In his report on the cerebellar atrophies (1936) HALLERVORDEN stressed the endogenous origin of these affections, but admits the cooperating influence of toxic factors on these diseases. HALLERVORDEN mentions also the significance of tumours outside the central nervous system. This is the same conclusion, which BIEMOND and BROUWER have drawn from their investigations on the primary parenchymatous degenerations of the cerebellum. The disturbance of the metabolism of the body, caused by a malign tumour, affects those parts of the central nervous system weakened from the beginning (abiotrophy in GOWER's sence). Especially in cases where the cerebellar symptoms are gradually developing in elderly patients and where an increase of protein with positive colloidal reactions are found in the spinal fluid, eventually with pleocytosis, one has to consider the presence of a malign tumour outside the central nervous system.

Summary.

In this article the result of a clinico-anatomical investigation is given, in which a malign tumour in the abdominal cavity was combined with degeneration in the cerebellum and in some regions of the medulla oblongata. The conclusion is reached that this observation is a new example of the toxic degeneration of the cerebellum, caused by a malign tumour outside the central nervous system.

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Zoology. — *The influence of lithium chloride and calcium chloride on viscosity and tension at the surface of uncleaved eggs of Limnaea stagnalis L.* By G. A. DE VRIES. (From the Zoological Laboratory, University of Utrecht). (Communicated by Prof. CHR. P. RAVEN.)

(Communicated at the meeting of November 29, 1947.)

Introduction.

Investigations on the tension at the surface and the viscosity of *Limnaea stagnalis* eggs in the uncleaved stage gave evidence of variations in both physical properties from the moment of oviposition till first cleavage (RAVEN 1945). Since Ca- and Li-salts have a great influence on the development of *Limnaea* eggs (RAVEN 1942; RAVEN & MIGHORST 1946), it was of great importance to investigate their direct effects on viscosity and tension at the surface of the eggs of this snail.

Material and methods.

Limnaea stagnalis eggs in the uncleaved stage were used for the experiments. Egg masses were obtained in the manner described by RAVEN & BRETSCHNEIDER (1942). For each experiment one egg mass, as large as possible, was used; it was ascertained by means of a binocular microscope that all eggs had a normal appearance. Only eggs taken from the middle of the egg mass were used, because the stage of development of the eggs is nearly equal there. The eggs were delivered from the adhering jelly as nearly as possible, but remained within their capsules. About 10 eggs were used to determine exactly the stage of development; 4 other samples, each of about 15 eggs, were transferred to the centrifuge tubes. Then distilled water was poured out on one of these samples, serving as a control, salt solutions on the other ones. Care was taken that there was plenty of fluid over the eggs. By shaking from time to time, adhering of the eggs was precluded and the contact with the surrounding medium rendered as intense as possible.

After a lapse of 20 minutes the eggs were centrifuged for 15 minutes in the same tubes at a velocity of $\pm$ 2750 revolutions per minute, giving a centrifugal pressure of about 1000 $\times$ gravity. Immediately after centrifugation the eggs were transferred to small glass containers and examined in transmitted light by means of a horizontal microscope. The eggs remained in the medium up to the end of the experiment. Measurings were performed by means of an ocular micrometer. Centrifuged eggs almost immediately orientate with their heavy yolk pole down. For each egg sample no more than 4 to 6 measurings were performed, to keep the error resulting from recovery to the original form as low as possible.

A total of 100 experiments, each consisting of 4 samples, have been carried out. The solutions used were: 3.0 %, 1.5 %, 0.5 %, 0.1 %, 0.08 %, 0.06 %, 0.05 %, 0.03 %, 0.02 % and 0.008 % $CaCl_2$; 1.5 %, 1.0 %, 0.6 %, 0.1 % 0.08 %, 0.06 %, 0.05 %, 0.03 %, 0.02 %, 0.01 %, 0.008 % and 0.006 % LiCl.

An attempt was made to express the relative viscosity in a quantitative manner. To this end, the centrifuged eggs were compared with a standard series of 11 photographs, numbered 0 to 10, and representing different degrees of stratification brought about by centrifuging (fig. 1). The number of this series to which the stratification of the eggs corresponded was called their "viscosity number" and considered as a measure of their viscosity. Viscosity number 0 means: stratification very distinct, viscosity low; viscosity number 10: stratification indistinct, viscosity high. I want to emphasize that my evaluations have been based primarily upon the occurrence of vacuoles or granules in the hyaloplasm zone, contrary to RAVEN's paper (1945), where the height of this zone has also been taken into account. Though, as a rule, both phenomena change concurrently, in some cases the two methods of evaluation may lead to slightly different results. As, however, the height of the hyaloplasm zone does not only depend on the viscosity, but also on the degree of stretching, i.e. on the tension at the surface, I thought it preferable to use only the first of the above-mentioned criteria.

The tension at the surface is expressed by the quotient $\frac{B}{L}$, i.e. the relation between the largest diameter B, perpendicular to the direction of the centrifugal force, and the largest diameter L, in the direction of this force (RAVEN 1945). For the sake of brevity, this quotient will be called the "tension index". The recovery to spherical form is very slow. We therefore may consider the value of this index at $\pm$ 5 minutes after centrifugation to be a measure of the tension at the surface at the moment, at which centrifugation ended.

The time was related to the developmental processes, as observed in untreated eggs of the same batch. In all instances, the time is given, at which centrifugation ended.

The experiments were performed at room temperature of $\pm$ 20° C. No attempt was made to keep it constant. Two periods of very hot weather caused a temporary rise to 32° C. This renders it difficult to compare different experiments; firstly, because there may be a direct influence of temperature on viscosity and tension at the surface; in the second place, because the rate of development increases with temperature. For the construction of the graphs experiments have been put together, performed at different temperatures. The elaboration of my results showed, however, that no regular influence of temperature upon viscosity and tension at the surface could be deduced from them; obviously, the effect of this factor is only small as compared with the individual differences between

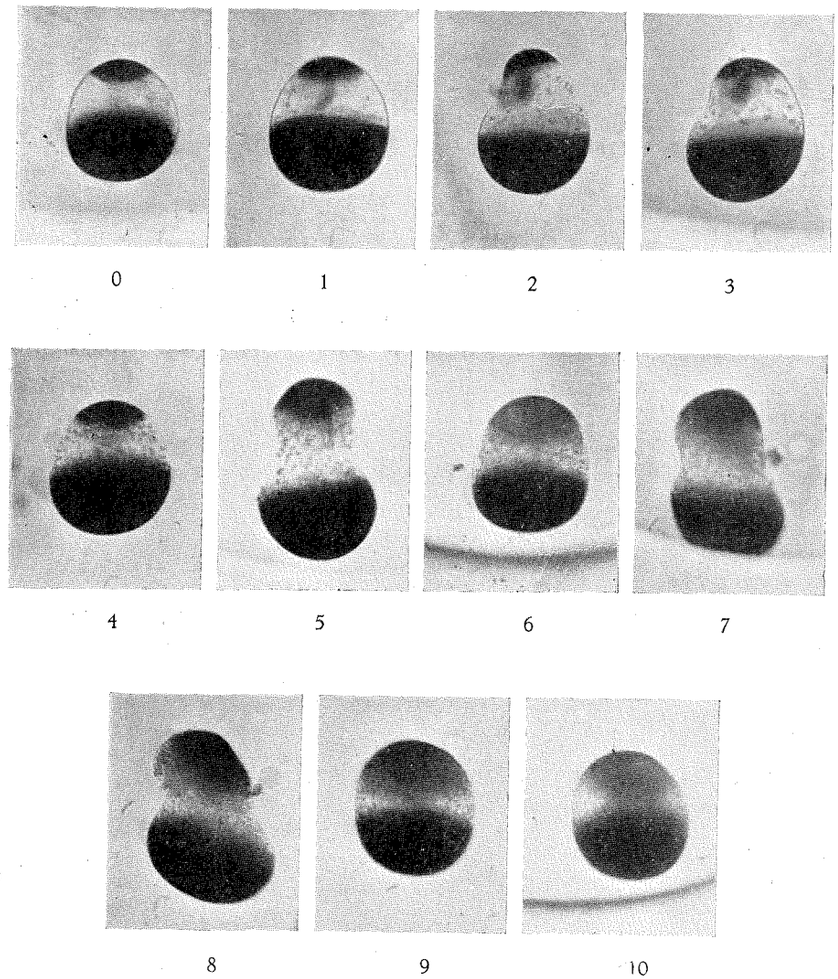

Fig. 1. Standard series of photographs of centrifuged eggs, representing viscosity numbers 0 to 10. Cf. text.

the batches. So, we may neglect this source of error in considering only the major trend of the curves. In the single experiments, the influence of this factor does not play a part, as the samples of one experiment were all at the same temperature.

In the graphs, the time between the two maturation divisions was taken as 60 minutes, the time between second polar body formation and first cleavage as 100 minutes.

In order to have a measure of the influence of the salt solutions on viscosity and tension at the surface, each sample was compared with its control. The difference between the viscosity numbers and tension indices of both will be called the "deviation"; it is taken positive, when the value of the salt-treated sample is higher, negative when it is lower than that of the control. From the deviations of all samples, treated with a same solution, an average deviation for this solution can be computed.

Viscosity.

In fig. 2, the viscosity of control samples has been plotted against time. When one compares this viscosity curve with the curve, given by RAVEN (1945, fig. 8), there is a rather good agreement. Before the first maturation

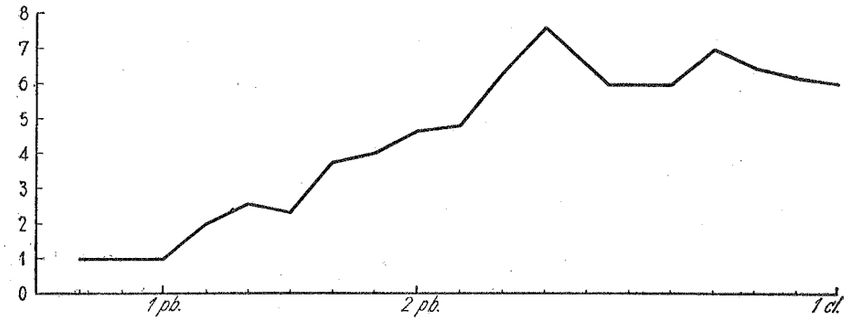

Fig. 2. Graph showing change of viscosity of normal eggs from oviposition till 1st cleavage. Abscissae: time. Ordinates: viscosity number.

division viscosity in both curves is low. Between the two maturation divisions viscosity rises earlier and to a greater height in my curve than in RAVEN's. Viscosity at the moment of the extrusion of the second polar body is "medium" in my curve, "low" in RAVEN's. This is due to a difference in evaluation of viscosity. RAVEN e.g. indicates the viscosity of his photographic pictures 8 and 9 as "rather low", whereas it is called "medium" by me (viscosity number 4). Evidently, this difference is due to the different methods of evaluating the viscosity, referred to above, RAVEN laying more stress on the fact that the height of the hyaloplasm zone has not yet diminished at this moment. After the extrusion of the second polar body there is in both curves a rapid rise to a maximum, after which there is a decrease. This drop of viscosity shortly before first

cleavage in much more pronounced in RAVEN's curve, than in mine. Again, this may be due partly to differences in evaluation. However, this does not suffice to explain the difference. The eggs of RAVEN (1945, fig. 7, egg 17 and 18) centrifuged 36 and 20 minutes before 1st cleavage, respectively, show a stratification corresponding to my viscosity numbers 1—3. The same holds true for the eggs pictured by Miss HEIKENS (1947, fig. 1, egg 1 and 2), centrifuged about 30 and 10 minutes before 1st cleavage. In my experiments, during this period an average viscosity of 6—7 was observed. In RAVEN's and Miss HEIKENS' experiments, the eggs had been centrifuged for 5 minutes at a velocity of 3800 revolutions per minute (1860 × gravity), whereas I centrifuged for 15 minutes at 2750 revolutions per minute (1000 × gravity). Probably, this difference in velocity and duration of centrifugation is responsible for the difference of the results. Furthermore, RAVEN's eggs were kept in tap water, whereas I centrifuged them in distilled water; perhaps, the difference in pH between these media has some influence on the viscosity of the eggs.

Table I shows the average deviations of viscosity number in the salt solutions. 1.0 %, 0.6 % and 0.1 % LiCl and 1.5 %, 0.5 % and 0.1 % $CaCl_2$ solutions all caused a significant decrease of viscosity. Greater dilutions of both salts had no effect, but with still greater dilutions there is a tendency to increase the viscosity; this increase is significant in the case of 0.01 % and 0.006 % LiCl and 0.05 % $CaCl_2$. The number of experiments for each

TABLE I.

Average deviations of viscosity number and tension index in various salt solutions.

		Number of experim.	Deviations of	
			Viscosity number	Tension index
LiCl	1.0 %	9	**— 2.3 ± 0.9**	**+ 0.15 ± 0.03**
	0.6 %	24	**— 2.3 ± 0.6**	**+ 0.09 ± 0.03**
	0.1 %	23	**— 1.4 ± 0.3**	**— 0.07 ± 0.02**
	0.08 %	14	— 0.1 ± 0.3	— 0.04 ± 0.04
	0.06 %	5	0 ± 0	— 0.01 ± 0.04
	0.05 %	5	0 ± 0.3	— 0.03 ± 0.02
	0.03 %	5	+ 0.2 ± 0.2	— 0.09 ± 0.07
	0.02 %	5	+ 1.0 ± 0.8	+ 0.03 ± 0.03
	0.01 %	5	**+ 0.6 ± 0.2**	+ 0.07 ± 0.05
	0.008 %	5	+ 0.2 ± 0.2	— 0.05 ± 0.03
	0.006 %	5	**+ 1.0 ± 0.3**	+ 0.09 ± 0.05
$CaCl_2$	1.5 %	27	**— 3.2 ± 0.5**	**+ 0.12 ± 0.02**
	0.5 %	23	**— 1.2 ± 0.3**	+ 0.02 ± 0.03
	0.1 %	20	**— 1.1 ± 0.2**	— 0.02 ± 0.03
	0,08 %	14	— 0.3 ± 0.4	+ 0.01 ± 0.03
	0.06 %	11	+ 0.5 ± 0.4	+ 0.02 ± 0.04
	0.05 %	13	**+ 1.0 ± 0.3**	— 0.06 ± 0.05
	0.03 %	8	— 0.4 ± 0.3	— 0.01 ± 0.02
	0.02 %	20	+ 0.3 ± 0.3	+ 0.02 ± 0.03
	0.008 %	5	+ 0.2 ± 0.2	— 0.03 ± 0.02

of these solutions is only small; however, when one takes together the experiments with 0.03 % to 0.006 % LiCl (25 experiments), there is a significant increase (0.6 ± 0.2). It seems, therefore, that this increase of viscosity at great dilutions is real.

With 1.5 % LiCl and 3 % $CaCl_2$, no stratification appeared; the eggs are dark and flattened perpendicularly to the centrifugal force. Apparently, these highly hypertonic solutions withdraw too much water from the eggs. Although the osmotic value of a 3.0 % $CaCl_2$ solution is higher than that of a 1.5 % LiCl-solution, the effect of the latter is much stronger. Variations of permeability may have played a part. One could try to explain the observation by assuming that $CaCl_2$ renders the egg more impermeable to water.

According to HARVEY (1945), stratification of *Arbacia punctulata* eggs by centrifugation is more rapid in $CaCl_2$ than in isosmotic NaCl or KCl solutions. The viscosity increases in the order: $CaCl_2 < MgCl_2 <$ seawater $<$ NaCl $<$ KCl. In my experiments, isosmotic solutions of $CaCl_2$ and LiCl did not differ significantly in their effect upon the viscosity of the egg.

Tension at the surface.

The curve in fig. 3, obtained by plotting the tension indices of the controls against time, shows a periodical variation of the tension at the surface of normal eggs in the uncleaved stage. It agrees rather well with RAVEN's curve (1945, fig. 8); the tension at the surface drops at both maturation divisions and before the first cleavage in both cases. However, in my curve the variations are somewhat less pronounced. This may be due to the difference in centrifugal force employed.

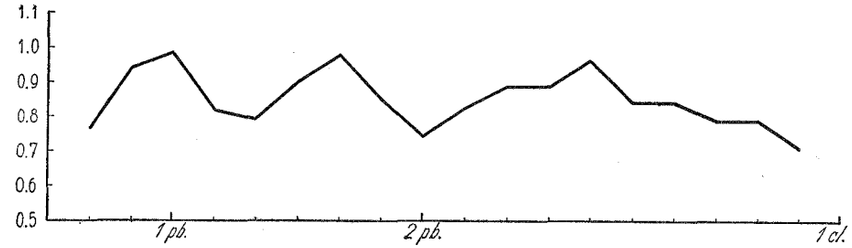

Fig. 3. Graph showing variations of tension at the surface of normal eggs from oviposition till 1st cleavage. Abscissae: time. Ordinates: tension index.

In table I, the average deviations of tension index in the salt solutions are given. There is a significant increase of tension at the surface in 1.0 % and 0.6 % LiCl and in 1.5 % $CaCl_2$; on the contrary, there is decrease of this tension in 0.1 % LiCl. Lower concentrations gave no significant effects.

According to HARVEY (1945), *Arbacia punctulata* eggs break with increasing rate in the following order: $CaCl_2 <$ seawater $<$ NaCl $<$ KCl.

Hence, the tension at the surface is increased in isosmotic $CaCl_2$ solution, decreased in isosmotic NaCl and KCl solutions. In my experiments, $CaCl_2$ increases the tension only at a rather high concentration (1.5 %), whereas LiCl gives an increase or a decrease according to concentration.

There is a typical influence of certain concentrations on the shape of the second polar body. The polar body is drawn out during centrifugation and gets the form of a carrot, the broad end turned towards the egg. This malformation took place in 1.5 % $CaCl_2$ and 1.0 % and 0.6 % LiCl, but not always. Control eggs in distilled water did never show a similar malformation of the second polar body. This phenomenon may be due to a decrease in tension at the surface of the polar body, though viscosity may play a part as well.

Discussion.

As mentioned above, the degree of stratification of the egg after centrifugation, especially the abundance of granules and vacuoles in the hyaloplasm zone, has been taken as a measure of viscosity.

The first question we have to answer is, whether a change in this respect, caused by the action of the salt solutions, really points to a change of viscosity. The altered degree of stratification in salt solutions might be due e.g. to a change in the specific gravity of the inclusions of the egg.

The vacuoles in the hyaloplasm zone, according to RAVEN (1945), arise from γ-granules, which have a high specific gravity immediately after oviposition, but become lighter by swelling and, then, remain in the hyaloplasm zone at centrifugation.

The swelling of the granules is due to an absorption of water by the eggs from the surrounding egg capsule fluid. By hypertonic solutions, water might be withdrawn from the vacuoles; in this way, their specific gravity would increase and at centrifugation they would accumulate in the centrifugal part of the egg. This would give rise to a decrease of the number of inclusions of the hyaloplasm zone and, therefore, to an apparent decrease of viscosity.

However, we have seen that a decrease of viscosity number is not only caused by hypertonic solutions, but also by 0.1 % LiCl and $CaCl_2$ solutions, which are considerably hypotonic to the eggs. Furthermore, the increase of viscosity at still greater dilutions cannot be explained in this way, either. We must conclude, therefore, that besides possible osmotic effects, real changes of viscosity under the influence of LiCl and $CaCl_2$ solutions have taken place.

In colloid chemistry, the effect of a decrease of viscosity by adding electrolytes to hydrophilous colloids is well known. The viscosity is increased by an electric charge. This phenomenon is called "the effect of electro-viscosity". The ζ-potential, or kinetically active difference in potentials, of the proteins is lowered by the first millimoles. When the ζ-potential is zero, the effect of electro-viscosity has disappeared. A further adding

of electrolyte may cause a reverse of charge, and consequently a rise of viscosity.

The egg protoplasm probably contains hydrophilous colloids for a greater part. Therefore, this factor may be of great importance. Other factors may play a part in lowering the viscosity as well, e.g. the influence of kations on the combination of the protein molecules.

In this connection, the experiments carried out by RANZI (1943—46) on the influence of certain salt solutions, among which LiCl, on the eggs of *Rana virescens* should be mentioned. He prepared an extract from *Rana* gastrulae and separated from it different protein fractions. In all experiments, the action of LiCl took place during 12 hours at 2° C; after that, relative viscosity was determined at 14.6° C by means of an Ostwald-viscosimeter. He found a rise of viscosity in concentrations from 0.42 % up to 2.1 % LiCl. Lower concentrations did not change viscosity. This fact certainly deserves attention in connection with the above-mentioned effect of electro-viscosity. The difference in results probably is not due to a reverse in charge, but perhaps to a process of denaturation during the 12 hours' stay in the solution at low temperature.

In my experiments, the *Limnaea* eggs remained undamaged, contrary to RANZI's *Rana* eggs. The egg surface plays an important part and can cause a limitation of the amount of ions passing. My experiments are, therefore, not comparable with RANZI's.

According to HARVEY and SHAPIRO (1941), the eggs of *Arbacia punctulata* and *Asterias forbesii* have a cortical layer with considerably higher viscosity than the interior plasm. By the presence of this layer the egg is inhomogeneous. It is supposed that the amount of $Ca^{\cdot\cdot}$-ions combined with proteins at the cell surface depends upon the $Ca^{\cdot\cdot}$-ion concentration in the medium and that it determines the firmness of the cell membrane (SHAPIRO 1941).

BUNGENBERG DE JONG (1935) considers the protoplasmic membranes as phosphatide autocomplex systems and can give an explanation of the tightening action of $Ca^{\cdot\cdot}$-ions in certain concentrations. Although tension at the surface is not mentioned, we could imagine that this property becomes higher by the increase of electric attractions. From his experiments with phosphatide coacervates it is evident that the tightening influence rapidly decreases in the order: $CaCl_2 > LiCl > NaCl > KCl$.

As already mentioned above, in *Limnaea* the egg surface probably is rendered more impermeable to water by Ca, while the tension at the surface increases.

The rate of elongation certainly will depend upon the degree of viscosity (RAVEN 1945), although in my experiments no relation can be traced. On the other hand, viscosity may depend upon the character of the membrane, e.g. tension at the surface and permeability.

HARVEY (1945) centrifuged unfertilized *Arbacia punctulata* eggs in isosmotic salt solutions and found a decrease of rate of stratification in

the following order: $CaCl_2 > MgCl_2 >$ seawater $> NaCl > KCl$. This would point to an increasing viscosity. The eggs break in two parts more easily in the same order. In $CaCl_2$, they stratify best and break with the greatest difficulty. The effect of breaking is due to an alteration of the surface.

These experiments are of course not quite comparable with mine. Anyhow, in sea urchins $CaCl_2$ causes a more distinct stratification and a rise of the tension at the surface, results, which I found also for *Limnaea* eggs with higher $CaCl_2$ concentrations. Whereas, however, in HARVEY's experiments monovalent kations had the opposite effect, in *Limnaea* the effect of the Li^+-ion on the viscosity is entirely comparable to that of the Ca^{++}-ion, while its effect on the tension at the surface agrees with that of Ca in hypertonic solutions, but is reversed in a slightly hypotonic solution.

Summary.

1. The influence of LiCl and $CaCl_2$ solutions on viscosity and tension at the surface of eggs of *Limnaea stagnalis* was studied by centrifugation.
2. The viscosity of the eggs is decreased by 0.1 %—1.0 % LiCl and 0.1 %—1.5 % $CaCl_2$ solutions; it is increased by 0.006 %—0.03 % LiCl and 0.05 % $CaCl_2$ solutions.
3. The tension at the surface is increased by 0.6 %—1.0 % LiCl and 1.5 % $CaCl_2$ solutions; it is decreased in 0.1 % LiCl solutions. Lower concentrations of both salts gave no significant effects.
4. The solutions which increased the tension at the surface of the egg apparently decrease this tension of the second polar body.

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Mineralogy. — *On cassiterite and garnet from the Kaokoveld, S.W. Africa.* By L. P. G. KONING. (Communicated by Prof. H. A. BROUWER.)

(Communicated at the meeting of November 29, 1947.)

Introduction.

In the present paper the results of the crystallographic and optical investigation of cassiterite and garnet from the Kaokoveld, S.W. Africa will be given.

These minerals have been sent to me for investigation by Mr. G. W. BRANDT from the West Africa Company, Grootfontein, Southwest Africa, for which I wish to express my gratitude.

The crystallographic measurements have been carried out with a two-circle goniometer. The collection of minerals contained no complete crystals. The size varied from 4—20 mm for the cassiterite and from 4—10 mm for the garnet.

The cassiterite is mainly yellowish and greenish brown coloured and somewhat transparent; sometimes, however, the mineral is black coloured. The prismatic faces are mainly greenish and yellowish brown and slightly transparent; the pyramidal faces are black. Mainly the black faces are lustreous.

The garnet is black coloured and opaque. The rhombendodecahedron {110} is highly lustreous.

a. *Cassiterite.*

1. Crystallographic investigation of the cassiterite.

Several crystals have been subjected to crystallographic measurements. The mineral is ditetragonal dipyramidal. The prisms are highly striated parallel to the *c*-axis.

Forms: *b*(010), *a*(100), *m*(110), *h*(120), *s*(111), *e*(011), see fig. 1. The forms *m*(110) and *s*(111) have the principle development.

The results of the crystallographic measurements are tabulated in table I.

TABLE I.

Face	Symbol	φ	ϱ
1 *b*	(010)	0°	90°
2 *a*	(100)	90	90
3 *m*	(110)	45	90
4 *h*	(120)	26 30′	90
5 *e*	(011)	0	33 55′
6 *s*	(111)	45	43 32