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KUKENTHAL and ZIEHEN) of the sulcus temporalis superior, the latter has not only pushed down the *b* sulcus, but it too has divided the lateral pli de passage as it were into two portions. In this drawing one could speak of two lateral plis de passage, which therefore run towards the occipital surface. But as this example is an exception, it cannot be counted of much worth. Yet it proves that where in the higher development the complex of sulci becomes more complicated, it can be of influence on the image of the plis de passage. The embryonal material could show us the way in this case.

From what is found in anthropoids follows that the ape-fissure on the whole is less developed than in semnopithecids and related monkeys. It gives the impression as if the sulcus begins to contract.

CONCLUSIONS.

1. The plis de passage in foetal life are lying on the surface.
2. They form, lying on the surface, the communication between the lobi parietalis and temporalis with the lobus occipitalis.
3. In semnopithecids, macacids and related monkeys only three plis de passage are known
 - a. gyrus annectens lateralis, lying between the *m* and *b* sulcus.
 - b. gyrus annectens interpositus, lying on the mantle surface, forming the continuation of the arcus parieto-occipitalis.
 - c. gyrus annectens medialis, forming the communication between the cuneus and praecuneus, lying above of the fissura calcarina.
4. When the ape-fissure is formed on the border of the parietal and occipital part (semnopithecus, macacus etc.), then the lateral and interposed-gyrus annect. are pushed totally or partially in the depth.
5. When the ape-fissure is formed on the occipital surface (ateles, nycticebeus tardigradus etc.), then it does not come in contact with the plis de passage and these therefore remain on the surface.
6. In the anthropoids the ape-fissure is considerably less developed than in semnopithecids etc.

Physiology. — “Quantitative determination of slight quantities of SO_4 . II. Contribution to macrovolumetrical analysis”¹⁾. By Prof. H. J. HAMBURGER.

(Communicated in the meeting of April 28, 1916).

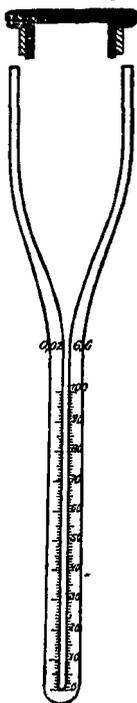
1. Introduction.

Repeatedly physiologists and clinicists find themselves confronted by the task of determining quantitatively very slight quantities of some substance and if no good titration method is available, the

¹⁾ A more detailed account will be given in “Biochemische Zeitschrift” 1916.

solution of the problem becomes practically impossible, at least when for instance daily, series of determinations have to be made. Chemists often experience these difficulties no less. This will be the reason why a quantitative micro-analysis begins to develop itself, to which all the principles of the macro-analysis are applied, but with miniature implements, the micro-scales of *Nernst* are then mostly used as a weighing-apparatus.

This method, however, has its drawbacks in being an analysis by weight, with all the difficulties attending it, which difficulties are still increased by the fact that a slight mistake materially affects the results. Besides, the accuracy of the macro-gravimetric method is anything but perfect. All this applies for instance to the quantitative determination of the potassium and that of the SO_4 . It need not surprise us, therefore, that our knowledge of the K- and SO_4 - economy leaves much to be desired.



As regards these two substances, however, I have succeeded in finding another method, which consists in the volume of the precipitate being determined instead of the weight; this is done in a glass apparatus, the top part of which is funnelshaped, the neck being a calibrated capillary tube (see fig. 1). I might call the instrument a *chonohaematocrite*¹⁾. The calibrated capillary part has a content of 0.04 cub. centimetres, and is divided into 100 equal parts. The column of the precipitate is read off after being centrifugated to constant volume.

This method combines accuracy with a simplicity hitherto unattained in the determination both of potassium and of SO_4 . The potassium method, I applied, has been described already²⁾, it was lately used by me to solve physiological problems which could not be settled by the available methods³⁾. I shall not dwell upon this potassium-method. It need only be stated that to the fluid containing the potassium-salt, a solution of sodium-

¹⁾ From $\chi\acute{o}\nu\eta$ (funnel) and haematocrite or blood-investigator, the name formerly given by *Hedin* to a calibrated capillary tube, which he used to determine the volume of the red blood-corpuscles in blood.

²⁾ HAMBURGER. *Biochemische Zeitschrift* **71** (1915) 415. *Recueil des Travaux chimiques des Pays Bas et de la Belgique*. T. **35**. (1916), 225.

³⁾ HAMBURGER, Die Permeabilität von unter physiologischen Verhältnissen kreisenden Blutkörperchen für Kalium, nach einer neuen Methode untersucht. (Zusatz geringer Mengen von CO_2 , Glukose, NaCl , KCl , NaOH und KOH). *Wiener Med. Wochenschrift* 1916 No. 14—15; Festnummer für Prof. S. EXNER.

cobaltidnitrite is added and all the potassium is precipitated as potassiumsodiumcobaltidnitrite, which with GILBERT we call cobalt-yellow. As we said, the precipitate is centrifugated until the volume remains constant and then read off. When the volume of cobalt-yellow supplied by a known K-solution has been determined, the amount of the unknown K-solution can be found. The imperative condition is satisfied that there should be a proportionality between the volume of cobalt-yellow and the amount of K, and that the result is also found to be independent of the rapidity of centrifugation.

2. *Difficulties in the quantitative determination of SO₄.*

The fact that SO₄ is precipitated by BaCl₂ is known to every one who has studied chemistry for half a year. The reaction is a classical instance of precipitate-formation. After the experiences met with in the case of K, it would be expected that slight volumes of SO₄ in a solution containing a sulphate, could be determined volumetrically without much difficulty by simply measuring the volume of the resulting BaSO₄. The results of the experiments, however, were entirely unsatisfactory. The volume was found to be influenced by: the temperature, the volume of the fluid, the amount of the excess of BaCl₂, the quantity of HCl and the presence of numerous substances which are met with in the sulphate-solution, nay even the manner in which the reagent was added.

What the literature taught me on the determinations of SO₄ by weight-analysis was far from encouraging. It is evidently among the worst that exists. When we read TREADWELL's book on quantitative analysis 4th Ed. (1907) p. 353, then it appears what great obstacles the usual gravimetric method encounters, even when BaCl₂ is added to H₂SO₄ the weight of the precipitate is not always the same. BaCl₂ is closed in, and that in amounts which depend for instance on the manner in which the BaCl₂ is added, by drops or at once. If we have to deal with a sulphate containing other salts, the matter becomes more complicated still. Besides the BaCl₂ being closed in, other salts are adsorbed; especially iron and calcium-salts are weighed with the precipitate, even after the BaSO₄ has been carefully washed. The quantity of HCl added is by no means immaterial, nor the dilution with water, which makes itself the more felt in proportion as the crystals are smaller.

A few years ago the matter was taken up again by M. J. VAN 'T KRUY'S¹⁾, who submitted it to a detailed systematic investigation; he

¹⁾ M. J. VAN 'T KRUY'S. Zeitschrift für anal. Chemie, **49** (1910), 393.

tried to arrive at the conditions under which the quantitative determination of SO_4 gives the most reliable results. "Wie einfach eine Bariumsulfatbestimmung auch scheint, so zeigt sich doch, dass in Wirklichkeit die Ausföhrung derselben um mehrere Ungenauigkeiten behaftet ist, die sogar trotz aller möglichen Vorsicht gewöhnlich nicht vermieden werden können," and then he gives a method which is rather complicated. Especially the presence of Calcium is objectionable.

After these remarks it will not surprise us that the physiology and the pathology of the sulphur-economy have been little studied until now, especially if we consider that, except in urine the quantity of SO_4 in bodyfluids is exceedingly small.

Therefore it was attempted to establish a microvolumetrical method for SO_4 in the same way as it had been done for K. - To known solutions of Na_2SO_4 , HCl and BaCl_2 were added, both in known concentrations. Then it was determined:

1. Whether, if the circumstances were the same, the same volume of precipitate was obtained.
2. Whether the volume of the precipitate is influenced by:
 - a. dilution of the fluid with water.
 - b. the concentration of HCl .
 - c. the quantity of BaCl_2 solution added.
 - d. the presence of other mineral substances which may be met with in animal fluids, such as NaCl , KCl , Ca , Mg , and phosphates.

It soon appeared that these factors strongly influenced the volume of the precipitate.

Mindful of what our K determinations had brought to light, we asked ourselves whether these unsatisfactory results were connected with or due to, the nature of the crystalline precipitate. Again we called in the help of the microscope. It was indeed discovered that whenever we started from the same volume of SO_4 and yet different volumes of BaSO_4 were arrived at, this was always attended with another microscopic view of the precipitate. Now there were needles, now columns, sometimes they presented an appearance of crosses; but not only the shape differed, considerable differences in size were also manifest. And all this at the same temperature. Hence it was advisable to find the conditions under which the crystals always had the same size and shape.

The investigations connected with the K determination had shown that in order to obtain useful results the crystals ought at any rate to be very fine. Here again it was attempted to satisfy this demand. *An addition of some acetone was one of the chief means by which this result was attained.*

It would be of little use here to give an account of the many difficulties experienced in this investigation.

Let us, therefore, relate the *modus operandi* which has given results that were under all circumstances satisfactory, that is to say by which we obtained 1. invariably the same volume of precipitate from the same quantity of SO_4 , 2. n -times a volume of precipitate from n -times a quantity of SO_4 , 3. a volume of the precipitate entirely independent of the presence of mineral substances met with in biological fluids.

3. *Method to be followed in the microvolumetrical determination of SO_4 .*

5 cc. of the fluid containing sulphate are mixed with $2\frac{1}{2}$ cc. of concentrated HCl 1:1; to this mixture are added 5 cc. of a BaCl_2 2 aq. sol. of 2.44%, in which 3 to 5 drops of acetone have been added.

Sulphate and reagent are mixed at room-temperature. The manner, however, in which the BaCl_2 is added to the HCl-sulphate solution is of the greatest importance if we wish to obtain satisfactory results. If this is lost sight of, if it is added quickly in one case and slowly in another, the two parallel-experiments may give entirely different results, and the microscopic investigation shows accordingly that the crystalline precipitate is not the same in these two instances. It seemed to me that this must be explained as follows: If the BaCl_2 -sol is added slowly, there will still be free SO_4 in the fluid at first. Now if we go on adding, the amount of dissolved SO_4 decreases; hence the proportion between the reagent and the SO_4 which has not been precipitated, is continually modified during the addition. Since the quantity of acetone materially affects the shape and size of the crystals most likely owing to its effect on the surface-tension, the way in which the BaCl_2 -sol. with the acetone are added, will likewise affect the volume of the precipitate. So important a part is played by the manner of adding that if in two parallel-experiments the BaCl_2 -solutions containing acetone, are seemingly added in the same way and immediately shaken, the volumes of the precipitate may still be different. In order to effect a rapid and perfect mixture we proceeded as follows:

In a tube of thick glass a narrower one is placed having a content of about $5\frac{1}{2}$ cc. (see fig. 2). On the outside of this narrow tube a glass column has been melted reaching to the cork which may shut off the wide glass tube. Into the space between the narrow

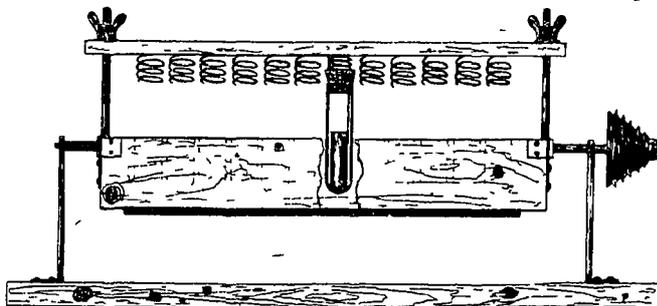


Fig. 2.

$\frac{1}{2}$ of actual size.

and the wide tube we bring $7\frac{1}{2}$ cc. of the hydrochloric sulphate solution. It does not reach the upper rim of the inner tube. Then the 5 cc. BaCl_2 -solution are brought into the inner tube by means of a pipette, mixed with 5 drops of acetone and the wide tube is closed with a stopper. This stopper is pushed in so far that the narrow tube is gently pressed against the bottom. Then this tube is placed in a frame which can be turned on an axis. This frame can hold twelve such tubes. When the frame is turned round the reagent suddenly bursts into the sulphate-solution so that in an extremely short space of time a perfect mixture is obtained. To make quite sure the frame is turned a few times. Experience teaches that thus a very fine precipitate is formed, the particles measuring on an average 0.0026 millimetres. The crystal shape at the usual magnitude (Leitz obj. 8, or 2) cannot be distinctly recognized; they are not needles, columns or crosses; to an untrained eye they suggest cubes that are not clearly cut.

The drawing shows that the apparatus can also be turned mechanically, for instance by an electromotor. The rotation may also be brought about by substituting for the disc an iron bar

Fig. 3. $\frac{1}{4}$ of the actual size.

perpendicular on the axis. A solid brass cylinder can be moved along the iron bar and fastened with a screw. It depends on the place of this cylinder how rapidly the frame with its tubes will turn round when it is loosened (Cf. the article in the "Biochemische Zeitschrift" 1916).

When the precipitate has been formed it can immediately be transferred to the chonohaematocrite. Therefore the small tube is taken out of the larger one and the latter is centrifugated for a short time, so that all the precipitate in it, is found on the bottom. Now that which is still found in the small tube must be removed

to the large one. To bring this about some of the clear fluid in the latter is taken into a pipette and all the precipitate in the small tube is washed into the large one. In the same manner the surface of the small tube is washed, so that all the BaSO_4 gets into the large tube.

We might wish to bring the contents of this tube ($12\frac{1}{2}$ cc.) into the funnelshaped part of the chonohaematocrite, but the latter holds only about $2\frac{1}{2}$ cc. Therefore we centrifugate; the greater part of the clear fluid is removed and the sediment is mixed with the rest of the fluid. After the capillary tube has been filled with mother liquor by means of a glass tube drawn out into a capillary, this turbid mass is brought into the funnel-shaped part. We now centrifugate, the clear fluid is removed from the funnel-shaped part and replaced by the BaSO_4 which had remained behind in the wide tube. It need hardly be said of course that the BaSO_4 adhering to the inner and outer surface of the small tube must be transferred to the wide tube by being washed with mother liquor. Now the whole is centrifugated until the volume remains the same, which takes about half an hour¹⁾.

We will now state the results of some experiments with some pure Na_2SO_4 solutions. They are all double-experiments.

4. *Results of some microvolumetrical SO_4 -determinations.*

TABLE I.
Proportionality between the volume of BaSO_4 precipitate and the SO_4 used.

7.5 cc. of HCl sulphate-solution +5 cc. of BaCl_2 -solution containing acetone.	Number of divisions of BaSO_4		
	average		calculated III
	I	II	
1) 2 cc. of Na_2SO_4 10 aq. 2% + $2\frac{1}{2}$ cc. of HCl 1:1 + 3 cc. of water	41	} 41	
2) 2 " " + $2\frac{1}{2}$ " + 3 " "	41		
3) 3 " " + $2\frac{1}{2}$ " + 2 " "	63	} 62	$\frac{3}{2} \times 41 = 61.5$
4) 3 " " + $2\frac{1}{2}$ " + 2 " "	61.2		
5) 4 " " + $2\frac{1}{2}$ " + 1 " "	82	} 82	$\frac{4}{2} \times 41 = 82$
6) 4 " " + $2\frac{1}{2}$ " + 1 " "	?		
7) 5 " " + $2\frac{1}{2}$ " "	103	} 102.5	$\frac{5}{2} \times 41 = 102.5$
8) 5 " " + $2\frac{1}{2}$ " "	102.5		

¹⁾ Compare the article: Une méthode simple pour le dosage de minimes quantités de potassium. Recueil des Travaux Chimiques des Pays-Bas et de la Belgique. Tome XXXV 1916, 225.

This table shows.

1. that the results of each pair of experiments agree satisfactorily (see column I).

2. that the volumes of the precipitates vary as the quantities SO_4 used (cf. columns II and III).

We will now consider to what extent an addition of NaCl influences the volume.

TABLE II.
Effect of NaCl on the volume of the $BaSO_4$ precipitate.

7.5 cc. of HCl sulphate solution + 5 cc. of $BaCl_2$ sol containing acetone.							Number of divi- sions of $BaSO_4$		
1)	2cc. Na_2SO_4	10 aq. 4%	+ 2½ cc. of HCl 1:1	+ 0,2 cc. of NaCl 9%	+ 2,8 cc. of H_2O			82	
2)	2	"	+ 2½	"	+ 0,2	"	+ 2,8	"	81.5
3)	2	"	+ 2½	"	+ 0,4	"	+ 2,6	"	81
4)	2	"	+ 2½	"	+ 0,4	"	+ 2,6	"	81
5)	2	"	+ 2½	"	+ 0,6	"	+ 2,4	"	81.5
6)	2	"	+ 2½	"	+ 0,6	"	+ 2,4	"	81.5
7)	2	"	+ 2½	"	+ 0,8	"	+ 2,2	"	81.5
8)	2	"	+ 2½	"	+ 0,8	"	+ 2,2	"	80.5
9)	2	"	+ 2½	"	+ 1	"	+ 2	"	80.5
10)	2	"	+ 2½	"	+ 1	"	+ 2	"	81

TABLE III.
Effect of Na_2HPO_4 12 aq. on the volume of the $BaSO_4$ precipitate.

7.5 cc. of HCl sulphate solution + 5 cc. of the $BaCl_2$ sol. containing acetone.							Number of divi- sions of $BaSO_4$		
1)	2cc. of Na_2SO_4	10 aq. 4%	+ 2½ cc. of HCl 1:1	+ 0,2 cc. of Na_2HPO_4 9%	+ 2,8 cc. of H_2O			82 1)	
2)	2	"	+ 2½	"	+ 0,2	"	+ 2,8	"	82 1)
3)	2	"	+ 2½	"	+ 0,4	"	+ 2,6	"	81
4)	2	"	+ 2½	"	+ 0,4	"	+ 2,6	"	82 1)
5)	2	"	+ 2½	"	+ 0,6	"	+ 2,4	"	82 1)
6)	2	"	+ 2½	"	+ 0,6	"	+ 2,4	"	81.5
7)	2	"	+ 2½	"	+ 1	"	+ 2	"	80.5
8)	2	"	+ 2½	"	+ 1	"	+ 2	"	80.5

1) The caoutchouc stoppers had let loose some small particles, which mixed with the white precipitate.

Evidently the NaCl has not affected the volume of BaSO₄.

A similar result was obtained when Na₂HPO₄ 12 aq. was added, (See table III p. 122).

Where the precipitate has not been polluted, *the volume of BaSO₄ has obviously not been modified by the phosphate.*

Finally an experiment to investigate the influence of the salts met with in animal substances.

TABLE IV.

Effect of a mixture of K, Na, Mg, Cl and Na₂HPO₄ on the volume of the precipitate.

7,5 cc. of HCl sulphate solution + 5 cc. of BaCl ₂ solution containing acetone.	Divisions of BaSO ₄
1) 2 cc. of Na ₂ SO ₄ 4 ⁰ / ₀ + 2 ¹ / ₂ cc. of HCl 1:1 + 3 cc. of H ₂ O	81.5
2) 2 " + 2 ¹ / ₂ " + 3 "	80.5
3) 2 cc. of Na ₂ SO ₄ 4 ⁰ / ₀ + 2 ¹ / ₂ cc. of HCl 1:1 + 1,2 cc. of KCl 3,6 ⁰ / ₀ + 0,8 cc. of NaCl 9 ⁰ / ₀ + 1,3 cc. Na ₂ HPO ₄ 9 ⁰ / ₀ + 0,8 cc. CaCl ₂ 3 ¹ / ₀ + 0,8 cc. MgCl ₂ 2 ⁰ / ₀ + 0,2 cc. H ₂ O	82.5 ¹⁾
4) like 3	82.5 ¹⁾

From this experiment it may be concluded that if the foregoing prescriptions (sub 3) are carried out, the volumetrical sulphate-determination gives highly satisfactory results, much more so than the gravimetric method. But we repeat on the ground of our extensive researches that the directions must be carefully attended to.

The prescriptions especially apply to the manner in which fluid and reagent are mixed. The size and shape of the crystals depend on it. Hence we are in the habit, when the mixture has taken place, of taking a drop of the turbid fluid with a capillary pipette and examine it under the microscope (Leitz obj. 8, Oc. 2). It becomes then evident at once without measurement whether the crystals are right. As we said before they suggest small cubes. Their surface must seem smooth. If the microscopic view does not satisfy this demand, if the crystals seem rough at the surface or if they have for instance double the diameter then the volume of the sediment is also found to exceed 81.5 divisions. Who has not yet seen the desired crystals may take as a criterion whether 2 c.c. Na₂SO₄ 10 aq. of 4⁰/₀ gave a volume of 81.5 divisions. Whenever this was the case, the crystals were as they should be. A preparation may be kept to serve for comparison. When kept, evaporation must of

¹⁾ The cork had lost some small particles, which polluted the column of BaSO₄.

course be prevented; for this purpose the preparation may be surrounded with a frame of soluble glass or paraffin wax as is usually done in histological technics.

5. *Some general observations.*

In chemical work other metals and acids than those we added will be met with in the fluid containing the sulphate. It will have to be determined by an intentional addition of these substances in what degree they influence the volume of the precipitate. These researches fall outside the scope of this paper. But it remains to be settled to what extent the noxious influence of proteid can be neutralized. Experience has taught us that this can be easily done by ultrafiltration. A filter, drenched with a 5% sol. of celloidin in alcohol and ether and subsequently treated with water easily keeps back the proteid.

As regards the *degree of accuracy of our method* we may give the following calculation: 2 c.c. of Na_2SO_4 10 aq. 4% contain 0.02386 grammes of SO_4 . The volume of BaSO_4 gives by these averages 81.5 divisions. *Each division* of our tube corresponds, therefore, to $\frac{0.02386}{81.5} = 0.000294$ grammes of SO_4 . Since in accurate work no greater mistake is made than one division, the method is *accurate to 0.000294 grammes of SO_4 .*

The object of this paper is twofold. First it intends to supply a simple method enabling us to make in an easy manner highly accurate determinations of very slight quantities of SO_4 . Secondly it aims at giving an impulse to the further development of a new method of quantitative analysis, which certainly deserves to rank with both the micro- and macro-gravimetric method, because it is simple and accurate, saves time, allows us to work with slight quantities, and is moreover particularly adapted to series of determinations. These qualities lately became manifest at the determination of potassium, and some years ago in the study of an equilibrium reaction¹⁾. The great obstacles to be encountered will especially consist in the difficulty to discover the conditions under which a precipitate is obtained giving at all times the same microscopic view. At the present moment this can be effected only empirically; indeed, as far as I am aware, next to nothing is known of the forces which underlie the varieties of crystal shape and crystal size of the same

¹⁾ HAMBURGER and ARRHENIUS. On the nature of precipitin-reaction. These Proceedings, meeting of May 26th 1906.

substance. Also from a physico-chemical point of view it will be desirable to start researches on this subject.

S U M M A R Y.

It was set forth that it is of importance:

1. To possess by the side of the macro- and microgravimetric analysis, another method which enables us to make quantitative determinations of very slight quantities of a substance in a simpler and more accurate way.

2. The method detailed here is based on the principle that after the reagent has been added, the precipitate formed is centrifugated in a calibrated capillary tube until the volume remains constant and can be read off. When the volume of BaSO_4 corresponding to a SO_4 -sol. of a known concentration has been determined, then it is possible to determine by means of this result the SO_4 -concentration of an unknown sulphate-solution.

3. In order to make a quantitative determination of SO_4 we always add $2\frac{1}{2}$ cc of HCl 1:1 (concentrated NCl diluted with an equal volume of water) to 5 cc of the SO_4 fluid, and to this mixture 5 cc of a BaCl_2 2 aq-solution of 2.44%, containing 3 to 5 drops of acetone. The precipitate formed is centrifugated until the volume remains the same.

Whether the 5 cc of fluid contains much sulphate or only a little and whether these 5 cc of fluid contain Na , K , Ca , Mg , Cl and PO_4 makes no difference whatever, as regards the results: an n -fold quantity of SO_4 gives an n -fold volume of BaSO_4 and the presence of the above-mentioned admixtures does not affect the volume of the precipitate.

One division = 0.0004 cc. of the BaSO_4 -solution corresponds to 0.000294 grammes of SO_4 . Mistakes greater than 0.000294 grammes of SO_4 are not made if the method described sub 3 is carefully followed.

Groningen, April 1916.

*Physiological Laboratory
of the University.*

Physics. — “*Direct optical measurement of the velocity at the axis in the apparatus for FIZEAU’s experiment*”. By Prof. P. ZEEMAN.

(Communicated in the meeting of May 27, 1916).

For the comparison with theory of the *absolute* values of the shifts of the interference fringes, which I determined for light of different colours in FIZEAU’s experiment, the magnitude of the velocity