purple in solution most effectually quenches. Thus the order of activity in the bleaching of the purple is as follows:—yellowish-green, green, blue, green-yellow, yellow, violet, orange and red. Between yellow-green and yellow the time of bleaching is considerable; it is less between yellow-green and green up to blue.

But intensity of light, or the quantity on the unit of surface, has an influence which renders the above classification very general and bespeaks for it some latitude. We may in connection with this subject draw attention to a practical point, which has already been referred to incidentally. The inconvenience of the preparation of the visual purple in the dark or in a dim twilight may be met by using a monochromatic light of slight decomposing powers. A glance at the previously mentioned orders of decomposing-activity of light of different colour will convince us that red would be the best light for the preparation of visual purple, were it not that in red light it is impossible to detect and avoid blood stains. But yellow light from a sodium flame, which takes about two hours to bleach a frog's retina, is a useful substitute.

Although rays from different regions of the spectrum differ in their powers of transforming visual purple, yet no visible ray fails to bleach it if the exposure be prolonged enough. Further, the rays differ among themselves in the *rate* at which they convert and bleach the retinal colours. White light, to which we refer as a standard, brings about the following transitions from purple:—*red-purple, pure red, orange, yellow, chamois-yellow* to *no colour whatever.*

On the other hand, the red rays of the spectrum produce a change through *pure red* and *orange* to the *palest yellow*, whilst taking an extraordinarily long time to do so. And the rays from the opposite end of the spectrum cause the purple to merge into a final stage which is not yellow but bright red or lilac. In other words, as the wave-lengths diminish less and less *visual* yellow is produced; or, perhaps, the yellow which *is* produced is bleached as quickly, or more quickly, than the still unaltered purple.

Influence of temperature upon the Visual Purple.

Light is not the only agent which affects the visual purple. When retinae are exposed to temperatures varying from about 50° C. to 76° C. the colour fades with a rapidity which increases with the temperature. At 52—53° in the absence of light it takes some hours to disappear, at 76° it disappears instantly. That a low temperature does not interfere with the action of light upon the purple-stained rods is shewn by the

fact that a temperature of -13° C. does not materially impede the bleaching by light.

Action of various chemical agents on Visual Purple.

Caustic alkalies, acids, alcohols, ether, and chloroform decolourize the retinae of recently killed frogs. On the other hand, many agents whose activity might have been presumed upon, such as ammonia, alum, the process of putrefaction, trypsin, are ineffectual in changing the visual purple.

When deprived of water the Visual Purple comparatively stable.

In describing the effect of various agents upon the visual purple that body has usually been under conditions which presupposed the presence of water. If, however, water be withdrawn from the structure or substance coloured with visual purple, though that substance continues to be affected by sunlight, the time during which the light must act is enormously increased.

Optograms.

The fact that the living retina possesses a colouring matter which is decomposed by light led Kühne very early to enquire whether it was possible, under certain circumstances, to obtain actual images on the retina, corresponding to objects which have been looked at. After his first experiments Kühne endeavoured to observe, on the retinae of rabbits, bleached spots corresponding to the images of external objects, but his endeavours failed. In the course of his researches Kühne discovered the remarkable fact which will be described in the succeeding section, viz. that there exist within the retina agents which are concerned in the restoration of the visual purple. Taking for granted that such agent or agents exist, it will follow that, in order to obtain on the retina a picture of external objects, the effect of the light would have to be so prolonged or so intense as to destroy the balance between the destruction of the visual purple and the power possessed by certain retinal elements to restore it.

Kühne took a coloured rabbit and fixed its head and one of its eye-balls at a distance of one metre and a half from an opening thirty centimetres square in a window shutter. The head was covered for five minutes with a black cloth, and then exposed for three minutes to a somewhat cloudy mid-day sky. The animal was then instantly decapitated; the eye-ball which had been exposed was rapidly extirpated by the aid of yellow light, then opened, and instantly plunged in a 5 per cent. solution of alum. Two minutes after death, the second eye-ball, without removal from the head, was subjected to exactly the same processes as the first, viz. to a similar exposure to the same object, then extirpated. On the following morning the milk-white and now toughened retinae of both eyes were carefully isolated, separated from the optic nerve, and turned; *they then exhibited on a beautiful rose-red ground a nearly square sharp image with sharply-defined edges; the image in the first eye*

was somewhat roseate in hue and less sharply defined than that in the second, which was perfectly white. The size of the images was somewhat greater than one square millimeter.

To the images obtained by following such a method as that described Kühne gives the name of *Optograms.* The process may be modified by taking the retinae from the alum solution and then drying them *in vacuo* in the dark. They are in this way rendered very resistant to the action of light.

Chemical facts relating to the Retinal Epithelium.

The retinal epithelium cells (formerly termed hexagonal pigment cells of the choroid) present most externally a covering of *neuro-keratin;* more internally they present a protoplasm wherein are found imbedded one or more nuclei, and still more internally that protoplasm presents large numbers of pigment granules. From this part of the cell proceed processes which make their way between the outer segments of the rods.

The protoplasm of these epithelium cells presents deposits which are described by Kühne as consisting of *Myeloidin,* besides a fat tinged with yellow colouring matter termed Lipochrin; in the more internal part of the cell and in the processes is found the dark pigment now termed Fuscin.

Myeloidin. By this term Kühne indicates the fact that the retinal epithelium cells contain deposits of a substance closely resembling, if not identical with, that forming the medullary sheaths of nerve fibres.

Fat. Fat is not a constant ingredient of retinal epithelium, being absent in that of man, the ox and the pig. In the frog it always is tinged of a deep golden or citron colour. It remains fluid at low temperatures, and is readily extracted by ether, benzol and carbon disulphide.

Lipochrin. This is a yellow colouring matter extracted by ether from the eyes of frogs, from which the retinae have previously been removed.

Lipochrin presents two absorption bands, the position of which differ according to the nature of the solvent. When dissolved in ether these bands are situated between F and G. This colouring matter seems to resemble somewhat a yellow colouring matter which has been named *lutein,* in consequence of its being readily extracted from the *corpora lutea* of cows. Lipochrin is slowly bleached in sunlight.

Fuscin.

The brown pigment of the retinal epithelium has usually, like other pigments of the same colour and appearance, been termed Melanin; of late Kühne has proposed to term it Fuscin. This

pigment occurs in the form of elongated, sometimes spindle-shaped, rods in the epithelium cells; these rods are protruded into the processes of the cell protoplasm.

Method of separating Fuscin—Mays' method. More than 500 hens' eyes are bisected and the posterior halves are placed whilst yet fresh in alcohol; they are then boiled in alcohol and afterwards extracted with boiling ether and water. They are then subjected to energetic trypsin-digestion for 24 hours. The pigment is then left in little masses which are collected on gauze and triturated with alkali with the object of separating nucleins. It is mechanically separated from adhering neurokeratin.

Properties. No chemical reagent dissolves fuscin, except concentrated acids and alkalies, and these only do so very gradually, or by the aid of heat. On long boiling in concentrated sulphuric acid fuscin dissolves, colouring the acid of a dark brown colour. By long digestion in caustic alkalies and their carbonates, fuscin dissolves.

In the presence of oxygen, fuscin is slowly bleached, apparently in consequence of an oxidation process; the sensibility of the pigment obtained from different animals appears to differ.

Fuscin is a nitrogenous body and, on ignition, leaves a small quantity of ash containing iron.

Action of Light upon the Visual Purple of the Living Eye.

Regeneration of Visual Purple.

Though when the eye is exposed to diffuse daylight the visual purple is not destroyed, by exposing frogs for considerable periods to direct sunlight the retinae are found to have been bleached. By allowing such frogs to remain in comparative darkness the colour is however soon restored. Amongst the earliest of Kühne's experiments were those which threw light upon the structures which retard the bleaching of visual purple or are concerned in its restoration.

If an equatorial section be made through a recently extirpated eye, and a flap of retina be lifted up from the underlying choroid to which the retinal epithelium cells are adhering, and if the whole be exposed to light, it will be found that the purple colour of the flap will be destroyed, whilst the colour of the rest of the retina will persist. If, however, the bleached portion of flap be carefully replaced, so that it is again in contact with the retinal epithelium cells, comp'ete restoration of the visual purple occurs. The restoration is a function of the living cells, and it appears to be independent of the *fuscin* which they contain. As it is absolutely dependent on the life of the structures which overlie the rods, it is natural that it should persist for a longer time after somatic death in the frog than in the rabbit.

While the epithelium at the back of the retina is the agent in the restoration of the visual purple, it is ascertained that it may impart something to the rods themselves, leading to "*auto-regeneration*," as Kühne terms it. Frequently when an isolated retina is bleached it will on being removed from the light regain somewhat of its purple colour; and similarly bile solutions of the visual purple, if they contain no ether, may also exhibit this "auto-regeneracy," especially if both retina and epithelium have been employed in making the solution.

Do the rods then contain a something out of which the visual purple may be regenerated, and are the epithelial cells the agents of this elaboration, withdrawing the supposed substance from the rods and working it up into visual purple?

Vision without Visual Purple.

In concluding this account of the visual purple it is expedient to point out what bearing, if any, the facts which have been described have upon our knowledge of the physiology of vision.

The most sensitive region of our eye—that which we turn upon any object which we wish to see with the utmost distinctness—contains cones only, and cones are just those elements of the bacillary layer which are destitute of visual purple. Again, many animals which are keen-sighted may be seen to have retinae which are quite free from this colouring matter.

Under these circumstances we cannot assert that these beautiful discoveries relating to the visual purple have succeeded in solving the tempting problem as to the mode in which light affects the retina. They, however, open up a promising field for speculation and hold out inducements to those who would pursue similar lines of enquiry. The changes in the visual purple are perhaps little more than accidental accompaniments of more important, though by our senses unseen, chemical processes—processes which may in reality be initiated by the undulatory movements of the ether and of which the results may be the real stimuli which normally throw the optic nerves into activity.

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