P. C. Colls, Junior Demonstrator of Physiology, King's College, London, made the following communication:

**On a Modification of W. G. Smith's Reaction Time apparatus.**

The following modification of Mr W. G. Smith's Reaction Time apparatus\(^1\) was devised while attempting to utilise it for class purposes. The result obtained is the same, namely that the chronograph writes only during the reaction time, and the modification avoids the somewhat complicated arrangement of wires and keys in the original apparatus and so is better suited for students. The following diagram represents the suggested modification.

\[\text{Diagram of proposed modification.}\]

The cell \(A\) is connected with the electro-magnetic tuning-fork \(B\) (provided with platinum contact) and with the chronograph \(C\). On the

\(^1\) *Proc. Physiol. Soc.* Nov. 17, 1894.
course of the wires are placed two short circuiting keys \( D \) and \( E \). Key \( D \) is closed and \( E \) is left open; the writing point of \( C \) is then at rest. The signal is the sound of the opening of key \( D \); the chronograph records time; the subject responds by closing the key \( E \) and this brings the chronograph once more to rest. If an electrical signal is required a double Du Bois Reymond key may be employed instead of \( D \). This is arranged so as not only to bridge the chronograph circuit, but also to complete the primary circuit of an induction coil. When this key is opened the chronograph is thrown into action and a break shock produced in the inductorium, the electrodes of which are placed on the subject's body.

The following diagram shows how this modification may be adapted for a large class. The wires from the tuning-fork may be led round a table and pass into chronographs at intervals; four are shown in the figure. Each chronograph is short-circuited by two keys, \( D \) and \( E \), \( D'' \) and \( E'' \), \( D''' \) and \( E''' \), which correspond to the keys \( D \) and \( E \) in figure 1.
On the Relation of Muscular Activity to the Regulation of Heat-Production. By M. S. PEMBREY.

The relation of muscular activity to the maintenance of a constant temperature can be studied by testing the response of an animal to changes of external temperature under conditions in which the control of the animal over its muscular system is varied. The response of the normal animal is compared with that exhibited by the animal under anaesthetics or after section of its spinal cord.

This investigation differs chiefly from that of other observers in the shortness of the consecutive periods in which the respiratory exchange was determined; thus it is possible to follow the immediate effect of changes of temperature.

Section of the Spinal Cord.

Example. Black and White Mouse, No. 9.

Twenty-four hours after section of the cord. There is complete paralysis below the shoulder-girdle. The mouse is in good condition. It was in the ventilated chamber, water-bath = 25°, for 30 minutes before the first period.
Consecutive periods of 10 minutes.

<table>
<thead>
<tr>
<th>CO₂</th>
<th>Temp. of water-bath</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0497 grm.</td>
<td>25°</td>
<td>Mouse active</td>
</tr>
<tr>
<td>-0500</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>-0516</td>
<td>9.5</td>
<td>Mouse very active</td>
</tr>
<tr>
<td>-0465</td>
<td>9.5</td>
<td>Mouse active</td>
</tr>
<tr>
<td>-0375</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>-0272</td>
<td>9.75</td>
<td>Mouse shivering</td>
</tr>
<tr>
<td>-0200</td>
<td>25</td>
<td>Mouse quiet</td>
</tr>
<tr>
<td>-0198</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>-0226</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>-0245</td>
<td>24.5</td>
<td>Mouse slightly active</td>
</tr>
<tr>
<td>-0351j</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>-0352j</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

The mouse was very active when taken out of the chamber, and commenced to eat directly it was placed in its cage.

*Note.* The section was purposely made low down in order that the mouse might breathe well, eat, cleanse itself and move about with its fore feet.

From the results it was probable that the mouse only responds in a manner similar to that of a cold-blooded animal when it is no longer able, by the activity of the few muscles over which it still has control, to keep up the temperature of its tissues.

Observations upon the temperature of the animal confirm this view.

**Anæsthesia from Ether.**

When the mouse is under ether the effect of a change of temperature is more marked. One example may be given.

**Black and White Mouse A.**

The mouse was in the ventilated chamber, water-bath = 25°, for 50 minutes before the first period.

Consecutive periods of 15 minutes.

<table>
<thead>
<tr>
<th>CO₂</th>
<th>Temp. of water-bath</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0437 grm.</td>
<td>25°</td>
<td>Mouse was sent under ether</td>
</tr>
<tr>
<td>-0392</td>
<td>25</td>
<td>Mouse under ether</td>
</tr>
<tr>
<td>-0290</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>-0308</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>-0185</td>
<td>13.5</td>
<td></td>
</tr>
</tbody>
</table>

The mouse was fully under ether when taken out of the respiration-chamber. It took about 15 minutes to come round.


Coagulation of Colloids. By J. W. Pickering.

(Preliminary Communication.)

In a previous paper I have pointed out that the experiments of Grimaux would if substantiated prove of considerable interest from a physiological standpoint. Owing to the kindness of that gentleman who has placed at my disposal specimens of his synthesised colloids I have been enabled not only to confirm but extend his observations.

The three principal substances under consideration are the colloids amidobenzoic A and B, which are formed by the action of phosphorus penta-chloride on meta-amidobenzoic acid, and the "colloide aspartique," which is formed by the action of phosphorus oxychloride on aspartic acid.

The product of the reaction in each case is a white amorphous friable powder which is readily soluble in ammonia.

On evaporation of the ammoniacal solution in a vacuum a mass of brownish plates remains; these are not unlike dried serum-albumin.

The plates are slightly soluble in distilled water in the cold, readily soluble after heating.

The solution is opalescent and does not coagulate on heating. But if one or two drops of a one per cent. solution of a soluble salt of either barium, strontium or calcium be added, pronounced coagulation commences on heating to 70° C. A few drops of a saturated solution of sodium chloride will also induce coagulation at 70° C.

If a more dilute solution of these salts be added the passage of a current of either CO₂ or SO₂ through the solutions will determine their coagulability.

Sodium sulphate and potassium acetate retard the coagulation; thus if one c.c. of a solution of potassium acetate be added to one c.c. of a one per cent. solution of either of the colloids under discussion, it is necessary in order to induce heat coagulation to add one c.c. of one per cent. calcium chloride instead of the one or two drops which are sufficient in the absence of the potassium acetate.

Similarly, the addition of an equal volume of glycerine to the solution prevents heat coagulation, but the passage of a current of CO₂ through the solution will restore coagulability.

The colloid C gives a typical violet reaction with copper sulphate and potash, a yellow with nickel sulphate and potash, a red brown with cobalt sulphate and potash.

1 This Journal, xiv. 347.
Each of the colloids gives a well-marked xanthoproteic reaction and each is precipitated by trichloracetic acid and by salicylsulphonic acid, the latter precipitate being coagulated on heating.

Intravascular injection into the rabbit of a one per cent. solution of either of the colloids will cause complete intravascular clotting.

In one experiment the clotting extended throughout all the larger veins of the body, and pronounced clots were found in each of the cavities of the heart, and in the aorta, after the injection of five c.c. of colloid A. A similar experiment was demonstrated to the meeting.


(Preliminary Communication.)

While studying the amount of work performed by, and upon, muscle under several conditions, I found much inconvenience in that we had no method, in most cases, for graphically representing the amount of work performed. After some consideration I therefore devised the following method of showing by successive areas the amount of work performed during successive contractions.

Suppose AB (Fig. 1) to represent the zero abscissa line, and that loads are measured as abscissae starting from O, and that at equal distances ordinates at right angles to this line are drawn representing exactly the heights, or some multiple of the heights, the muscle has raised the different weights. DE is one of these ordinates, and is placed at 6 units of measurement from the zero O. In my experiments the unit of measurement represents 4 grammes; and the magnifi-

![Fig. 1.](image-url)
cation of the recording apparatus is 4. Hence the diagram represents that the muscle raised a weight of 24 grammes through a height \(\frac{1}{4}DE\). Hence the work performed during the contraction was \(\frac{1}{4}DE \times OD\).

A point \(A\) is next taken 4 units of measurement to the negative side of \(O\) and a vertical \(AC\) is drawn there. On this \(AF\) is marked off equal to \(DE\), and \(FO\) is drawn and produced to cut \(ED\) produced in \(G\). The parallelogram \(GN\) is then completed and its area exactly represents the amount of work performed by the muscle during that contraction. For since the two triangles \(ODG\) and \(OFA\) are similar, \(AF \times OD = AO \times DG\). But \(AF \times OD\) is 4 times the work performed and as \(AO = 4ND\), the work = \(ND \times DG\).

In this way we obtain a series of rectangles, one for each contraction, and as they are all on equal bases their areas and therefore the work each represents are directly proportional to their heights. They therefore form a series at once appealing to the eye, and enabling us to pick out all the essential points that we wish to learn. The figure obtained for a series of contractions is similar to the diagram given below (fig. 2). It is there seen that the maximum amount of work, produced during a contraction, occurred at the sixth contraction.

![Fig. 2.](image)

From the first to the sixth there was a gradual increase of the work produced, and from the sixth onwards a gradual decrease.

The contractions are registered by the photographic method. A long aluminium bar is suspended at one end on two horizontal pivots, the free end being supported by the muscle. This bar is divided into 100 equal divisions, and a carrier weighing 100 grammes slides along it.

At the fixed end is placed a plane mirror, the external surface of
which is silvered. The plane of this reflecting surface is at right angles to the direction of the bar and in the plane of the points of support. A transverse slit, which is strongly illuminated, transmits light to this mirror, which throws an image upon a long vertical slit fixed in one face of a light-tight box. In this box is fixed vertically a sensitive plate travelling horizontally along rails in front of the slit. This plate is moved by hand so as to bring a fresh piece of it behind the vertical slit for each contraction. The plate is placed at a distance from the mirror equal to twice the length of the aluminium bar. Then, as the reflected beam is moved through twice the angle that the bar is moved, the recorded contraction is 4 times the height of the contraction. The successive loads are effected by moving the carrier along the aluminium bar towards the muscle, and, as the bar is divided in 1/10ths, and the weight of the slider is 100 grms., each division nearer the muscle means an increase of 1 gramme to the load.

The muscle is made to contract by a stimulation applied directly, and from a constant current.

W. D. HALLIBURTON, F.R.S., made a short communication on some further work he had carried out in connection with nucleo-albumin. The substance he had previously called cell-globulin was shown by further analysis to be nucleo-albumin, whether it is prepared from the thymus gland or from the stromata of red corpuscles. He also added some particulars regarding the nucleo-albumin of red marrow; this substance appears to be especially remarkable for the high percentage of phosphorus it contains. Full analytical details will be shortly published.
On the Physiological Action of Extract of the Suprarenal Capsules. By G. Oliver, M.D. and E. A. Schäfer, F.R.S.

(Second Preliminary Communication.)

In the communication which we made to this Society¹ twelve months ago, we showed that when an extract, whether prepared with water, alcohol, or glycerine, of the suprarenal bodies of the calf, sheep or dog was injected—even in very small quantities—into a vein of a dog or rabbit the following pronounced physiological effects were produced in a few seconds:

1. Extreme contraction of the arteries, which was shown to be of peripheral origin.
2. A remarkable and rapid rise of the arterial blood-pressure, which took place in spite of powerful cardiac inhibition, and which became further augmented when the vagi were cut.
3. Central vagus stimulation, so pronounced that the auricles came to a complete standstill for a time—although the ventricles continued to contract, but with a slow independent rhythm.
4. Great acceleration and augmentation of the contraction of the

auricles and ventricles after section of the vagi—the auricular augmentation being specially marked.

(5) Respiration only slightly affected—becoming shallower.

A large number of observations made on dogs, cats and rabbits since last March have confirmed these results; and have enabled us to determine others, an outline of which we now submit to the Society.

This research naturally divides itself into two branches—one dealing with the physiological effects, and the other with the chemical constitution of suprarenal extracts. The latter has been undertaken by Messrs Moore and Nabarro of this laboratory, who themselves contribute the results of their observations to the Society.

As in our earlier experiments, watery decoctions of the glands in normal saline have been chiefly employed by us. The suprarenals experimented with have been derived from the calf, sheep, dog, cat, guinea-pig, and man. The physiological results produced have been precisely the same whatever the source of the glands, except in two taken from cases of Addison's disease, which will be afterwards referred to.

(1) Effect of a continued intravenous injection. As a rule when the intravenous mode of administration was adopted—as in the dog and rabbit—a definite small quantity of the extract representing a known weight of the fresh gland was injected. In exceptional instances however a continuous inflow of a 5% solution of the extract in normal saline has been employed. In these instances the physiological effects already described were maintained during the whole time the injection lasted, but without the development of other apparent symptoms, and without causing death. We have in this way administered large doses of the extract to the dog, thereby producing the most violent cardio-vascular disturbance, without causing a fatal result.

(2) Effect on the kidney. In our former communication the inference as to the extreme contraction of the arteries was derived from observations on the blood-pressure, from the use of the limb plethysmograph, and from the arrest of the flow of normal saline through the vessels of a frog caused by the addition of a small quantity of suprarenal extract. Several observations with the oncometer have confirmed this conclusion and have shown that it is to be extended to the vessels of the kidney; for the tracings have shown a well-pronounced reduction in volume of that organ during the suprarenal effect on the circulation.

(3) Depressor nerve. We have observed that stimulation of the depressor nerve does not produce the usual reduction of the
blood-pressure while the effects of the suprarenal injection last: that is to say, if the depressor nerve in the rabbit is stimulated at the height of the pressure caused by intravenous injection of suprarenal, the usual fall of blood-pressure is not produced, nor is any depressor result to be obtained until the blood-pressure has returned to the normal or thereabouts. The depressor result reappears simultaneously with the Traube-Hering curves, if these are seen at all in the tracing. (We have already shown in our previous communication that these curves are abolished while the effect of the extract lasts.)

(4) Effect on cardiac muscle. We have invariably found in the dog, cat, and rabbit, after section of the vagi, that the heart’s action is remarkably accelerated and augmented. In view of this powerful stimulation of the heart in mammals we were somewhat surprised to find a comparatively small effect produced by the extract on the frog’s ventricle. We have not found that solutions of less than 1% suprarenal extract in Ringer’s circulating fluid will with certainty affect the frog’s ventricle, recording its pulsations in a heart-plethysmograph. The following results were, however, obtained with this and with stronger solutions—up to 5%:

(a) Reduction of diastole—with consequent acceleration;
(b) The abolition of groups of contractions, and the setting up of continuous pulsation; and
(c) The arrest of the ventricle in systole.

Inasmuch as we found that this extreme effect of the extract was not prevented or antagonized by potassium chloride we conclude it was not due to lime salts in the extract; for Ringer has shown that the calcium effect upon the contraction of the frog’s ventricle is counteracted by potassium. Moreover the individual contractions do not show the characteristic lime effect (delay of diastole). On the contrary each individual contraction remains normal; although the acceleration produced by the drug may be ultimately sufficient to prevent the completion of the diastole, and the contractions may thereby be caused to run together.

(5) Effect on the skeletal muscles. The paralysing effect of the subcutaneous injection of the extract (equal to 1 or 2 grains of the fresh gland) in the frog, referred to in our first communication, has not been observed in other animals experimented on in this way, namely, in the rabbit, guinea-pig, and cat—except in lethal doses in the rabbit. We have, however, observed in dogs subjected to the intravenous injection of the extract that when the muscles were electrically stimulated
through the nerve supplying them, a modification of the normal contraction was apparent—the relaxation being delayed, as in the case of frog’s muscle.

This effect was, moreover, not only observed while the suprarenal rise of the blood-pressure was being recorded, but was traceable for some time after that rise had passed away. We infer therefore that the active material is probably taken up by, and remains for a time stored within, the muscular tissues.

(6) Effect on secreting glands. We have not obtained any definite effect upon the secretion of the submaxillary gland as the result of injecting suprarenal extract into the blood. Nor have we found the chorda tympani any less active in promoting the secretion of the gland in an animal the blood vessels of which are contracted by the extract.

(7) Comparison between the cortex and medulla. We have found that when two extracts are prepared of equal strength—say 1 to 100 of normal saline—one of the cortex, and the other of the medulla of the perfectly fresh gland, the intravenous injection of the former will not produce the characteristic cardio-vascular disturbance, while that of the latter in the same dose will induce it in a marked degree. It is, however, somewhat difficult to prepare the cortical extract perfectly free from a trace of the medulla; so that it may happen, as we have found, that a comparatively large dose of cortical extract (e.g. equal to 8 milligrammes of the fresh cortex) may produce a slight physiological effect: but not more than that of a much smaller portion (e.g. equal to 0·2 milligrammes or \( \frac{1}{300} \) grain of the fresh medulla) of the medullary extract.

We therefore conclude that the active principle of the extract is present in the medulla only: the effects which we have obtained from extract of cortex being small and inconstant, and probably to be explained by accidental contamination or post-mortem diffusion.

(8) The suprarenals of man. We have experimented with suprarenals derived from three subjects—one in which the glands were healthy, and two others in which they were diseased (cases of Addison’s disease). The healthy organs provided an extract of great physiological activity; whereas the diseased adrenals afforded one which gave a purely negative result.

(9) Pre-digested suprarenal extract. In view of the oral administration of the extract as a remedy it seemed desirable to ascertain whether peptic digestion impaired its active properties. A little of the watery extract of the gland was added to artificial gastric juice
(pepsin + 0·2 \%/ HCl) and exposed to a temp. of 40° C. for 24 hours. The intravenous injection of a small quantity of this and of an equal portion of the same extract diluted at the time to the same extent with 0·2 \%/ HCl produced identical physiological effects. Injection of an equivalent amount of acid as a control produced no effect. This observation was repeated with the same result.

It does not, therefore, seem likely that stomachic digestion will seriously lessen the physiological properties of the extract.

(10) How is the extract eliminated or disposed of? Some experiments were made with the view of elucidating this question. Is the active principle destroyed in the blood? This seemed not improbable, inasmuch as Mr Moore has found that alkalies and oxidation destroy the activity of the extract. We have, however, observed that when allowed to stand in freshly-drawn blood (a) with free exposure to the air, or (b) with complete exclusion of air for 22 hours, the extract possessed the same activity as when preserved in exactly the same manner in normal saline. We have likewise ascertained that clamping the renal vessels or the suprarenals themselves does not prolong or perceptibly modify the duration of the effect produced on the blood-pressure by the intravenous injection of the extract. Inasmuch as we have observed an altered contraction of the muscles to persist after the subsidence of the cardio-vascular disturbance set up by the injection it seems probable, as already hinted, that the active principle of the extract passes out of the blood into the muscles, and remains there for a time.

We have shown that in Addison's disease the adrenals may become totally devoid of the physiologically active material. If these bodies are to be regarded as eliminators of toxic materials rather than as producers of materials which are of definite physiological value, the toxic materials they should remove or destroy might be expected, in cases in which their function is in abeyance, to pass out by the kidneys. We have, however, found that an extract prepared from the urine in Addison's disease has precisely the same effect when injected into a vein as that of an extract prepared from normal urine. In fact all the evidence we have obtained leads us to view the function of the suprarenal bodies\(^1\) as secretory rather than destructive, and the secreted product as being in all probability of great physiological importance for maintaining the tonicity of the muscular tissues in general, and especially of the heart and arteries.

\(^1\) At least the medulla.
We must again express our indebtedness to Messrs. Willows, Francis and Butler for the liberal supplies of fresh suprarenal glands with which they have provided us.

**On the chemical nature of a physiologically active substance occurring in the suprarenal gland.** By B. Moore, M.A.

(From the Physiological Laboratory, University College, London.)

The experiments of which a preliminary account is given in this communication were undertaken at the suggestion of Prof. Schäfer, with the object of determining the chemical nature of, and if possible isolating a substance discovered by him and Dr G. Oliver in the suprarenal glands, which possesses a well-marked physiological action.

The first experiments were made with a glycerine extract of which one part was equivalent to one of the fresh glands. A portion of this extract was treated with ten times its volume of absolute alcohol, filtered from the resulting precipitate, and evaporated to a syrup at a temperature of 70°C. This residue was dissolved in normal saline, tested physiologically by venous injection and found to be very active. The precipitate caused by the alcohol was also extracted with normal saline and the extract injected and found to be inactive. Boiling the original extract, or a solution prepared by treating it with alcohol as described above, did not destroy any of its active properties; also on subjecting it to the action of acids or alkalies for a few minutes, then neutralizing and injecting, the extract was found to be still active. But it was shown by subsequent experiments that boiling for three or four hours with water alone, or heating to 40°C with even very dilute alkalies for a similar period, completely destroys the physiological activity of the substance, while heating to 40°C for a like time with different acids, varying in strength up to 10%, left it quite untouched.

A water extract of the gland was prepared by mincing the glands, covering with water for a few hours, boiling for a few minutes, and then filtering. This filtrate was evaporated to dryness at a temperature of 70°C and a brown-coloured residue obtained, of a sticky wax-like consistency when warm, becoming brittle on cooling, and very deliquescent. This residue was found to be very active. It was extracted with various organic solvents, viz. ether, chloroform, carbon-bisulphide, amyl alcohol, benzene and ligroin, as well as with mixtures of these solvents,

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1 *Proceedings of the Physiological Society, March 10, 1894.*
but the active principle was not dissolved by any of them. Repeated extraction with boiling absolute alcohol, gave a brown-coloured wax similar to the original residue; even in hot absolute alcohol this wax, which it is impossible to dry completely, is very feebly soluble, and in a later experiment it was shown that the fresh glands dried at 40° C. and then, after several extractions with anhydrous ether, placed under absolute alcohol had a mere trace of the active principle extracted from them even after several days' standing. The water extract, boiled and filtered, was saturated with ammonium sulphate, which caused a scanty precipitate, the filtrate from this diluted with its own volume of water gave a strong effect, a control experiment showing that an equal amount of half saturated ammonium sulphate solution had no such action. The precipitate caused by saturation was extracted, injected and found to be inactive.

The water extract was distilled in a vacuum at a temperature between 50° and 60° C. and the distillate was found to be inactive. The brown residue remaining was also distilled in vacuo and the portion coming over below 100° C. and that between 100° and 180° C. collected separately and tested, as well as an extract of the charred residue, all three were found inactive. The brown residue which softens and becomes semi-fluid between 60° and 80° C. chars rapidly when heated above 150° C.

Some fresh glands were dried at 40° C., then, after several extractions with ether, and after standing for some weeks under absolute alcohol, were extracted with a small quantity of distilled water, and the filtrate allowed to dialyse into distilled water through parchment paper. The dialysate was strongly active.

This dialysate contains some substance possessed of a powerful reducing action. Such a substance has already been described, as occurring in the gland, by Vulpian¹, and his observations have been confirmed and extended by Virchow², Arnold³, and Krukenberg⁴.

Vulpian found that it is confined almost exclusively to the medulla of the gland, that it gives a dark green or blue colour with ferric chloride, passing through purple to a dark red on the addition of ammonia, or sodium carbonate; with various oxidizing agents, such as chlorine, bromine or iodine water, peroxide of hydrogen or alkalies in the presence of oxygen, it gives a rose-red colouration, which is discharged by sulphuretted hydrogen or ammonium sulphide. It is

¹ Compt. rend., T. 43, 1856. ² Virchow’s Archiv, Bd. 12, 1857.
insoluble in organic solvents, alcohol, ether, benzene, &c.; soluble in water and dilute acids. It is also, as shown by Vulpian, peculiar to the suprarenals, as its reactions are not given by any of the other glands; he also found it after death in the blood of the suprarenal vein, and supposes that it is during life supplied to the blood by the gland.

I have found that the solubilities of the active principle are exactly the same as those of this reducing agent. Experiments, in which portions of the cortex and medulla were carefully separated and made into extracts, demonstrated that the active principle is also confined to the medulla. As stated above, heating with dilute alkalies destroys the active substance, it also oxidizes the reducing agent. Oxidation of an active extract for a few minutes with peroxide of hydrogen developed a rose red colouration and at the same time, as shown by a control experiment, very materially weakened the physiological effects of the extract.

These considerations point to the fact that the active principle and the body giving these peculiar reactions are probably identical; and in all cases where extracts possessing active physiological properties were afterwards tested chemically for this reducing agent it was invariably found to be present, while in those cases in which no physiological effect was obtained the reducing agent was found absent by chemical tests.

The action of various precipitants was tried on the dialysed extract.

Platinic chloride gave no precipitate. A small precipitate was obtained by the use of this reagent in an alcoholic extract, but on decomposing it with sulphuretted hydrogen, filtering, neutralizing and injecting no effect was obtained, and the original filtrate from the platinic chloride, after removing the excess of that reagent gave a strong effect. A similar result was also obtained with mercuric chloride.

Potassio-mercuric iodide also gave no precipitate.

On adding phospho-molybdic acid, the solution turned green and a blue precipitate fell, both evidently due to the reduction of the reagent.

Nitrate of silver gave a white precipitate, which rapidly turned black from reduction, even when kept in the dark, and on heating a silver mirror is rapidly obtained.

The various extracts were found not to reduce Fehling's solution even after boiling with mineral acids, and yielded no crystalline product when heated with phenyl hydrazine.
On passing a solution of the brownish resin obtained by evaporating the water extract to dryness through animal charcoal, to decolourise it and obtain a purer product, a colourless filtrate was obtained, yielding on evaporation fern-shaped masses of crystals, but on testing a solution of these no physiological effects were obtained. Nor could the active principle be re-obtained from the charcoal by boiling with water. On trying the effect of smaller quantities of charcoal it was found that the solution instead of being decolourized became more deeply coloured. Probably the active principle is first oxidized by the charcoal and afterwards decolourized.

The results obtained in the course of the investigation up to the present may be briefly stated as follows:—

The active principle is soluble in water, and in dilute alcohol; its solubility decreases with the percentage of alcohol present, until with absolute alcohol it is almost insoluble. It is insoluble in ether, chloroform, amyl alcohol, carbon bisulphide, benzene or ligroin.

It is not attacked by acids nor by boiling for some minutes, but is destroyed by alkalies, by oxidizing agents and by continued boiling.

It is not precipitated by excess of alcohol, by saturation with ammonium sulphate, by mercuric chloride, potassio-mercuric iodide, or tannic acid.

It does not reduce Fehling's solution alone, nor after boiling with mineral acids, nor does it form a crystalline compound with phenyl hydrazine.

It is not volatile either alone or with water vapour. It dialyses freely through parchment paper, and the highly active dialysate so obtained is completely free from proteids.

The Proteids of Suprarenal Capsules. By D. N. Nabarro.

The investigation, of which the following is a preliminary communication, was started at the instigation of Prof. Schäfer with the view of ascertaining the nature of the proteids derivable from the suprarenal bodies.

The materials employed for examination were the suprarenals of calves. They were extracted with $5\%$ $\text{MgSO}_4$ solution, within 12 hours after death. The proteids obtained are for the most part of the nature of globulins and nucleo-albumins, that is, they are, with one exception, precipitated by saturation with $\text{MgSO}_4$. The temperatures of heat
coagulation are 56° C., 65° C. and 75° C. One albumin is present in small amount, coagulating at 71° C. The proteid coagulating at 56° C. is a globulin. It leaves no trace of a residue on digestion with artificial gastric juice. That coagulating at 65° C. leaves a very small residue on digestion; the proteid coagulating at 75° also leaves a very small residue.

The extract of the glands made with 5% MgSO₄ gave, on acidifying slightly with acetic acid, a considerable coagulum at 40—41° C. On repeating the experiment with a second extract, the precipitation began at a still lower temperature, viz. about 35° C. On careful neutralization of some of the same extract and warming, a thick precipitate occurred at 40° C. Whether this is a precipitation of a globulin or of a nucleo-albumin I am not at present in a position to assert definitely, but that it is probably of the nature of nucleo-albumin would appear from the fact that a considerable residue is left upon gastric digestion.

No pepsin or peptone is present in these glands.

**Experiments on the conditions of coagulation of fibrinogen.**

By E. A. Schäffer, F.R.S.

*(Preliminary Note.)*

It was shown by Hammarsten that solutions of fibrinogen prepared by half-saturating blood-plasma with NaCl and purified by repeatedly washing with half-saturated solution of NaCl, redissolving and reprecipitating give a coagulum of fibrin on the addition of solution of Schmidt's fibrin-ferment prepared from blood by precipitation with alcohol. This solution contains as is well known a proteid or nucleo-proteid (Halliburton, Pekelharing) which is regarded as the actual ferment, besides inorganic salts, including soluble lime salts.

It follows therefore from this experiment, and the conclusion has been accepted by most physiologists, that for the formation of fibrin from fibrinogen the presence of an organic "ferment" is necessary, and it has further been made abundantly clear from the experiments of Green, Ringer and especially of Arthus and Pagés that the presence of lime is equally essential to fibrin formation.

Recently it has been argued by Lilienfeld¹ that nucleo-albumin acts in promoting the formation of fibrin from fibrinogen by splitting up the fibrinogen into a substance which he calls "thrombosin" and a

globulin, and that it is this new substance thrombosin which by combination with lime forms fibrin. Lilienfeld further finds that the ferment action of nucleo-albumin is due to the nucleic acid it contains, and he asserts that other acids such as acetic are competent to act in a similar way, i.e. to produce thrombosin from fibrinogen. In illustration of this he gives the following experiment. "Fibrinogen is prepared pure by Hammarsten's method and dissolved in dilute NaCl, acetic acid is added and produces a precipitate; this Lilienfeld terms thrombosin: the precipitate is dissolved up (after washing with H₂O) in a little dilute sodium carbonate solution: the addition of calcium chloride produces a coagulum of fibrin."

I have repeated the above experiment and have found, as Frederikse and others have before me, that the statement is correct. I am not however prepared to admit without further evidence (1) that "thrombosin" is chemically different from fibrinogen, (2) that fibrin-ferment (i.e. nucleo-albumin) does not enter into the reaction.

1. *I fail to find any evidence in Lilienfeld's paper that thrombosin is different from fibrinogen.* For a solution of fibrinogen in dilute carbonate of soda will also yield a coagulum on the addition of CaCl₂ alone. This is contrary to a statement of Lilienfeld, but it is unquestionably true. The rapidity of formation of the coagulum is, *ceteris paribus*, as great as in the case of the so-called thrombosin, that is to say, if sufficient lime salt be added and if the solution of fibrinogen be strong the coagulum forms instantly, and the clot has all the appearance and properties of fibrin. With a strong solution a mere trace of lime will produce some coagulum,—in fact it is difficult so thoroughly to get rid of all traces of lime from the glass vessels employed as to avoid the formation of some fibrin on standing.

2. *It is not proved that nucleo-albumin does not enter into the reaction.* For however many times fibrinogen may be washed with half saturated NaCl, redissolved and reprecipitated, it is impossible to prepare a product which does not leave a residue on digestion with artificial gastric juice. Lilienfeld has himself shown that the digestion-residue of fibrinogen contains phosphorus, and has inferred that

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1 Nevertheless Lilienfeld denies the existence of nucleo-albumin in the solution of Schmidt's fibrin-ferment (as asserted by Pekelharing) and confirms Halliburton's statement that the proteid is a cell-globulin. Halliburton has however himself since admitted the correctness of Pekelharing's assertion (*Physiol. Soc.* Feb. 16, 1895).

2 Also on the addition of *boiled* solution of Schmidt's alcohol precipitate of blood.

3 This is equally true of the fibrin formed from such "pure" fibrinogen, although in this point my results differ from those obtained by Lilienfeld.
fibrinogen may itself be a nucleo-albumin. But it does not appear to
me that the amount of residue obtained warrants this inference. It
seems more probable that the source of the phosphorus-containing
digestion-residue is nucleo-albumin (i.e. fibrin-ferment) which has
clught to the fibrinogen, and that the presence of this nucleo-albumin
explains the fact that fibrin becomes formed from Hammarsten's
fibrinogen on the addition of CaCl₂.

With regard to the necessity of lime in promoting the change of
fibrinogen into fibrin it must be admitted that soluble salts of lime play
a most important part in the transformation. It is however not the
case that the formation of fibrin in fibrinogenous fluids such as blood-
plasma will not occur in the presence of excess of soluble oxalates, as is
inferred by Arthus and Pagés from their experiments. In support
of this statement I bring forward the following observation.

Dog's blood was mixed, as it flowed from an artery, with solution
of potassium oxalate in excess and put aside in a cold place for the
corpuscles to subside. After two days the supernatant plasma was
poured off from the subjacent mass of corpuscles and blood-platelets
and was filtered. The filtrate, which contained a considerable amount of
potassium oxalate, was again put aside for a few days, when a thick film
of coagulum was noticed at the bottom of the otherwise clear fluid.
Examined with the microscope this coagulum was found to present all
the appearances and reactions of fibrin, and I have under the microscope
a stained preparation which the members of the Society will at once
recognize as exhibiting the characteristic felt-work of fibrin-filaments.
These however have been formed in a fluid containing abundance of
dissolved oxalate of potassium, and if we assume (as appears to be
conclusively proved by recent research) that lime is a necessary com-
ponent of fibrin, we are driven to the conclusion that fibrinogen can
slowly take up lime and become converted into fibrin even in the
presence of soluble oxalates.

The Exchange of Blood Gases in the Brain and in the
muscles in states of rest and of activity. By Leonard Hill
and D. N. Nabarro.

(Preliminary Communication.)

A series of experiments has been carried out by us in order to
investigate the comparative metabolism of the brain and the muscles.
The research was suggested by the fact that the blood which flows out
from the torcula Herophili is far less venous in colour than that of the femoral vein. In each experiment successive samples of blood were collected from the carotid artery, the torcula Herophili and the deep femoral vein.

The gases were extracted for analysis by the pump described by one of us (L. Hill) in the *Journ. of Physiol.* 1894. The samples of blood weighed 8—9 grms. and were collected in small bulbs which had been rinsed out with oil, and the rate of flow was noted in each case. The blood was immediately transferred from these small bulbs to the vacuum froth-bulbs of the pump, defibrinated by shaking with mercury and the gases then extracted. This procedure was followed because it was impossible to draw the blood directly from the torcula into the vacuum bulbs. By comparative experiments it was found that the percentages of the blood gases were unaltered by this method of procedure. Blood taken from the carotid artery either in the small bulbs or directly into the vacuum bulbs yielded similar results, i.e. within the limits of error of the pump.

The gases were extracted from all the samples of blood taken, within two hours of collection, and the vacuum froth-bulbs containing the blood were kept in the cold until the time of extraction.

The animals employed were dogs; and the experiments were carried out under chloroform or under morphia narcosis.

Samples of carotid blood were collected before and after the collection of the samples of venous blood, in order to eliminate variations in the general state of the animal. In the condition of rest the gases of the carotid blood remained remarkably constant during the course of an experiment (see Exp. II.).

The state of activity was provoked by the intravenous injection of the essential oil of absinthe, and samples of blood were obtained in the conditions of tonus and clonus. During the fit the gases of the carotid blood vary much more than in the state of rest, and this fact has hitherto increased the difficulty of obtaining uniform comparative results in activity.

The general results of more than thirty experiments, now completed, are that:—

(1) The metabolism of resting muscles, as measured by the gaseous exchange, is very much greater than that of the resting brain (Exps. I. and IV.).

(2) The metabolism in the muscles in the state of activity is enormously increased (Exp. III.).
(3) The metabolism of the brain during an epileptic fit is somewhat but not constantly increased (Exp. IV.).

The first and second of these results are fully established by our experiments; the third requires still further investigation. These results are opposed to those of Mosso on heat formation in the brain. The latter cannot be regarded as the seat of very active metabolism.

**Examples of Experimental Results.**

All the numbers are reduced to 0°C. and 760 mm. pressure and corrected for tension of water vapour.

I.

<table>
<thead>
<tr>
<th></th>
<th>Normal Artery</th>
<th>Normal Torcula</th>
<th>Normal Deep Femoral Vein</th>
<th>Differences</th>
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<td></td>
<td></td>
<td></td>
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<td>Art. and Vein</td>
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<td>64.43</td>
<td>63.59</td>
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<tr>
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<td>O</td>
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<td>13.74</td>
<td>6.94</td>
<td>+ 3.03 + 9.83</td>
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<tr>
<td>N</td>
<td>2.79</td>
<td>2.17</td>
<td>2.33</td>
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The difference between the artery and the deep femoral vein is more than twice that between the artery and torcula in the condition of rest.

II.

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<tr>
<td>Total</td>
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<td>57.08</td>
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<td>14.86</td>
<td>+ 0.07</td>
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<td>2.85</td>
<td>- 0.37</td>
<td>2.15</td>
<td>1.95</td>
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This experiment shows how constant the results remain in the condition of rest. The second pair of samples was taken some minutes after the first pair.

III.

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<tr>
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<td>-</td>
<td>2.29</td>
<td>3.02</td>
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The fit vein samples flowed at almost three times the rate of normal vein, so that the fit differences must be trebled and thus become very large.
**SOCIETY, MARCH 16, 1895.**

**IV.**

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<tbody>
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<td>65.77</td>
<td>65.20</td>
<td>—</td>
<td>—</td>
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<tr>
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<td>—</td>
<td>2.35</td>
<td>2.23</td>
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</table>

**The Influence of Saline Media on Tubifex Rivulorum.** By SYDNEY RINGER, F.R.S., and ARTHUR G. PHEAR.

In a communication shortly to be published in this *Journal* we have drawn attention to the influence of salts in sustaining the structural integrity of tadpoles. We now record the results of further investigations on the influence of saline media on tubifex rivulorum. As with tadpoles, so with tubifex, when placed in distilled water the animals in a few hours begin to disintegrate, and in 24 hours disintegration is complete, all structure being lost and only débris being left. Certain salts employed in appropriate strengths arrest or entirely obviate this disintegrating action of distilled water. Salts may protect against disintegration which are quite unable to sustain the functional activity of a frog's heart. Of all salts we have found those of calcium the most efficacious, notably the acid phosphate and the bicarbonate of calcium. Very small quantities of calcium salts suffice to maintain structure and life,—so minute as to produce merely a haziness with ammonium oxalate after standing for some minutes.

**Calcium salts.** The acid phosphate and the bicarbonate are the only two which sustain efficiently; the former is the better of the two. It was prepared by adding tribasic calcium phosphate to distilled water, and allowing the mixture to stand for 24 hours. The clear supernatant fluid was then removed, containing in solution a minute quantity of the acid phosphate of calcium, produced by the action of the carbonic acid present in the distilled water on the tribasic phosphate. This solution supports the integrity and life of tubifex as well as tap-water. *Calcium bicarbonate* solution was prepared by passing well washed carbonic acid through lime water until the milkiness first produced became almost dissolved. After standing for some time the clear overlying fluid was removed, and found to support life in tubifex when added to distilled water in the proportion of 60 c.c. to 140 c.c., and in stronger solutions.

In this proportion the reaction of the solution was feebly alkaline.
Calcium hydrate, sulphate, nitrate, and chloride support life indifferently, a few organisms living three or four days. Of calcium hydrate, solutions only are efficient of neutral or slightly acid reaction; in these the lime is doubtless converted into the bicarbonate. The calcium sulphate solution was prepared by allowing distilled water to stand on sulphate of calcium for several days.

**Sodium salts.** Of sodium hydrate only very weak solutions (1 in 50,000 to 1 in 30,000) sustain, and these imperfectly; the neutral or faintly acid solutions are the best; alkaline solutions dissolve the organisms rapidly. Sodium bicarbonate sustains four or five days in solutions of 1 in 5000 to 1 in 2000. Neutral or faintly alkaline solutions are the most efficient. With larger quantities movement is arrested in 24 hours; but the shape and colour of the organisms are retained, and they recover completely if placed in tap-water. Trisodium phosphate solutions of neutral or faintly acid or alkaline reaction (1 in 4000 to 1 in 2000) sustain movement till the fourth day. In these doses structure is preserved after motion is arrested. Higher and more alkaline solutions cause a rapid dissolution of the animals. Disodic phosphate solutions of neutral or nearly neutral reaction (1 in 5000 to 1 in 2000) permit sluggish movement till the fifth day. Stronger solutions though arresting movement preserve integrity, and recovery ensues on placing the animals in tap-water. Monosodic phosphate solutions are very inefficient,—hardly better than distilled water; stronger solutions arrest movement, but obviate disintegration, and allow of recovery in tap-water. Sodium chloride though unable to sustain movement yet in strong solutions (1 in 2000 and stronger) keeps the organisms intact, and they recover when placed in tap-water. Sodium nitrate is quite inefficient.

**Potassium salts.** Potassium hydrate. Solutions of this in distilled water of neutral or almost neutral reaction support life imperfectly for four days. Alkaline solutions rapidly dissolve the animals. On the second day the solutions of potassium hydrate became acid, doubtless from absorption of carbonic acid, more than sufficient to convert the hydrate into the bicarbonate. Potassium bicarbonate is very inefficient; in a weak solution of faintly acid reaction sluggish movement persisted for three days; in strong solutions (1 in 280) the animals became motionless in 5 hours. Tripotassium phosphate solutions possess very imperfect sustaining powers; in neutral or faintly acid solutions slight movement was observed on the morning of the fourth day; in strong solutions the animals rapidly became dissolved. Dipotassic phosphate displays slight sustaining powers in neutral or nearly neutral solutions;
strong solutions cause rapid arrest of movement, but disintegration is avoided, and recovery takes place in tap-water. *Monopotassic phosphate* fails to maintain movement. *Potassium chloride* is quite inefficient to support movements. In the weaker solutions the animals disintegrate; in higher solutions integrity is preserved, and movement is restored readily by tap-water.

Certain salts then obviate disintegration. Of these some, as will be shown, are of highly poisonous nature. Of many of these poisonous salts however the percentage requisite to maintain integrity is so small as to be probably quite innocuous. Other poisonous salts preserve integrity and movement in comparatively large doses; such are the oxalates of sodium and potassium. Of these salts we do not suppose absorption to occur. With intact and living skin or mucous membrane the physical laws of osmosis are held in abeyance, and a selective function is possessed and exercised by the live tissue. If a sufficiently strong solution of any salt be employed, this selective function is paralysed, osmosis occurs, the salt gains access to the organism, which becomes motionless and in a short time dies.

The nature of this protective influence over integrity is obscure. In many of our experiments it appears that solutions of neutral or nearly neutral reaction are the most efficient. Reaction however though important is not the sole factor. Some salts, of themselves neutral, and therefore leaving unmodified the acidity of distilled water powerfully protect. Such salts are the magnetic oxide of iron and potassium ferrocyanide. Moreover calcium salts are extremely competent to sustain, to a great extent, independently of their reaction. Distinctly acid solutions of calcium phosphate, and strongly alkaline solutions of calcium bicarbonate sustain as well as tap-water.

Again, it may be asked:—in the maintenance of integrity and movement, do salts act as a whole, or by virtue of one or other of their constituents? Although we cannot claim to have settled this question, yet some experiments devised to elucidate the matter may be of sufficient interest to be recorded. Carbonic acid is a common constituent of calcium, potassium and sodium bicarbonate, and occurs also in distilled water. Yet the last is incapable of supporting integrity; and the first three though all efficient in this respect yet vary widely in their degree of efficiency. Moreover tap-water saturated with carbonic acid gas acts in a very different manner on tubifex from distilled water similarly treated. Tubifex when introduced into either become almost immediately motionless; movement however rapidly
returns to those placed in tap-water, and in a few hours they are seen to be again perfectly active. Those in distilled water on the other hand remain motionless, and while retaining their shape in the course of twenty-four hours become opaque and granular looking—a peculiar appearance which we have learnt to associate with the action of acids.

The action of potassium ferrocyanide may here be described. This salt is very efficient to maintain, in solutions varying from 1 in 400,000 to 1 in 28,000; the lowest of these permitted a small amount of disintegration: but in all the animals were active in their movements on the fifth day. The solutions were slightly acid, all to the same degree, the acidity being that of the distilled water employed. Stronger solutions while maintaining integrity hampered and even arrested movement; complete recovery ensued on placing the animals in tap-water. Sodium ferrocyanide acts similarly to the potassium salt. Potassium ferricyanide resembles the ferrocyanide in supporting life and movement well. Of the constituents of potassium ferrocyanide, potassium has been shown to sustain movement imperfectly in the form of the hydrate, bicarbonate and phosphate. Iron in the form of the magnetic oxide perfectly supports life and movement even in minute quantities. Pure magnetic oxide of iron free from lime (which is often present in commercial magnetic oxide) was shaken up with distilled water and allowed to stand for five days. The clear supernatant fluid was removed and found to contain about 1 part of Fe₂O₃ to 500,000₁. This minute quantity is very efficient in sustaining integrity and motion. It might be supposed that the iron acted by combining with the carbonic acid in the distilled water, on the assumption that it is the carbonic acid that destroys. This is disproved by the fact that the iron water was quite as acid as distilled water, and yet this solution sustained thoroughly well. Potassium cyanide imperfectly supports movement for three or four days in doses from 1 in 150,000 to 1 in 28,000. Stronger solutions with marked alkaline reaction quickly dissolve the tubifex. Hydrocyanic acid even in very minute quantities (1 in 2,000,000) sustains movement some hours longer than distilled water. Stronger solutions are no better than the weaker ones. It appears then that all the constituent parts of potassium ferrocyanide support integrity and life, although iron is the most effectual.

In the case of oxalates the acid constituent appears to play a more

₁ The analysis was made by Mr B. Moore, in the Physiological Laboratory of University College.
important part than the sodium or potassium. **Sodium oxalate** in solutions of 1 in 20,000 to 1 in 2000 preserved activity for more than four days. Higher strengths rapidly arrested movement, but preserved integrity, and the addition of calcium chloride to the solution was followed by recovery. In solutions of **neutral potassium oxalate** (1 in 25,000 to 1 in 4000) movement continued till the third day. **Potassium binoxalate** in weak solutions sustained languid movement to the third day. **Oxalic acid** in minute quantities proved very efficient. Solutions containing 1 in 200,000 to 1 in 50,000 sustained well; on the fifth day the animals were clustered and active. Larger quantities speedily kill, but the animals retain their shape, becoming of a dull opaque white colour. No sustaining properties have been discovered in **phosphoric acid**. It seems then that the constituent concerned in maintaining integrity varies with different salts, in some cases being the acid element, in others the base. With calcium phosphate and calcium bicarbonate and the corresponding salts of sodium and potassium, the preservation of integrity appears to be due to the calcium, sodium and potassium respectively; whilst with sodium oxalate and potassium oxalate the oxalic acid is the more potent constituent.

Brief reference must be made to the property displayed by calcium salts, oxalates and iron in protecting tubifex (and tadpoles) from the poisonous effects of other salts. The presence of calcium phosphate or bicarbonate in solutions of potassium chloride which by themselves would arrest movement in about three hours entirely obviates this paralysing action of potassium chloride. The oxalates of sodium and potassium and the magnetic oxide of iron possess similar properties, but to a slighter degree. These salts permit movement to continue for some days in a solution of potassium chloride; with lime salts under similar circumstances movement persists indefinitely. But calcium salts, as we have seen, antagonise the action of potassium salts in another way. Tubifex which have been rendered motionless by potassium chloride, on the addition of calcium chloride completely recover. The paralysed animals retain their shape and colour, and it must be supposed that the potassium salt is absorbed and arrests function by its direct action on the tissues of the organism. Revival in these cases must be due to an antagonism, effected in the tissues, on the part of the lime salt over the paralysing action of the potassium chloride. Magnetic oxide of iron and oxalate of sodium or of potassium are powerless in this respect; they appear to act solely by preserving integrity and preventing absorption, and are unable to antagonise the action of potassium chloride on the tissues.