Collected papers / edited by W. D. Halliburton.

Contributors

King's College, London. Physiological Laboratory.
Halliburton, W. D. 1860-1931.
Lyle, Herbert Willoughby, 1870-1956
King's College London

Publication/Creation

[London?] : [King's College London?], 1900.

Persistent URL

https://wellcomecollection.org/works/j4yepxhy

License and attribution

Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

KING'S College LONDON

KING'S COLLEGE, LONDON.

PHYSIOLOGICAL LABORATORY.

COLLECTED PAPERS.

No. IV.

EDITED BY

W. D. HALLIBURTON, M.D., F.R.S.

PROFESSOR OF PHYSIOLOGY, KING'S COLLEGE, LONDON.

1900.

CONTENTS.

[*Reprinted from the Journal of Physiology.*
Vol. XXII. No. 5, *March* 17, 1898.]

AN INTESTINAL PLETHYSMOGRAPH. By ARTHUR EDMUNDS, B.Sc. (*Lond.*). (Three Figures in Text.)

(*From the Physiological Laboratory, King's College, London.*)

In the course of the investigations by Drs Halliburton and Mott into the physiological action of choline and allied products of the disintegration of nervous tissues, it was considered advisable to study the vasomotor changes taking place in the intestinal area, and I therefore devised an apparatus, by means of which this could be effected.

A few experiments on intestinal plethysmography were made by Bayliss[1]; his method consisted in taking a thistle funnel covered at its wide end with a flexible membrane and filled with warm oil. The stem of the funnel was then connected with a recording apparatus and the funnel carefully lowered, by means of a rack and pinion, on to the surface of a loop of intestine supported on a glass slide.

A more extended series of observations, on the vasomotor changes taking place in the intestine, has been recently made by Hallion and François Franck[2]. Their method is to isolate a piece of intestine by dividing it between double ligatures and then to draw the loop of intestine, which is still connected by its mesentery, through a tubulure in the bottom of a glass vessel shaped like an inverted bell jar. A wide flexible piece of rubber tubing several centimeters in length is fixed to the neck of the tubulure and encircles the mesentery, around which is wrapped a pad of the animal's own omentum. The glass vessel is then partially filled with tepid salt solution and its wide end closed by a large rubber cork, through which a thermometer is passed. Passing also through this cork, but not reaching the surface of the liquid, is a tube connected with a Marey's tambour, by means of which the movements of the intestine are recorded.

The apparatus which I have devised has the advantage over either

[1] Bayliss. This *Journal*, xiv. p. 303. 1893.

[2] Hallion and François Franck. *Archives de Physiologie*, (5) viii. 2, pp. 478 and 493. 1896.

of these methods of being very much simpler, while it is possible to obtain with it equally, if not more, satisfactory results. It has been shown by Schäfer and Moore[1] that air is far preferable to salt solution or oil as an oncometric medium and therefore air is the medium employed in the present apparatus. In its simpler form this consists of a hemispherical basin four inches in diameter and about two inches deep, to the margin of which is fixed a flattened rim about three-quarters of an inch wide. At the bottom of this basin is an elliptical opening about one inch long by half an inch wide, which leads into a projecting tubular portion about an inch long; near the rim, a glass tube is inserted for the purpose of connecting the oncometer with the recording apparatus. This is an ordinary Marey's tambour and the connection is best made by means of a piece of rubber pressure tubing; a side tube is interposed to allow of the pressure being equalised on both sides of the tambour membrane; during the progress of an experiment this is kept closed by a clip.

To apply the instrument, an incision about an inch and a half long is made in the linea alba, preferably below the umbilicus, as this prevents ejection of viscera during violent contraction of the abdominal muscles. The apparatus is then supported in a retort ring over the abdomen of the animal and the intestine drawn through the elliptical opening into the basin. When as much of the small intestine as possible has been drawn through the opening, the ends are cut between ligatures and the attached ends returned to the abdomen, thus leaving within the basin a long loop, consisting of practically the whole of the small intestine, attached merely by peritoneum and its enclosed structures. The space around this pedicle is then carefully packed with cotton-wool soaked in vaseline and finally with vaseline itself, the top of the vessel being closed finally by a glass plate luted on with vaseline. Before placing this last in position, it is advisable to moisten its lower surface in the centre with salt solution; this prevents condensation of moisture on this surface and allows one to see the intestines during the whole of the experiment.

Such a basin may be made very conveniently from stout gutta percha, which has the advantage of being readily moulded into any shape required; the instrument in question was made by softening sheet gutta percha in hot water and pressing it with well wetted fingers into a wet mortar; the whole operation may be very conveniently conducted under a hot water tap.

[1] This *Journal*, xx. p. 1. 1896.

I might here add that I have made several plethysmographs of gutta percha after the pattern of Schäfer and Moore's instrument and find that when, as in the present case, the apparatus is entirely outside the animal, this material may be considered as practically rigid, but that when the apparatus is returned to the abdominal cavity, it becomes sufficiently yielding at the temperature of the body to allow of its being slightly compressed by contraction of the abdominal muscles.

Another and a more accurate form of the instrument consists of two concentric copper hemispheres connected at their circumference by a flat brass ring (see Fig. 1), which serves the same purpose as the rim of the

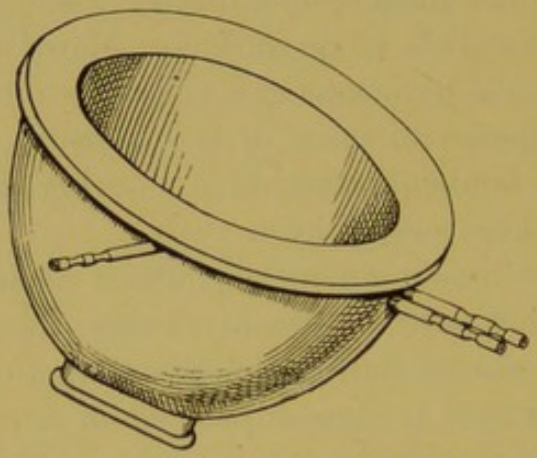


Fig. 1. External view of intestinal plethysmograph.

gutta percha basin, viz. that of allowing a glass plate to be luted on with vaseline or, preferably, a mixture of vaseline and a small quantity of paraffin. Into the space thus formed, two tubes are inserted (Fig. 2, *c*),

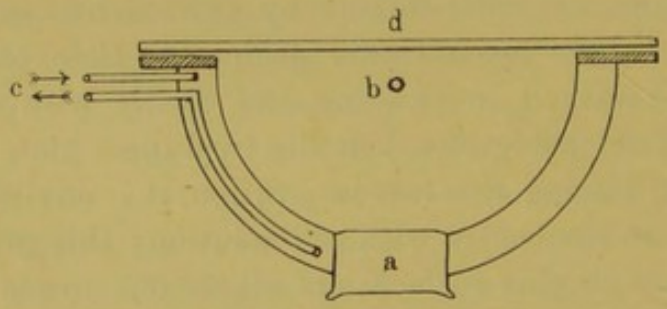


Fig. 2. Section of intestinal plethysmograph.

a. Opening for mesentery and blood vessels.
b. Opening of tube leading to recording tambour.
c. Tubes for water jacket.
d. Glass plate covering the plethysmograph.

one going to the bottom and the other leading from the top of the apparatus, so that a current of warm water may be circulated between the two hemispheres.

As in the case of the gutta percha basin, there is an elliptical opening in the bottom of the inner vessel connected with a short wide tube, which passes through the outer one and projects for a short distance beyond it, while from near the circumference a tube passes from the inner vessel for the purpose of connection with the tambour.

The method of keeping the apparatus at a constant temperature is to pass water at a constant pressure through a tube heated in a water bath and then through the instrument. To do this one takes an inverted bell-jar fitted with a cork through which passes the stem of an ordinary glass funnel, the wide end of which is thus within the bell-jar; through the cork a wide glass tube is also passed. Water from the supply pipe is allowed to flow into the funnel at such a rate that there is always an overflow, which escapes by the wide tube. The stem of the funnel is then connected by means of india rubber tubing, with a loop of metal tubing, immersed in a fairly large vessel of boiling water; this vessel must be provided with a cover through which the ends of the loop pass in order that a constant amount of tubing may be exposed to the hot water. About 12 in. of $\frac{3}{8}$ in. gas-piping will answer excellently; a greater length of tubing presents so much surface to the water that it is very difficult to keep the bath at the boiling point. The water is then conducted by rubber tubing to the pipe leading to the top of the water jacket, where it circulates between the two hemispheres and flows out by the other pipe, which is connected with an india rubber tube furnished with a screw clip, by means of which the rate at which the water flows, and hence the temperature, can be regulated. After the water has been flowing for a few minutes, the temperature will be found to keep quite constant. This I have proved by control experiments.

This form of the apparatus has the advantage that the intestines can be drawn from the abdominal cavity directly into a warm chamber, thus minimising shock and preserving the intestine in a comparatively normal condition.

For most purposes the simple gutta percha basin answers perfectly well, especially if the animal be kept on a hot bath and carefully covered with cotton-wool, but, whenever it is desired to avoid shock, the water-jacketed apparatus is preferable.

With either form of apparatus, it is possible to get a very satisfactory tracing, with a considerable excursion of the lever, the heart-beats and the respiration waves being both clearly marked. Moreover the intestine being cut, movements of the abdomen do not affect the

instrument. Fig. 3 is an example of a normal tracing taken from a cat anæsthetised with A.C.E. mixture and ether, the second line is the

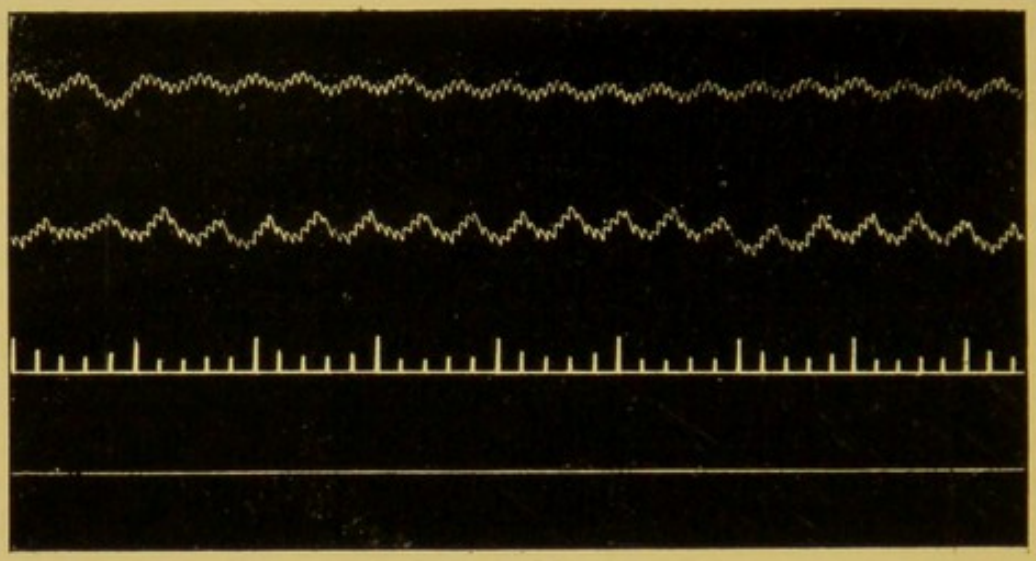


Fig. 3.

Upper line. Carotid blood-pressure.
Next line. Intestinal plethysmogram.
Next line. Time in seconds.
Last line. Abscissa of blood-pressure.

plethysmogram, the next is the carotid blood-pressure. The two writing points were exactly vertical under one another. Drs Mott and Halliburton will deal with the results they have obtained with the instrument in a future paper.

[*Reprinted from the Journal of Physiology.*
Vol. XXII. No. 5, March 17, 1898.]

THE PRECIPITATION OF CARBOHYDRATES BY NEUTRAL SALTS.

By R. A. YOUNG, M.D., B.Sc. (*Lond.*), M.R.C.P., *Lecturer on Physiology at the Middlesex Hospital Medical School*[1].

(*From the Physiological Laboratories of King's College, London, and the Middlesex Hospital Medical School.*)

The researches of Nasse[2], Hofmeister[3], Wenz[4], Halliburton[5] and others into the action of neutral salts on proteid solutions have greatly increased our knowledge of the various proteids and their solubilities, and have afforded at the same time one of the most generally useful means of separating the individual proteids from mixtures, and of obtaining them in a comparatively pure condition.

It has long been known that many other colloid substances including certain carbohydrates can also be precipitated from their solutions by saturation with neutral salts ("salted out," to adopt the convenient German expression "Aussalzen"); in fact Neumeister[6] goes so far as to state "that all non-diffusible substances are capable of being salted out from their solutions, as are also certain other substances such as picric acid and the urates."

The recognized value of this means of precipitation as applied to proteids seemed to warrant some more extended investigation of the action of neutral salts on carbohydrate solutions, and especially to determine if the method can be used to separate the various carbohydrates and their digestion cleavage products from one another.

[1] Towards the expenses of this research a grant was made by the British Medical Association on the recommendation of the Scientific Grants Committee of the Association.

[2] Nasse and Krüger. *Pflüger's Archiv*, XLI. S. 504. 1887.

[3] Hofmeister. *Archiv f. Exper. Path. u. Pharm.* XXIV. S. 247. 1888; *ibid.* XXV. S. 1. 1889.

[4] Wenz. *Zeit. Biologie*, XXII. S. 1. 1886.

[5] Halliburton. This *Journal*, V. p. 172. 1884.

[6] Neumeister. *Lehrbuch d. physiol. Chem.* I. S. 22. Jena, 1893.

Accordingly the following research was undertaken at the suggestion of Professor Halliburton.

Previous work in this direction may be very briefly summarised, being confined chiefly to a few observations made during the course of researches into the action of neutral salts on other substances.

Nasse[1], who worked for the most part with proteids and albuminoids, stated that soluble starch, glycogen, glycogen-dextrin, inulin, and the soluble iodide of starch were also precipitated by ammonium sulphate and magnesium sulphate.

Neumeister[2] stated that glycogen is "completely precipitated by saturation of its solutions with ammonium sulphate."

Pohl[3] applied the salt precipitation method systematically to the vegetable gums, and found that the majority were precipitated by one or more salts, gum arabic being the only exception.

Halliburton[4], referring to the precipitation of colloid carbohydrates by salts, makes the statement, "I have found that glycogen is precipitated by saturation with ammonium sulphate or magnesium sulphate, but not at all or only very slightly by sodium chloride."

My own observations may be conveniently considered under the following headings:—

I. Methods.
II. The crystalline carbohydrates.
III. Starch and its cleavage products, the dextrins.
IV. Glycogen.
V. Inulin.
VI. The iodine reaction and the iodine compounds.
VII. Theoretical considerations on salt precipitation.
VIII. General summary.

I. **Methods.**

Most of the substances experimented with are soluble in water with comparative ease, especially on gentle heat, except in the case of starch, of which pastes of various strengths were made as described in the section devoted to it.

The solutions used were in all cases neutral in reaction, and made

[1] Nasse and Krüger. *loc. cit.*
[2] Neumeister. *Zeit. Biol.* XXIV. S. 279. 1888.
[3] Pohl. *Zeit. f. physiol. Chem.* XIV. S. 151. 1890.
[4] Halliburton. *Text-book of Chem. Physiology and Pathology*, p. 106. London, 1891.

by dissolving the substance as a rule in hot water, filtering while hot, and then allowing to cool to the ordinary room temperature, at which unless otherwise stated saturation was always effected.

The strength of solution most frequently employed was from 1–2 %, although in some cases much stronger solutions were also investigated.

The chief salts used were, ammonium sulphate, magnesium sulphate, and sodium sulphate.

Observations were also made with other salts, including sodium chloride, ammonium chloride, potassium sulphate, potassium iodide, and sodium phosphate, in most cases with very little, if any result.

Saturation was effected by adding successive quantities of the finely pounded salt to solutions of carbohydrates in flasks or test tubes, and shaking. Excess of the salt was in all cases added, and then the solution often allowed to stand over the excess of salt for some hours or even days. As a rule precipitation is completed in twenty-four hours.

In a few cases the saturations have been effected at higher temperatures, but without any effect on the result obtained except in the case of sodium sulphate. This salt has its maximum solubility at 33–34° C., and if saturation be carried out at this temperature, it is a very effective precipitating agent, much more so than at ordinary temperatures. At this temperature it can be used for most purposes for which ammonium sulphate is used, sometimes with advantage, as it acts quickly; its disadvantages are the great quantity of salt which has to be added, and the filtration and washing of the precipitate on a warm-water filter.

If as the result of saturation of any solution with a neutral salt a precipitate occurred, it was collected on a filter and then well washed with a saturated solution of the salt used in precipitating it, at the temperature at which saturation was effected, and then its solubility and reactions were tested.

If necessary any salt remaining in the precipitate may be removed by dialysis before a solution is made, but this in most cases is not necessary, as the salt does not interfere with any of the ordinary reactions, except in the testing of the point at which precipitation by alcohol commences.

The chief reactions investigated for the different precipitates were —solubility, the iodine reaction, action of tannic acid[1], of alcohol, and

[1] Tannic acid precipitates glycogen, starch, and the earlier erythrodextrins, but not erythrodextrin III, nor the achroodextrins.

behaviour with Fehling's solution before and after heating with dilute acids.

In some cases, especially with starch and its cleavage products, attempts were made at fractional precipitation. The methods adopted are described in the section devoted to starch and need only be briefly referred to here.—The most useful were—

(i) To add ammonium sulphate to half-saturation, and after allowing to stand for some time, to filter off any precipitate, and then completely saturate the filtrate with the same salt.

(ii) First saturate with magnesium sulphate, and then with sodium sulphate either at the ordinary room-temperature or at 33° C.

If in any case a double saturation is to be carried out, or if it is important to be quite certain that all of a given substance is precipitated, it is best to add more of the first salt to the saturated filtrate and filter again before saturating with the second salt.

That the salt precipitation method has disadvantages even with proteids is well recognized. It is somewhat tedious, especially if attempts be made to remove all the salt from the precipitates obtained. With proteids, it is difficult to apply to strong solutions owing to the difficulty in filtering off the precipitated substance from the semi-solid mass which is obtained on adding the salt[1]. This difficulty I have not encountered in dealing with carbohydrate solutions, the precipitate in nearly all cases having filtered off very readily. On the whole, I think the disadvantages are less than with proteids.

The chief difficulties I have found have been first—the difficulty in removing all traces of a substance soluble in a saturated solution of the salt used, from one which is precipitated, *e.g.* the erythrodextrins or sugar from soluble starch, but this can be effected by repeated washings with a saturated solution of the salt used for precipitation. The second difficulty is that of removing all the salt, above referred to. The presence of the salt, however, in no way interferes with the iodine reaction.

The advantages of the salt precipitation method are its simplicity, its cheapness, and the ease with which precipitation is in many cases effected, so that it is of value to confirm and extend the results of the alcohol method of separation.

[1] Neumeister. *Lehrbuch d. physiol. Chem.* 1. S. 22. Jena, 1893.

II. **The Crystalline Carbohydrates.**

The ordinary crystalline carbohydrates, dextrose, lævulose, cane sugar, maltose, and lactose were all investigated, but as was expected with uniformly negative results as far as precipitation was concerned[1].

III. **Starch and its Cleavage-products.**

Brown and Morris[2], in one of their investigations on this subject point out the great variations in the accounts of the dextrins given by different observers, and state, "This is mainly owing to the great difficulty in obtaining the dextrins in anything like a pure state, to the alteration and separation which they undergo during purification, and lastly to the very few known processes to which when isolated they can be submitted in order to determine their properties, and their points of resemblance or difference."

I have therefore made a large number of experiments on the action of neutral salts on starch and the earlier products of its hydrolysis.

This section of my paper may be divided as follows:

(*a*) Starch paste.
(*b*) Soluble starch.
(*c*) The erythrodextrins.
(*d*) The achroodextrins.
(*e*) Comparison with the products obtained by other observers.

(*a*) **Starch paste.** Potato starch was used almost exclusively in my experiments. This may be purified as described by Brown and Heron. It is washed with distilled water, then with ·5 % potassium hydrate, then with 1 % hydrochloric acid, then with distilled water till the washings are neutral in reaction, and then dried at 35–40° C. A

[1] Several of them, however, form compounds with neutral salts, most of which are capable of being crystallised; such are:

Dextrose in combination with sodium chloride and sodium bromide.

$$2C_6H_{12}O_6.NaCl.H_2O$$
$$C_6H_{12}O_6.NaCl$$
$$C_6H_{12}O_6.2\,NaCl.$$

Galactose yields a crystalline compound with sodium chloride. With cane sugar several such compounds can be formed with sodium chloride, sodium bromide, and sodium iodide, *e.g.*

$$C_{12}H_{22}O_{11}.NaCl.H_2O.$$

With maltose no such compounds have been prepared. See Watt's *Dictionary of Chemistry* (Muir and Morley), IV. p. 530 *et seq.* London, 1894.

[2] Brown and Morris. *Journ. Chem. Soc.* XLVII. p. 528. 1885.

weighed quantity of this (generally about 1–2 %)[1] is then rubbed into a thick paste with distilled water, and poured into boiling water, or better still into water kept at the temperature of gelatinisation of the starch used[2] in order to avoid any conversion of the starch into soluble starch.

Starch paste is completely precipitated by ammonium sulphate or magnesium sulphate, and also by half saturation with ammonium sulphate if allowed to stand for some hours or days. Sodium sulphate at ordinary temperatures gives partial precipitation, which becomes complete if saturation is carefully effected at 33° C.

It is not precipitated by sodium chloride or ammonium chloride.

(*b*) **Soluble Starch.** This was prepared by (i) boiling starch paste prepared as described in the last section, but of much greater strength (10–20 %) with dilute sulphuric acid on a water bath till it became quite limpid, and then neutralising with some alkali, generally the base of the salt which was to be used in the saturation experiment, *e.g.* if ammonium sulphate were to be used, the sulphuric acid was neutralized with ammonia, or (ii) by the action of malt extract[3] or pancreatic extract on a similar starch paste. Both of these ferments liquefy the starch paste with great rapidity, even a strong paste being rendered quite limpid in a few seconds, if kept at about 40° C., or a somewhat higher temperature in the case of the malt extract. As soon as the process of liquefaction is complete, the solution is boiled to prevent further action.

Soluble starch is completely precipitated by ammonium sulphate, by magnesium sulphate, and by sodium sulphate at 33° C., but not by sodium chloride.

Sodium sulphate at ordinary temperatures renders a solution of soluble starch opalescent, but does not cause precipitation unless

[1] Brown and Heron used 3·5 grms. in 100 c.c. Roberts used 1 grm. in 100 c.c.

[2] The following table by E. Lippmann, *Chem. Centralblatt*, 1861, p. 859, is of value in this matter:

Source of the starch.	Temperature of swelling up.	Commencement of gelatinisation.	Perfect gelatinisation.
Rye	45° C.	50° C.	55° C.
Maize	50°	55°	62·5°
Barley	37·5°	57·5°	62·5°
Potato	46°	59°	62·5°
Rice	54°	59°	63°
Wheat	60°	65°	67·5°

[3] Prepared as described in Halliburton, *Essentials of Chem. Physiology*, 1896, p. 123. Digest 10 grammes of powdered malt with 50 c.c. of water at 50° C. for three hours and then filter.

allowed to stand for some days, and then much less than in the case of starch paste itself.

Soluble starch can also be completely precipitated by half saturation with ammonium sulphate if the mixture be allowed to stand for a day or more; this fact is of great use in separating soluble starch from mixtures.

(*c*) **The Erythrodextrins.** In investigating these substances I made use of several solutions. (i) Prepared by acting on starch paste with dilute acids till a purple or red coloration only was obtained with iodine. (ii) Digestions of starch paste with malt extract allowed to act till purple or red was obtained with iodine. In testing such a product with iodine it is necessary to boil a portion in a test tube, cool and then add iodine, since the rapid change which the iodine reaction undergoes in the presence of malt extract is apt to be misleading—*e.g.* a digestion which gives a red reaction with iodine may on testing after boiling and cooling give a purple. (iii) Solutions of commercial erythrodextrin, which as is well known contains unaltered soluble starch and some sugar, although some samples I have obtained have only contained a trace of the former.

In all cases on saturation with either ammonium sulphate or magnesium sulphate, one very readily obtains a fairly copious precipitate, and the filtrate on testing with iodine invariably gives a rich reddish-mahogany coloration without the least trace of blue, and hence in a preliminary account of my experiments read before the Physiological Society[1] I stated that erythrodextrin is not precipitated by neutral salts. Since that time I have been able to separate two precipitates, both of which in solution give reddish colorations with iodine, and hence I now propose to distinguish that erythrodextrin which is not precipitated as erythrodextrinIII.

The precipitate obtained by saturation of any of the above solutions after being well washed with saturated solution of ammonium sulphate or magnesium sulphate, whichever was used in precipitating it, can be readily dissolved in water, especially on gentle heat. Such solutions on cooling gave a very characteristic reaction with iodine, beginning with a pure blue, becoming purple and ultimately dark red as more and more iodine was gradually added. This reaction was obtained no matter how carefully the precipitate was washed, and is therefore not due to contamination with erythrodextrinIII. This precipitate I

[1] *Proc. Physiol. Soc.* King's College, Feb. 1897 (This *Journal*, XXI.).

at first regarded as being composed entirely of soluble starch, especially after discovering that according to Musculus and Grüber[1] soluble starch gives a reaction varying from blue to purple or even port wine colour according to the concentration. However, on comparing the amount of precipitate obtained with ammonium sulphate and magnesium sulphate respectively, when applied to samples of the same solution of the mixed products prepared by any of the three methods above described, I noticed that the amount of precipitate was always less when magnesium sulphate was used than when ammonium sulphate was the salt employed. On saturating the filtrate from a magnesium sulphate saturation with sodium sulphate at 33° C., I obtained a small quantity of a substance which gave a bright red-purple to red-brown coloration with iodine; and which I propose to call erythrodextrinII.

I was able to show that the excess of the ammonium sulphate precipitate over that obtained with magnesium sulphate was at any rate partly due to this substance, in the following way—the precipitate obtained from commercial dextrin by ammonium sulphate was well washed with a saturated solution of that salt, then dissolved in water and saturated with magnesium sulphate, when it was only partly precipitated, the filtrate giving an iodine reaction similar to that of erythrodextrinII., and being precipitated, though not completely, by further saturating with sodium sulphate at 33° C.

The magnesium sulphate precipitate when dissolved in water still gave the typical blue—purple—red sequence of colours when tested with iodine, though more purple than in the case of the ammonium sulphate precipitate when similarly tested.

A little later I found, as previously stated, that soluble starch could be completely precipitated by half-saturation with ammonium sulphate if the mixture were allowed to stand for some hours. Applying this process to the mixed dextrins obtained by any of the above methods, I was able to separate the factor which gave the blue reaction—*i.e.* soluble starch; the filtrate no longer gives blue, no matter how dilute a solution of iodine be used. Applying this process of half-saturation to a solution of the well-washed magnesium sulphate precipitate I obtained with the filtrate a peculiar purplish-red iodine reaction without any preliminary blue. This substance I call erythrodextrinI. It is very readily precipitated on completing the saturation with ammonium sulphate, and also to some extent by the half-saturation, especially if allowed to stand for some days.

[1] Musculus and Grüber. *Zeit. physiol. Chem.* II. S. 177 *et seq.* 1878—1879.

I therefore conclude that between soluble starch and achroodextrin there are a series of erythrodextrins, the earlier ones being precipitated by saturating their solutions with neutral salts, the end member or members not being so precipitated.

(*d*) **The achroodextrins.** This dextrin, or rather collection of dextrins, was prepared by allowing diastase or dilute acid to act on starch paste till beyond the 'achromic point'—that is, until a portion of the digestion after boiling and cooling gave no coloration with iodine. The action was then stopped by boiling in the case of the diastase experiment, by cooling and neutralisation in the case of the dilute acid. The dextrins were then precipitated by alcohol, collected, well washed—then dissolved in water, and boiled on a water bath to expel the alcohol. The solutions were then cooled and tested in the ordinary way by saturation. In all my experiments I obtained a slight cloudiness over the excess of salt in the case of ammonium sulphate, but no trace with any other salt. This cloud ultimately settled down to form a few flocculi. These were collected, washed, and a solution made, but owing to the small quantity obtained, it was difficult to apply tests to it satisfactorily. It seemed to be converted into a reducing substance on boiling with acids, and therefore to be possibly a small portion of the achroodextrin. I endeavoured to discover if it were some accidental impurity. It certainly is not proteid, and the alcohol used for its precipitation was gum-free. One hesitates however to regard it as achroodextrin because of the generally accepted opinion that all the achroodextrins are later products of hydrolysis than the erythrodextrins, and therefore presumably of less molecular weight and less likely to be precipitated.

(*e*) **Comparison with products obtained by other observers.** The difficulties in the investigation of the products of hydrolysis of starch become very evident in the very extensive literature of this subject, which teems with contradictions, varying theories[1] and a bewildering profusion in nomenclature. The greatest amount of recent work has however been done on the later products—the achroodextrins and the reducing sugars—maltose, glucose, and the much criticised isomaltose of Lintner and Düll.

[1] I have omitted any theoretical considerations as to the nature of the process by which the different products are split off and as to their relation to one another chemically, which may be found fully discussed in Brown and Morris, *loc. cit.*; Lintner and Düll, *Berichte d. Deutsch. chem. Gesellschaft*, XXVI. S. 2533, 1893; Ost, *Chemiker Zeitung*, XIX. S. 1504. 1895.

My own work has of necessity been confined to the earlier products owing to the limitation of the activity of the method to those bodies, in which it differs from the alcohol method of separation, and hence the interest of comparing my products with those of other observers.

Soluble starch. A soluble variety of starch has long been known, and is generally regarded as the simple hydrate of starch itself. It is no doubt the chief constituent of the products variously named as follows:—amidulin by Schulze[1]; soluble starch by Béchamp[2], O'Sullivan[3] and Musculus[4]; amylodextrin by W. Nägeli[5], and the products described under the latter name by Lintner and Düll[6] Bülow[7], and others[8]. Probably in most of the earlier observations it was contaminated with erythrodextrins, and hence the discrepancies in the published accounts of its reactions. For this substance I have retained the name soluble starch as being more descriptive, and not committing myself to any hypothesis as to its nature. I have found half-saturation with ammonium sulphate the most convenient method of separating it from the mixed products of hydrolysis. This I have found more convenient and more complete than the method of freezing used by Lintner and Düll.

The dextrins. Among the more important attempts to separate these bodies may be mentioned those of:

Griesmayer[9] in 1871 into dextrin I. and dextrin II.

O'Sullivan[10] in 1872 into α-dextrin and β-dextrin, to which products Brücke[11] in the same year gave the names of erythrodextrin and achroodextrin, because of their reactions with iodine.

In 1878–79 Musculus and Grüber[12] described the following series of products of hydrolysis of starch—soluble starch, erythrodextrin, achroodextrins α, β and γ, and maltose. At the same time they stated that they had not been able to obtain erythrodextrin pure.

[1] Schulze in 1840.

[2] Béchamp. *Comptes rendus*, xxxix. p. 653. 1854.

[3] O'Sullivan. *Journ. Chem. Soc.* xxv. p. 579, 1872; *ibid.* xxxv. p. 770. 1879.

[4] Musculus and Grüber. *Loc. cit.*

[5] W. Nägeli. *Beiträge zur Kenntniss der Starkegruppe.* Leipzig, 1874.

[6] Lintner and Düll. *Loc. cit.*

[7] Bülow. *Pfluger's Archiv.* lxii. S. 131–155. 1895.

[8] According to Brown and Morris the amylodextrin of Walter Nägeli is however quite a distinct substance; at any rate its method of production is a somewhat special one.

[9] Griesmayer. *Annalen* 160, S. 40.

[10] O'Sullivan. *Loc. cit.*

[11] Brücke. *Wien Acad. Ber.* [3] 65, S. 126.

[12] Musculus and Grüber. *Loc. cit.*

In the following year, Musculus and Meyer[1], in a paper on erythrodextrin give it as their opinion that this substance is in reality a mixture of soluble starch and achroodextrin, in varying proportions.

In 1890 Scheibler and Mittelmeier[2] investigated some of the properties of erythrodextrin, especially its phenyl-hydrazine compound, but state that they regard erythrodextrin as a series, and that no method of separation of its members is known.

In 1893 Lintner and Düll[3] in studying the action of diastase on starch described the following series of products, amylodextrin, erythrodextrin, achroodextrin, maltose and isomaltose.

In 1895 the same authors investigating the products of hydrolysis of starch by oxalic acid described the following series:

Amylodextrin, giving a blue reaction with iodine, and corresponding to the soluble starch of other authors.

ErythrodextrinI., giving with iodine a "red-violet" reaction.

ErythrodextrinII *a*, giving with iodine in dilute solution a pure red-brown reaction, blue if concentrated, especially if H_2SO_4 is present. In dilute solution only the red-brown coloration appears. This is of great importance as distinguishing it from soluble starch.

ErythrodextrinII *β*, giving with iodine a pure reddish-brown, even with concentrated iodine in the presence of sulphuric acid.

AchroodextrinsI and II.

Maltose and isomaltose.

The results of my own experiments seem to be strikingly similar to those obtained by Lintner and Düll by the alcoholic method, since my products agree with theirs sufficiently closely in reaction; I have however not been able to obtain sufficient of the second erythrodextrin to get the iodine reaction they describe with it. Most of my own experiments were complete before I was aware of Lintner and Düll's observations and are therefore free from bias on that account.

In 1895 Bülow[4] obtained a soluble starch with somewhat different properties to that described by the last authors, but this is possibly due to the method of preparation he used, viz. heating with potash.

In the same year Ost[5] discussing whether the erythrodextrins

[1] Musculus and Meyer. *Zeit. physiol. Chem.* IV. S. 451. 1880.

[2] Scheibler and Mittelmeier. *Ber. d. Deutsch. chem. Gesellschaft*, 1890, S. 3060.

[3] Lintner and Düll. *Ibid.* XXVI. S. 2523, 1893; *ibid.* XXVIII. 2, S. 1522. 1895.

[4] Bülow. *Loc. cit.*

[5] Ost. *Chemiker Zeitung*, XIX. S. 1504. 1895.

described by Lintner and Düll are pure individuals, says that in his opinion they are purified as far as possible with our present methods, but that he holds the view of Musculus and Meyer as the correct one with regard to erythrodextrins—that they are not chemical individuals, but mixtures of soluble starch and achroodextrins—that is, that all dextrins are achroodextrins.

This view, in the light of my own experiments, seems to me no longer tenable, and I hold it as definitely proved that these are true erythrodextrins. Musculus and Meyer's conclusion was probably due to the fact that their soluble starch was incompletely purified and contained erythrodextrins.

As to whether the products obtained by Lintner and Düll and myself are chemical units, it is impossible to say at present. On theoretical grounds it is highly probable that there is a long series of intermediate dextrins between soluble starch and maltose, and it is quite possible that any or all of the products obtained are themselves mixtures.

I have been unable to investigate the maltodextrin described by Herzfeld[1] and subsequently investigated by Brown and Morris[2]. Lintner and Düll regard it as one of the achroodextrins.

IV. Glycogen.

Solutions of glycogen, usually about 1 % in strength, were made by dissolving finely powdered glycogen in hot water and filtering. The solutions were in all cases strongly opalescent and gave the ordinary glycogen reactions.

Glycogen is completely precipitated at ordinary temperatures by:—

ammonium sulphate,
sodiomagnesic sulphate,
magnesium sulphate.

It is not precipitated by half-saturation with ammonium sulphate, nor by complete saturation with sodium chloride.

It is not precipitated by saturation with sodium sulphate at the ordinary room temperature, nor at 100° C., but is readily and completely precipitated by saturation at 33° C., as the following experiment shows. Some glycogen solution was taken in a flask, and sodium sulphate added to saturation, the solution remained opalescent and no trace

[1] Herzfeld. *Ueber Maltodextrin.* Halle, 1879.
[2] Brown and Morris. *Journ. Chem. Soc.* XLVII. 527. 1885.

of precipitation occurred. It was then put into a water bath and gradually heated to 33° C., more sodium sulphate being added. As more and more salt dissolved, the solution gradually became turbid, and in a very short time large flocculi were thrown down. The mixture was then filtered on a warm-water filter at 33° C.—the filtrate was found to be quite clear, non-opalescent and glycogen free.

In a repetition of the same experiment, instead of filtering it was allowed to stand and cool, some of the sodium sulphate soon crystallised out, and after a very little while the glycogen had all redissolved.

The fact that glycogen can be completely precipitated by salts differentiates it from the erythrodextrins, of which only the earlier ones are precipitable; erythrodextrinIII, which gives the nearest iodine reaction, not being precipitated.

I have found ammonium sulphate a very ready and rapid means of separating glycogen from its solutions, and the precipitate is flocculent and easily filtered off.

V. **Inulin.**

Inulin dissolves readily in water on heating. It first swells up, then dissolves and forms a solution which can be filtered easily. The experiments on this substance are more difficult since there is no colour test for inulin. Inulin itself gives no reduction with Fehling's solution, and one has to test for it by converting it into lævulose by boiling with dilute acid.

Inulin is not precipitated by saturation with sodium chloride, nor by sodium sulphate at 33° C.

Both magnesium sulphate and ammonium sulphate throw down a finely flocculent precipitate from inulin solutions; this precipitate when collected, washed and tested gives the inulin reactions, but the filtrate is not inulin free, so that precipitation is only partial.

Magnesium sulphate acts more readily than ammonium sulphate in precipitating inulin; if however the precipitate be filtered off in the latter case, a still further precipitation occurs on allowing the filtrate to stand for some days, but even then precipitation is not complete.

VI. **The Iodine Reaction.**

At first I used two different solutions of iodine and compared the results obtained—one, a solution of iodine with potassium iodide in water, of a colour rather darker than sherry; the other a solution of

iodine in alcohol, of the same colour. The colorations obtained with the two solutions were very closely similar, except that those with alcoholic iodine are nearly always darker in tint than those obtained with the potassium iodide solution—*e.g.* a solution giving a reddish purple with the latter, would give a deeper purple, or a violet with the former. The potassium iodide solution gives the reaction more readily, so that the colorations described are those obtained with that solution, which I have used much more than the alcoholic solution.

It is very important to use a dilute solution, and to add it carefully drop by drop, not only to be able to appreciate the difference in colour, but also to be able to watch the sequence of colour, when a mixed reaction is obtained; for instance, when soluble starch is present in an erythrodextrin solution. In such a case it is an advantage to use a solution of iodine so weak as to be only faintly tinged, for a reason which will appear a little later. At the outset it was important to determine whether the presence of neutral salts affected the iodine reaction, and therefore control experiments were made with iodine and solutions of the carbohydrates containing more or less of the salts used, and in the case of some of the precipitates the salt was dialysed away and the reaction compared with solutions containing salt. In no case did the presence of salt interfere with the reaction, except that in saturated solutions the iodine compound was frequently precipitated very shortly after its formation. So far from hindering the iodine reaction many neutral salts favour it. I have often found that in salt-free solutions several drops of iodine solution have to be added before any coloration is obtained, while solutions containing salt as a rule give it at once.

Meineke[1] has investigated the action of a large number of neutral salts on the blue reaction of iodine with starch, and finds that it is the exception for them not to favour the reaction—*i.e.* it is easier to obtain the reaction in their presence, sometimes very much easier. He finds that the soluble metallic iodides are the most effectual, then the chlorides, and then the sulphates. He also investigated the influence of the quantity of salt on the reaction, and determined for each salt the proportion which gives the greatest intensity of the reaction. The presence of the salts only very rarely influences the colour of the reaction.

Similarly with glycogen, the presence of salts does not hinder the

[1] Meineke. *Chemiker Zeitung*, XVIII. S. 157. 1894.

reaction; Külz[1] and Vogel state that the coloration with iodine is obtained more readily after the addition of sodium chloride; Nasse[2] also states that the glycogen reaction is increased by sodium chloride, ammonium chloride and sodium acetate.

Concerning the significance and value of the iodine reaction and its use as a means of identification and separation there is considerable difference of opinion.

Mylius[3] regards the so-called "iodides" as definite compounds, and says that a soluble inorganic iodide is a necessity for their production, and that hydriodic acid enters into the molecule of the iodide of starch which he represents as $(C_{24}H_{40}O_{20}I)_4HI$.

Duclaux[4] deprecates the use of iodine as a distinguishing reagent, and quotes Musculus's observations on soluble starch to show that the reaction varies with the concentration. In the light of my observations and those of Lintner and Düll there can be little doubt that the soluble starch of Musculus and Grüber contained some erythrodextrin, which would account for the variations described. On the other hand one must allow that the iodine reactions show considerable variations in colour, according to the solution of iodine used, and to the condition of the iodide compound, whether in solution, or in the form of a precipitate. There is certainly, however, a substance giving a blue coloration with iodine, which is blue under all circumstances; this substance is soluble starch; another gives a mahogany-red with iodine when in solution, which I have called erythrodextrinIII.

The intermediate substances give iodine reactions of less constant and definite colours, and it is certain that they have not been obtained in so pure a condition.

Again, all the iodides can be precipitated by salts, and although their colour in the precipitated condition may be different from that in solution, yet on re-solution they give the same coloration, and present all the properties of the original iodide before precipitation.

The iodine reaction of each substance may now be considered separately.

Starch—gives a blue coloration and precipitate. On heating, the coloration disappears first, the precipitate later. On cooling, the blue coloration only returns unless more iodine is added. If the precipitate

[1] Külz and Vogel. *Zeit. Biologie*, xxxi. S. 108. 1895.
[2] Nasse. *Pflüger's Archiv*, xxxvii. S. 582–606. 1885.
[3] Mylius. *Berichte d. Deutsch. chem. Gesellschaft*, xx. S. 688.
[4] Duclaux. *Ann. Institut Pasteur*, viii. 863–867. 1894.

be filtered off, the soluble iodide can be readily precipitated by salts—especially ammonium sulphate, and by sodium sulphate on standing, and even by sodium chloride; thus the iodide is more easily precipitated than starch itself.

Soluble starch. One obtains a clear, bright blue solution if pure soluble starch be tested with iodine: this can be readily precipitated as a fine blue powder by ammonium sulphate. If soluble starch be present in a mixture with erythrodextrins it seems to have a much greater affinity for the iodine than the latter, which do not give any red coloration till the soluble starch is saturated. If a very weak solution of iodine be added carefully drop by drop to such a mixture, one obtains a pure clear blue at first, even when there is only a very small quantity of soluble starch present, and a very large quantity of erythrodextrin; as more and more iodine is added, the coloration passes through purple or violet to a dark red or to a reddish brown, in which no trace of blue can be detected. It is obviously important to add the iodine very carefully or to use a very dilute solution, otherwise the preliminary blue may be overlooked.

This reaction I have confirmed by mixing small quantities of soluble starch with erythrodextrin solutions. It has been of great value, for if with any product I obtain a mixed reaction beginning with blue, I have at once inferred that it is a mixture of erythrodextrins with more or less soluble starch according to the intensity of the initial blue reaction. This fact was observed by Brown and Heron[1], who state that erythrodextrin has a less affinity for iodine than either soluble starch or achroodextrin; Lintner and Düll also emphasize the great affinity of soluble starch for iodine.

ErythrodextrinI. A solution of this substance was prepared from either commercial erythrodextrin, or a starch digestion by saturating with magnesium sulphate, washing with saturated magnesium sulphate solution, dissolving the precipitate obtained in water, and removing the soluble starch by half-saturation with ammonium sulphate. The filtrate gives a bright reddish purple coloration (almost magenta), without any preliminary blue, no matter how carefully the iodine be added. This iodide is precipitated on saturating with ammonium sulphate as a darker reddish purple precipitate.

ErythrodextrinII. Prepared by saturating the mixed products with magnesium sulphate, filtering off the soluble starch and erythro-

[1] Brown and Heron. *Journ. of Chem. Soc.* xxxv. p. 641. 1879.

dextrinI. so precipitated, and then further saturating with sodium sulphate at 33° C.

A solution of the precipitate so obtained gives a reaction with iodine varying from bright reddish purple to mahogany red. I have only been able to separate small quantities of this substance, and cannot therefore be certain of its purity. It is of course possible that it may be chiefly made up of erythrodextrinI. which has escaped precipitation by the magnesium sulphate, this is however I think improbable as I have saturated with magnesium sulphate as many as three times before using the sodium sulphate.

ErythrodextrinIII. The filtrate after saturation with ammonium sulphate of commercial erythrodextrin, or of a starch digestion giving red or purple with iodine, invariably gives a pure reddish brown (mahogany) coloration with iodine. If the salt be not dialysed away previously, the iodine compound is rapidly precipitated, the following being the sequence of events—the red brown solution becomes opaque, turbid, brownish black or even greenish in tint, and on standing a dark blue precipitate is thrown down. If this be collected on a filter and dried it goes reddish brown again; if the dried substance be then dissolved in water it gives a blue solution, which instantly changes to the red brown characteristic of this erythrodextrin, the solution now behaving as an ordinary solution of iodide of erythrodextrinIII. These variations are probably due to variations in the hydration of the substance.

Glycogen gives the well-known reddish brown or port wine coloration with iodine, very closely similar to that of erythrodextrinIII. It disappears on heating, reappears on cooling, and is readily precipitated by salts, completely by ammonium sulphate at ordinary temperatures and sodium sulphate at 33° C., partially by magnesium sulphate, and not at all by sodium chloride or sodium sulphate in the cold.

VII. **Theoretical considerations on salt precipitation.**

Two chief suggestions have been put forward to endeavour to explain the precipitation of colloids by neutral salts—the first, that a loose compound of the salt and the colloid is formed and that this compound is insoluble.

There seems to be no direct evidence of such a combination, and the well-known facts that the properties of the precipitated substance are not in any degree altered by the precipitation and that it dissolves

in water again as readily as the original substance, and dissolves again on dilution of the solution from which it was precipitated, tend to negative this hypothesis, or at least to indicate that if such a combination occur it is an exceedingly loose one.

The second suggestion, and that most generally adopted by those observers working with neutral salts, is that the precipitation depends on the 'water-attracting power' or 'water-withdrawing power[1]' of the salt used.

The salts are thus supposed to withdraw the means of solution from the colloid, which is hence thrown down, and the process is regarded as analogous to the dehydration of alcohol by potash, and the driving off of dissolved gases by the addition of salts.

As far as I have been able to discover, Nasse[2] first adopted this hypothesis, though he suggested that the former of the two theories was the more likely in the case of carbohydrates.

Pohl[3] in his work on the gums adopted the water-attraction hypothesis, and developed it by suggesting that the power of being precipitated is a function of the size of the colloid molecule, in the sense that bodies which are easily precipitated have a larger molecular weight than those which are precipitated with difficulty or not at all.

The disadvantage of this theory is that the chief method of investigating "water-attraction power" is by studying the precipitating power on colloids, or in other words, we know very little more of the properties of the salt upon which water-attraction power depends than of the process of colloid precipitation itself.

The problem of the salt precipitation of colloids may with advantage be considered from two points of view: first, regarding the colloid solution, and its power of being precipitated, and endeavouring to find any factors associated with it; secondly, regarding the salt and its power of precipitating, which we may provisionally call, water-attracting power.

The nature of colloid solutions is not known, it has been suggested that in them the solvent deals with little masses[4] instead of with molecules as in ordinary solutions; according to another view they are intermediate between emulsions and true solutions. They vary

[1] *Wasserattractionsvermögen*, Nasse; *Wasserentziehende Kraft*, Hofmeister; *Wasseranziehende Kraft*.

[2] Nasse. *Pflüger's Archiv*, XLI. S. 504. 1887.

[3] Pohl. *Loc. cit.*

[4] *Micellar-lösungen* (Nägeli).

widely in their solubility, some like starch forming pastes, or very viscid solutions, if the term solution may be applied to them at all; others like soluble starch, being readily dissolved by gentle heat, yielding clear, non-viscid, non-opalescent solutions, which however are easily supersaturated and deposit the substance on standing or cooling to 0° C. Concerning the relation between this property and the precipitability by salts it seems that the more easily soluble the colloid, the less easily is it precipitated. This is well seen in the increasing difficulty in "salting out" the members of the following series—starch, soluble starch, the erythrodextrins. It also seems highly probable that the capacity of being salted out of solution varies with the molecular weight, in the sense that the greater the molecular weight of the colloid, the more easily is it precipitated.

It is also of interest to determine in this connection whether crystallised proteids differ from the ordinary forms in their relations to neutral salts. Osborne[1] has shown that the crystallised globulins derived from plant tissues, like Brazil nut, castor-oil bean, and hemp seed are not precipitated by saturation with sodium chloride, but are completely precipitated by saturation with ammonium sulphate, and either partially or completely by magnesium sulphate, that is, though not so easily precipitated as ordinary globulins, they are still capable of precipitation. These results were confirmed by Chittenden and Mendel[2] for the phytovitellin of hemp seed.

Concerning the precipitating power or water-attracting power of salts, large numbers of observations have been made, including those of Nasse (*loc. cit.*) and of Halliburton[3], who investigated the action of a large number of salts on the proteids of serum. The most laborious observations are those of Lewith[4] and Hofmeister[5], in the course of an extensive series of experiments into the physical and physiological actions of salts. Hofmeister concludes that salts of monobasic acids possess a smaller water-attracting power than that of dibasic acids and those of higher basicity; and that in the case of monobasic acids it gets less with increasing molecular weight, and the influence exerted by the bases is practically the same for an equivalent of the alkalies, magnesium and calcium.

[1] Osborne. *American Chem. Journ.* xiv. p. 672.

[2] Chittenden and Mendel. This *Journal*, xvii. p. 50. 1894–1895.

[3] Halliburton. This *Journal*, v. 173. 1884.

[4] Lewith. *Archiv f. exper. Path. u. Pharm.* xxiv. S. 1. 1888.

[5] Hofmeister. *Ibid.* xxv. S. 27, 1889; summarised by v. Limbeck, *ibid.* xxv. S. 74. 1889.

My own observations have led chiefly to negative conclusions. There seems to be no relation between power of precipitation and the following factors—water of crystallisation, molecular weight of the salt, and the solubility, as the appended table shows[1].

Precipitating power on starch.	Salt.	Water of crystallisation.	Molecular wt. of anhydrous salt.	Solubility at 15° C.
Complete	Am_2SO_4		132	74·2
Complete	$MgSO_4$	7 H_2O	120	33·8
Partial	Na_2SO_4	10 H_2O	142	13·20 (50·76 at 33° C.)
Partial	K_2SO_4		174	10·3
Nil	NaCl		58·5	35·9
Nil	KCl		74·5	33·4
Nil	NH_4Cl		53·5	35·2
Nil	KI		166	140·2

At present no satisfactory explanation of the process of salt precipitation has been given. It is possible that the osmotic pressures of the dissolved substances may be a factor in the process. Van't Hoff[2] suggests that the osmotic pressure of a dissolved substance may possibly be regarded as the expression of a "water-attracting power," and a similar suggestion is made by Arrhenius[3] with regard to the variations in concentrated solutions.

It is interesting to note that Pfeffer[4] found the osmotic pressures of the colloids dextrin and gum very low in comparison with those of the salts he investigated.

VIII. General Summary.

The general results of my work may be summarised briefly as follows:—

1. The salt precipitation method can be quite as easily applied to the colloid carbohydrates as to proteids.

2. It can be used as a method of separating them from one another, quite as easily as the alcohol and freezing methods, and yielding results

[1] Comey's *Dictionary of Chem. Solubilities*. London, 1896.
[2] Van't Hoff. *Zeit. f. physikal. Chem.* I. S. 485.
[3] Arrhenius. *Ibid.* X. S. 64.
[4] Pfeffer. *Osmotische Untersuchungen*. Leipzig, 1877.

which are interesting to compare with the results obtained by those methods.

3. Ammonium sulphate and magnesium sulphate are the most generally useful salts. Sodium sulphate at 33° C. is a precipitating agent of great power, and is sometimes very convenient.

4. None of the crystalline carbohydrates are precipitated by neutral salts, but crystalline compounds of the sugar and certain salts can be prepared.

5. Starch and soluble starch are both very readily precipitable by salts, and this method affords a ready means of separating them from the later products of hydrolysis.

6. Erythrodextrin is a series of bodies quite distinct from either starch or the achroodextrins, in opposition to the views of Musculus and Meyer, and of Ost.

7. Of the erythrodextrins, one at any rate is not precipitated by salts; the precipitate obtained by saturation of mixed erythrodextrins can be fractionated into two.

8. These three products, which I have distinguished as erythrodextrins I, II, and III, give reactions closely similar to the products obtained by Lintner and Düll by other methods.

9. Achroodextrin gives a very slight precipitate on saturation with ammonium sulphate but it is doubtful if this is actually achroodextrin. At any rate the main bulk of the achroodextrins are not precipitable by salts.

10. Glycogen is readily and completely precipitated by neutral salts, especially by ammonium sulphate, or by sodium sulphate at 33° C. Either of these methods affords a ready means of obtaining it from its solutions, and distinguishing it from that erythrodextrin which gives the most closely similar reaction with iodine (erythrodextrinIII).

11. Inulin is partly precipitated from its solutions by magnesium sulphate and ammonium sulphate, the former being more active in this respect.

12. All the soluble iodine compounds of starch, soluble starch and the erythrodextrins are precipitated by salts as a rule more easily than the original carbohydrate.

13. The colour of the iodine compounds varies with the concentration of the iodine, and with the state of the compound itself whether solid or in solution.

14. Soluble starch gives blue with iodine under all conditions, and has a greater attraction for the iodine than the erythrodextrins, so that

a small trace of it can be detected even in the presence of a large excess of erythrodextrin, if the iodine solution used be weak, and added carefully drop by drop.

15. One of the erythrodextrins (erythrodextrinI,) gives a red-purple coloration with iodine after all the soluble starch has been removed.

16. There is no evidence as to the existence of a combination of the colloid precipitated and the salt precipitating.

17. The colloid precipitating power or "water attracting power" of a salt does not seem to depend on its molecular weight, nor its solubility.

February 12th, 1898.

[*Reprinted from the Journal of Physiology.*
Vol. XXII. No. 5, March 17, 1898.]

HYDROLYSIS OF GLYCOGEN.

By M. CHRISTINE TEBB.

(*From the Physiological Laboratory, King's College, London.*)

Most of the researches which have been carried out on the hydrolysis of glycogen have been directed towards determining the nature of the final product. In the case of hydrolysis produced by acids, the final result is dextrose[1]; in the case of hydrolysis with enzymes[2] there are differences; thus ptyalin, amylopsin and malt diastase produce maltose, mixed with more or less of an isomeric sugar with the same reducing power, called isomaltose; the liver enzyme[3] and that obtained from blood-serum[4] produce dextrose. In the formation of sugar from starch there are intermediate substances, the dextrins. These substances are probably numerous, and their characters are variously described by different investigators. There stand out, however, three principal members of the dextrin group, viz.—amidulin or soluble starch, erythrodextrin and achroodextrin. These dextrins, like the original starch and unlike the final sugars, are precipitable by alcohol; the dextrins which are formed last requiring a higher percentage of alcohol to cause their precipitation.

The question now arises in connection with glycogen whether a similar chain of dextrins exists there also; and of the numerous papers which I have consulted I can only find two which touch on this point. One of these is by Musculus and von Mering[5], the other by Seegen[6]. The former paper describes an achroodextrin which has some reducing power on Fehling's solution; this was obtained by the action of malt diastase and saliva on glycogen prepared from liver by Brücke's

[1] Max Cremer, *Zeitschr. f. Biol.* xxxi. p. 181, 1894. This observer confirms the older workers in finding that the principal end product is dextrose. He also found a small quantity of isomaltose.

[2] For recent work see Külz and Vogel. *Ibid.* p. 108.

[3] Pavy, *Physiology of the Carbohydrates*, 1894.

[4] Bial, *Pflüger's Archiv*, lii. p. 137. 1892.

[5] *Zeitschr. f. physiol. Chem.* ii. p. 403. 1876.

[6] *Pflüger's Archiv*, xix. p. 106. 1879.

method. Seegen acted on glycogen with saliva, pancreatic juice and acids, and in addition to the final result (sugar) obtained two dextrins; one, an achroodextrin which appears when opalescence disappears, and which is changed into sugar by the further action of the ferment or acid; and the other, which he calls dystropo-dextrin, appears after prolonged action, and is not further changed into sugar.

No observer, so far as I can discover, has described an erythrodextrin stage during the transformation of glycogen to sugar.

The colour which erythrodextrin gives with iodine is so much like that which glycogen gives with that reagent that the detection of either in the presence of the other is a matter of difficulty. Some recent work by R. A. Young[1] in this laboratory has however provided a new method of separating the two substances. He has found that whereas glycogen is completely precipitated by saturating its solutions with such neutral salts as sodium sulphate at 33° C. or ammonium sulphate, the final erythrodextrin, obtained by the hydrolysis of starch, remains in solution. I have therefore, at Prof. Halliburton's suggestion, attempted a reinvestigation of the subject. I have acted on glycogen with acids and ferments; I have paid attention to the intermediate dextrins rather than to the final products of the hydrolysis; and in the separation of the dextrins I have used the new method of Young.

Action of dilute acids on glycogen[2].

In these experiments I have used dilute hydrochloric acid and also dilute sulphuric acid. When the action continues for a short time there is evidence of the existence of two members of the dextrin group, which correspond to the substances obtained in the early stages of the hydrolysis of starch; one of these corresponding to soluble starch I propose to call *soluble glycogen*; the other is *erythrodextrin*.

The following experiments will serve as typical of a number performed.

(i) Four grammes of glycogen were dissolved in 200 c.c. of boiling water and 200 c.c. of boiling 2 per cent. hydrochloric acid were added. The opalescence became less at once; and in three minutes the contents of the flask were clear. After rapid cooling the fluid was neutralized with caustic soda; it was still perfectly clear, gave a good red colour

[1] *Proc. Physiol. Soc.* Feb. 13, 1897. This *Journal*, XXI.; also XXII. 1898, p. 401.

[2] The glycogen I used was obtained from mammalian liver.

with iodine and reduced Fehling's solution. After concentration to a small bulk on a water bath at 100° C., the fluid was dialysed in presence of thymol into running water until free from sodium chloride. This took forty-eight hours. A clear, yellowish fluid was left. This was heated with crystals of ammonium sulphate till the latter began to recrystallize; a gummy precipitate then began to separate out, and was collected with a glass rod. The precipitate consists of the substance I propose to call *soluble glycogen*. The fluid, after filtration, was again heated with fresh ammonium sulphate at 100° C., cooled, and filtered so as to ensure entire precipitation of the soluble glycogen. The filtrate was dialysed for two days into running water, in presence of thymol, concentrated, and again dialysed for a day. This removed all the ammonium sulphate. It was once more concentrated, and on the addition of alcohol a white flocculent precipitate came down. This was collected on a filter and dried below 100° C. It consists of an *erythrodextrin*.

The precipitate of soluble glycogen collected on the glass rod in the manner above described was dissolved in water and gave a perfectly clear solution. In order to get rid of as much erythrodextrin as possible, the soluble glycogen was repeatedly (four times) precipitated with ammonium sulphate and redissolved in water. The aqueous solution was then treated with spirit, and the gummy precipitate collected on a glass rod, dissolved in water and dialysed for two days. It was then once more precipitated with strong spirit and the white flocculent precipitate collected and dried below 100° C.

The following table shows the main characters in which the substances under investigation resemble and differ from each other:

	Aqueous solution	Alcohol causes	Saturation with ammonium sulphate causes	Iodine causes
Glycogen	opalescent	a flocculent precipitate	precipitation	A red colour, which disappears on heating and reappears on cooling
Soluble glycogen	clear		precipitation	
Erythrodextrin from glycogen	clear		no precipitation	

(ii) The same result was obtained when sulphuric acid (1 per cent.) was used instead of hydrochloric acid.

(iii) By the more prolonged action of acid on glycogen the clear fluid after the lapse of a certain time (1¼ to 2 hours) ceases to give any colour with iodine, but it nevertheless contains a dextrin, which is precipitable by alcohol, and is therefore an achroodextrin. This confirms the previous statements of Musculus and von Mering and of Seegen. This dextrin is not precipitated by saturation with ammonium sulphate.

Having, by the use of large quantities of glycogen, obtained a good supply of soluble glycogen, erythrodextrin and achroodextrin, I proceeded to investigate the amount of alcohol necessary to precipitate each. In addition to this I performed analogous experiments with the similar intermediate products of the hydrolysis of starch. I obtained at first somewhat irregular results, and found that the cause of the irregularity was variation in the temperature of the room, heat increasing the solubility of the carbohydrate. From June 28th to July 20th I repeated my experiments and worked in a room of which the temperature varied but little, from 65° to 68° F. and may be regarded as fairly constant. The following statement of the amount of alcohol necessary for precipitation is therefore true for this temperature only.

Starch paste[1] begins to get cloudy when 5 per cent. of alcohol is present; it is entirely precipitated when the percentage of spirit is raised to 27.

Soluble starch begins to get cloudy with 12 per cent. of alcohol, and it is entirely precipitated when the percentage of spirit is raised to 60.

Erythrodextrin (Young's Erythrodextrin iii.), prepared from commercial dextrin by Young's ammonium sulphate method, and dialysed free from the salt, begins to be precipitated with 45 per cent. alcohol; most is precipitated when 70 per cent. of alcohol is added; but the last traces do not come down until 90 per cent. of spirit is present.

The following are the numbers obtained in a similar way by experiments with glycogen and its dextrins.

Glycogen. The opalescence of the solution renders the commencement of precipitation rather difficult to observe; but when 35·5 per cent. of alcohol is present the solution is distinctly more cloudy, and when the percentage reaches 37 a precipitate separates out at once. Glycogen is almost entirely precipitated by 50, and completely by 55 per cent. of alcohol.

Soluble Glycogen. The first permanent cloudiness appears when 44 per cent. of alcohol is present, and the substance is entirely precipitated when the percentage is raised to 50.

Erythrodextrin from glycogen. The first permanent cloudiness appears when 44 per cent. of spirit is present; but the substance is not entirely precipitated until the percentage reaches 90, though most of it comes down at 80 per cent.

[1] Starch paste was rendered clear by allowing a 1% solution to stand in a tall vessel 24 hours; the upper portion of the fluid was then found to be quite clear.

Achroodextrin from glycogen begins to be precipitated by about 65 per cent., and is completely precipitated by 90 per cent. of alcohol.

These numbers may be compared in the following table.

Starch and its products	Percentage of alcohol necessary to commence precipitation	Percentage of alcohol necessary to complete precipitation
Starch paste	5	27
Soluble starch	12	60
Erythrodextrin	45	90
Glycogen and its products		
Glycogen	35·5	55
Soluble glycogen	44	50
Erythrodextrin	44	90
Achroodextrin	about 65	90

We see from this table that there is a progressive increase in the amount of alcohol necessary to produce precipitation as the hydrolysis proceeds. The close agreement between the erythrodextrin from starch and that from glycogen is also noteworthy; it would however be unsafe in the present state of our knowledge concerning the constitution of these substances to be certain that the two are identical.

Action of Amylolytic Enzymes (Ptyalin, Amylopsin, Malt-diastase) on Glycogen.

In this part of the investigation I have used dilute saliva prepared in the usual way, Benger's Liquor Pancreaticus[1] and malt-extract[2]. I have performed a large number of experiments and never had any difficulty, when the solution became clear, in detecting and collecting an achroodextrin the properties of which are similar to that obtained by the use of acids; but I have generally failed to obtain any evidence of the formation of either soluble glycogen or erythrodextrin. In fact the liquid ceases to give any iodine reaction before its opalescence entirely disappears[3].

[1] For fresh and active preparations of this Liquor Pancreaticus I am indebted to Mr Benger.

[2] This was made by extracting 10 grammes of ground malt with 50 c.c. of water at 50° C. for 3 hours.

[3] The presence of the proteid in the extracts to some extent masks the iodine reaction; the addition of egg albumin acts similarly; excess of iodine is necessary under these circumstances. But even allowing for this possible source of error the above statement is correct.

I have used glycogen solutions of different degrees of strength; I have varied the amount of enzyme added; I have stopped the process after short and long intervals by boiling. In order to isolate the resulting products I have attempted fractional precipitation by alcohol, and I have used methods of precipitation by ammonium sulphate and sodium sulphate; the use of sodium sulphate involves a little more trouble than the use of ammonium sulphate since the saturation and subsequent filtration must be performed at a temperature of 33°—34° C., the temperature of maximum solubility of the salt; but, as Dr Young pointed out to me, it gives more trustworthy results.

But in nearly all cases the result so far as soluble glycogen and erythrodextrin are concerned has been a negative one. I have sometimes observed a trace of reddish colour on adding iodine after the removal of all glycogen; but this colour was not constantly produced, and the substance to which it was due was certainly not present in sufficient quantity to make further study of it possible.

One is therefore driven to the conclusion that the earlier products of hydrolysis are so rapidly formed and so evanescent that it is not possible at present to isolate the substances in question. In this the action of amylolytic enzymes differs markedly from that of mineral acids.

Dystropo-dextrin. This name was given by Seegen to a variety of achroodextrin which is not further convertible into sugar. I find that dilute acid acts on a solution of glycogen less readily than on starch-paste of the same strength, but in both cases there is ultimately complete disappearance of all substances which are precipitable with alcohol; in other words there is no dystropo-dextrin.

With amylolytic enzymes however it is different. If saliva be added to glycogen, and digestion allowed to go on in a flask for several days, there always remains some dextrin which has not been converted into sugar. In one experiment I allowed 5 grammes of glycogen in 1 per cent. solution to digest 5 days with saliva; the dextrin was then precipitated by alcohol to remove the greater part of the sugar and redissolved in water; it was treated with fresh saliva for 4 days, and after a second precipitation with alcohol, and subsequent solution in water, the dextrin was again digested with saliva during 6 days. Thus the whole period of digestion was 15 days; and yet there was a considerable quantity of dextrin which was not converted into sugar.

The circumstances of the experiment prevented any attempt at accurate estimation of the quantity of dextrin obtained by each

precipitation with alcohol; but I believe that the quantity obtained at the first precipitation (after 4 days of salivary action) was not much if at all greater than that obtained at the end of the experiment.

That this dystropo-dextrin is a different substance from the achroodextrin formed in the earlier stages of hydrolysis is shown by the fact that more alcohol is necessary to precipitate it than is required to precipitate the achroodextrin formed in the earlier stages of hydrolysis. A specimen of the achroodextrin obtained from digestions which had lasted from a few hours to a day began to precipitate in solutions containing from 45 to 50 per cent. of spirit; specimens of the achroodextrin (dystropo-dextrin) obtained from digestions which had lasted for several days did not come down until 64 to 66 per cent. of spirit was present.

Analogous results were obtained with saliva and starch paste. Starch however is acted on by saliva more readily than glycogen, and the second form of achroodextrin (dystropo-dextrin) is produced from starch in a shorter time than is required to cause disappearance of the first variety from digested glycogen. The alcohol precipitation of the first variety commences at 45, and of the second variety at about 68 per cent.

Corresponding results were obtained with pancreatic extract. I have however done most of my work with saliva because it contains so little proteid material.

Throughout all such prolonged experiments thymol or chloroform, usually the latter, was employed to render the mixtures antiseptic.

Action of the Liver Enzyme on Starch and Glycogen.

By extraction with glycerine, Claude Bernard[1] obtained from liver a ferment which converts glycogen into sugar, but he did not describe the properties of the sugar. Miss Eves[2] extracted from liver a ferment which is active on both starch and glycogen, and states that the product of action was certainly not dextrose, but a sugar of less reducing power; she considers it very probable that she was dealing with the ordinary amylolytic ferment (converting starch and glycogen into maltose) obtainable from most tissues of the body.

In some work which I did with Dr L. E. Shore's cooperation in Cambridge in 1893, I used a method which is different from that

[1] *Comptes Rendus*, LXXXV. p. 519. 1877.

[2] This *Journal*, V. p. 342. 1884.

employed by other observers, and was suggested by some experiments of Brown and Heron[1].

A preliminary account of my results has already been published[2]. Pig's liver was rapidly dried at 35°—40° C. and finely shredded, and the sugar present initially or which had formed after death was removed by dialysis. The shredded tissue was then added to solutions of starch and glycogen and digestion allowed to go on at 37° C. for periods varying from 4 to 22 hours. The resulting sugar was removed by extraction with alcohol or by dialysis. Digestion was always carried on in neutral or faintly alkaline media and chloroform was used as an antiseptic. In some specimens of the liver the blood had been washed out previously to drying, but this made no difference in the result. The result in all cases was that dextrose was formed; it was identified by its osazone.

The following may be taken as a typical experiment. Ten grammes of dried liver, free from blood and sugar, were allowed to act on a solution of glycogen for 22 hours. The sugar produced was removed by dialysis, and on adding phenyl hydrazine to a portion of the dialysate, typical crystals of phenyl glucosazone were obtained. The reducing power of another portion was estimated; and after boiling the solution with 2 per cent. hydrochloric acid the reducing power was found to have increased but slightly—in the ratio 65 to 68. This change I attributed to admixture with a small amount of dextrin or possibly maltose. In the light of the experiments I have recently performed, the presence of dextrin seems to be the more probable explanation, especially as I have found dried liver[3] to be active in converting maltose to dextrose.

Extracts of the dried tissue were also made by soaking it in a 5 per cent. solution of sodium sulphate; and these were found to be active in the same way.

The following experiment is typical of several I have performed.

Fifty grammes of dried liver (pig) was soaked at 37° C. in 300 c.c. of 5 per cent. sodium sulphate for 19 hours, when by means of centrifugalization and subsequent filtration a clear yellow fluid was obtained. This was dialysed for two days, when it was found to be free from sugar. Of this extract 50 c.c. were added to 50 c.c. of 4 per cent. glycogen solution and digestion was allowed to go on for 21 hours at 37° C.

[1] *Roy. Soc. Proc.* xxx. p. 393. 1880.

[2] *Proc. Cambridge Philosophical Soc.* VIII. p. 199. 1894.

[3] This *Journal*, xv. p. 426. 1894.

The digested fluid yielded, with phenyl hydrazine, crystals of dextrosazone.

The sugar was separated by concentrating the fluid on a water-bath and treating with rectified spirit. After evaporating off the alcohol and taking up the residue with water, the reducing power of the fluid was determined, and again after boiling with 2 per cent. hydrochloric acid, which showed an increase in the ratio of 4 to 3; hence some of the sugar originally present must have been dextrose.

Since the publication of these experiments, the subject has been brought into prominence by the appearance of Pavy's work on the Physiology of the Carbohydrates. Noël Paton[1] confirms Pavy's statement that the final product is dextrose; and Bial[2] has arrived at the same conclusion. Pavy and Bial are both inclined to the view that an enzyme is responsible for the change. Noël Paton[3], in his most recent contribution to the controversy that has arisen, asserts that no amylolytic enzyme can be extracted from the liver after death. In this connection, I would call attention to the fact just stated that I have found sodium sulphate extracts of the dried liver active in converting glycogen and starch into dextrose.

I also find in my notes (Aug. 21st, 1893) an experiment which confirms Pavy's statement concerning the activity of liver coagulated by alcohol. The note describes a specimen of dog's liver, dried and shredded, and two months and a half later placed under alcohol where it remained for six months. It was at the end of this time found to be active in converting starch and maltose into dextrose.

In the experiments I have been performing lately I find that the livers, which I had dried and bottled five years ago, still produce the same effect so far as the final product (dextrose) is concerned. I have also investigated whether or not dextrins are formed as intermediate products. Using the methods previously described I found distinct evidence of the presence of erythrodextrin in the early stages of hydrolysis of glycogen; but though this substance is present more constantly than in the cases where saliva, pancreatic extract and malt extract are used, its amount is extremely small, so that I was not able to investigate its properties further. Achroodextrin is also formed, but owing to the presence of some proteid it was difficult to determine the percentage of alcohol necessary to cause the commencement of precipitation. Very

[1] Noël Paton. *Philosophical Transactions*, vol. 185, p. 233. 1894.

[2] Bial. *Pflüger's Archiv*, LV. p. 434. 1894.

[3] This *Journal*, XXII. p. 133. 1897.

prolonged digestion of either starch or glycogen by the liver fails to transform the whole of the dextrin into sugar; in other words we have in this instance also distinct evidence of Seegen's dystropo-dextrin.

Summary.

1. In the hydrolysis of glycogen produced by mineral acids, intermediate substances of the dextrin class are formed, which may be termed soluble glycogen, erythrodextrin and achroodextrin. The final sugar formed is dextrose, and prolonged hydrolysis converts all the intermediate dextrins into sugar.

2. The intermediate dextrins may be separated by Young's salt saturation method, which is a valuable means of discriminating between erythrodextrin on the one hand and glycogen and soluble glycogen on the other.

3. In the hydrolysis of glycogen, produced by the amylolytic enzymes of saliva, pancreatic extract and malt extract, the only dextrins which could be separated in amount sufficient to work with subsequently are of the achroo-variety. Though by the use of Young's ammonium sulphate or sodium sulphate method occasional evidence of the presence of a small quantity of erythrodextrin was obtained, the earlier products of the hydrolytic process are as a rule so rapidly changed that it was not found possible to isolate them in amount sufficient for subsequent study.

4. In the case of the liver enzyme, the intermediate dextrins resemble those produced by the amylolytic enzymes, except that a small amount of erythrodextrin is constantly found in the earlier stages of the hydrolysis of glycogen. The liver enzyma differs from ptyalin, amylopsin and malt-diastase in the nature of the final product obtained, which is, chiefly at least, dextrose and not maltose.

5. By the prolonged action of the enzymes enumerated above a form of achroodextrin (called by Seegen, dystropo-dextrin) is produced which resists further conversion into sugar. This requires for the commencement of its precipitation a larger percentage of alcohol than that required for the precipitation of the achroodextrin formed in an early stage of the hydrolytic process.

February 17th, 1898.

[*From the Proceedings of the Physiological Society*, Feb. 12, 1898.]

Preliminary Account of the Effects upon Blood-pressure produced by the intra-venous Injection of Fluids containing Choline, Neurine or allied Products. By F. W. Mott, M.D., F.R.S. and W. D. Halliburton, M.D., F.R.S.

(*Second communication.*)

In the communication on this subject published last year[1], we showed that cerebro-spinal fluid removed from cases of brain atrophy (particularly from cases of general paralysis of the insane) produces a fall of blood-pressure. We further stated that the material in the fluid that produces this effect is not proteid, nor is it of inorganic nature. It is precipitable by phospho-tungstic acid, and therefore probably alkaloidal. We also showed that dilute solutions of choline hydrochloride produce the same effect upon blood-pressure. The related alkaloid neurine produces a different effect, a preliminary fall of pressure being usually followed by a marked rise.

The work we have carried out since then, may be briefly summarised as follows:

1. The substance in the cerebro-spinal fluid which produces the effect is choline. Crystals of the platinum double salt, which are characteristic octahedra, have been prepared from the fluid and were exhibited to the meeting.

2. The fall of blood-pressure which occurs is partly of cardiac origin. There is slowing of the heart, but this is often not very marked, and as tracings with Barnard's cardiometer showed is accompanied with an increased output.

3. The main cause of the fall is vascular dilatation. This was investigated by the use of air-plethysmographs. In the case of the limbs, there is no evidence of active dilatation; the volume of the limb is in fact slightly diminished; this appears to be secondary to the general fall of arterial pressure. The same is true for the kidney; the lever of the kidney oncometer falls with the blood-pressure. In the case of the intestines, however, there is marked vascular dilatation.

[1] *Proc. Physiol. Soc.* Feb. 13, 1897.

The intestine oncometer we used, we owe to the ingenuity of Mr Arthur Edmunds, B.Sc., who has given us a good deal of assistance throughout our work. The instrument was exhibited in action to the meeting, and will shortly be described in a separate paper by Mr Edmunds.

4. Although the principal interest in our work centres round the fact that the toxic material in the specimens of cerebro-spinal fluid, in question, is choline, we have made a good many experiments on neurine as well. The primary fall in the arterial pressure is of cardiac origin; the slowing of the heart and deepening of the respiration are very marked symptoms. Usually this is followed by a rise of pressure due to constriction of peripheral vessels. In some cases this latter phase is absent; and the heart remains permanently slowed and the animal may die. In some few cases using small doses (1 or 2 c.c. of a 0·1 per cent. solution of the hydrochloride) there is only the second phase, a rise of pressure with peripheral constriction of the intestinal and kidney blood-vessels.

5. We have, thanks to the kindness of Dr Patrick Manson, had the opportunity of investigating the action of the blood removed by venesection from a case of Beri-beri. This is a nervous disease, accompanied by great cardiac failure, vascular depression and œdema. A saline solution of the alcoholic extract of the blood produces a marked fall of blood-pressure with cardiac depression, and dilatation of the peripheral vessels of the splanchnic area.

[*From the Proceedings of the Physiological Society*, Feb. 18, 1899.]

Abnormal conditions of the circulatory system of the frog.

By H. Willoughby Lyle, M.B.

In a frog (*Rana temporaria*) which was being used for class experiments, it was found that on removal of the sternum, two pulsating organs were to be seen, and each appeared to be surrounded by its own pericardium. The smaller pulsating organ was situated in the normal position of the heart; the larger one was lying under the floor of the buccal cavity, just behind the right half of the mandibular arch. Contractions of the smaller organ preceded those of the larger, and there was no pause observed between the contractions of the two.

The pericardium was then opened longitudinally, and it was found that what appeared to be two distinct pericardia was really a single cavity with a slight transverse constriction; the pericardial cavity contained a single heart which appeared to consist of four distinct parts lying in the position as indicated in the accompanying figure.

The sinus venosus was a thin-walled sac, round in shape, lying just in front of the liver; it received posteriorly the posterior vena cava, and mid-dorsally a small vein formed by the junction of the right and left pulmonary veins. In front of the sinus venosus was a chamber, oval in shape and with thicker walls than those of the sinus venosus; the colour and shape of this accessory chamber resembled that of the ventricle; the sinus venosus opened into it on its posterior aspect, and it opened into the auricles anteriorly; there were no blood-vessels connected with this chamber. When this accessory chamber was opened, an incomplete septum was discovered running in a longitudinal direction. The next chambers in order were the auricles; externally there was no sign of a depression to indicate that there were two; on dissection, however, an incomplete interauricular septum was found which was continuous with the septum in the preceding chamber. The walls of the auricles were normal in thinness; opening into the right auricle was the right anterior vena cava, and opening into the left auricle was the left anterior vena cava. The two auricles opened into the single ventricle which lay with its apex pointing forwards, and to the right towards the right half of the mandibular arch. The truncus arteriosus came off from the left side of the ventricle near the left part of the auriculo-ventricular septum, it ran forwards and

towards the middle line where it divided into right and left branches as usual, and these again into the three aortic arches, the carotid, the systemic and the pulmocutaneous. The slight external constriction in the pericardium corresponded with the junction of the accessory chamber and the auricles; there was, however, no constricting band pressing upon any part of the heart. The larger part of the heart was lying forwards and to the right, beneath the buccal cavity.

The lungs, the pulmonary arteries and veins appeared to be smaller than usual; on the other hand the cutaneous arteries and veins were large and engorged with blood, and it appeared as if the main part of the respiratory process was carried on by the skin.

The anterior abdominal vein was formed normally by the union of the two pelvic veins, and divided as usual into right and left branches to enter the right and left lobes of the liver respectively. The renal portal veins were also formed in the usual manner, and the left one entered the outer side of the left kidney, but the right one divided into three branches, one to the outer side of the right kidney, a second branch crossed the kidney dorsally, and the third branch crossed the kidney ventrally; both these latter opened straight into the posterior vena cava.

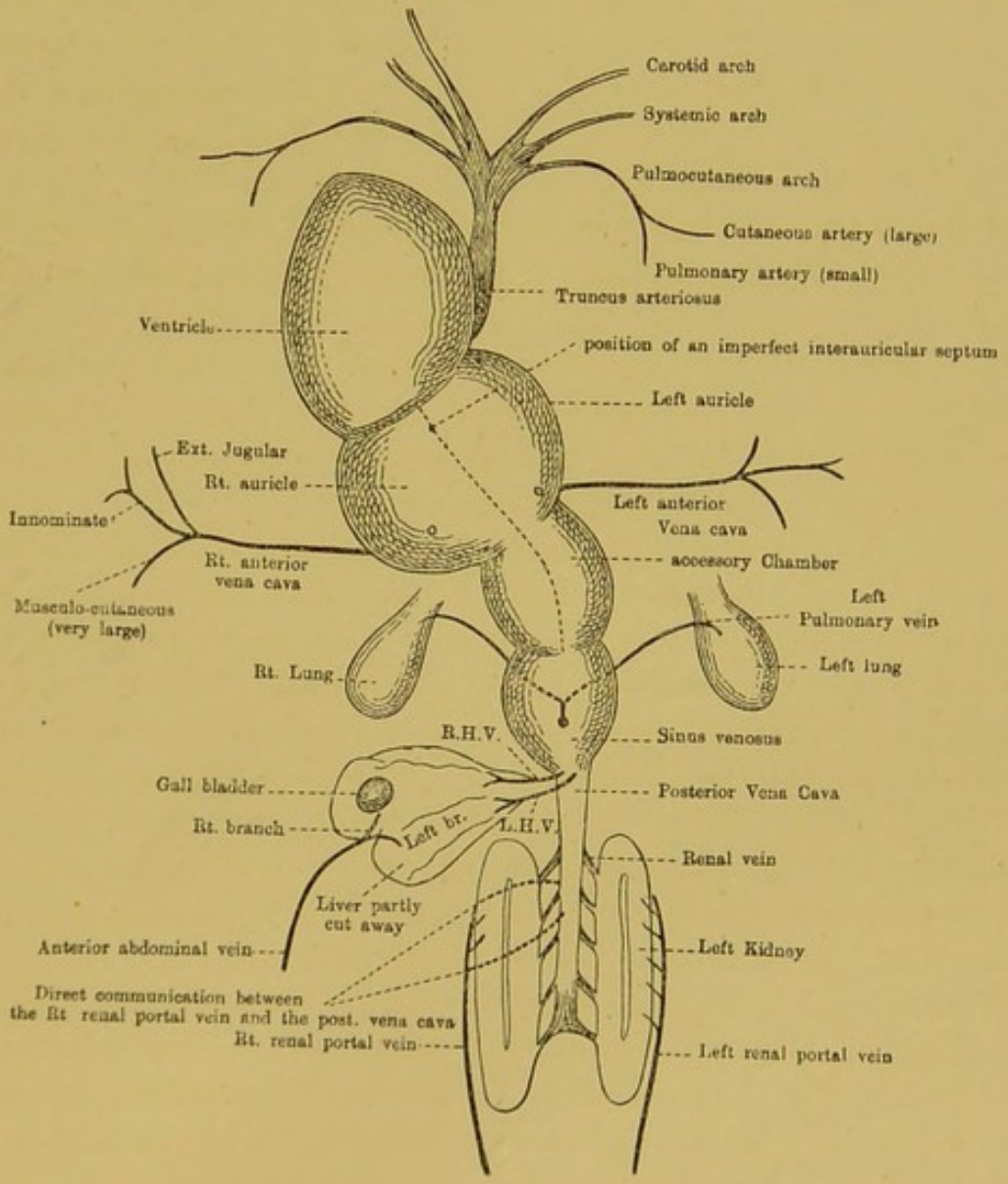
The other abnormal conditions observed were, that the head was broader than usual, probably due to the fact that it contained the larger portion of the heart, the right testis was normal in size, but the left one was only about a quarter of its proper size.

When the specimen was fresh, the contractions of the heart were observed to commence at the sinus venosus and travel in a peristaltic manner over the accessory chamber, auricles and ventricle, the contractions of the various parts following each other without any pause, but after the frog had been kept in a moist chamber for 24 hours, it was observed that the sinus venosus and accessory chamber contracted five or six times before the auricles would contract at all, and it appeared as if the accessory chamber were filling up the auricles so that an auricular systole might be brought about; when, however, they did contract, the ventricular systole rapidly followed.

A clamp was introduced between the sinus venosus and the accessory chamber, with the result that the sinus venosus continued to contract, but the accessory chamber, the auricles and ventricle rested. A second clamp was then introduced between the two auricles and the ventricle, with the result that the accessory chamber and auricles still rested, but the ventricle commenced to contract rhythmically.

The accessory chamber presented the appearance of a ventricle in shape, thickness of walls and in colour; indeed before the dissection was performed, the heart appeared to be a double one. Professor Hughes of King's College, to whom I showed the specimen, suggested that there may have been some transverse band pressing upon the walls of the auricles, and that the accessory chamber was simply a portion of the two auricles with hypertrophied walls to overcome the obstruction; there were no signs, however, of any fibrous band in the pericardial cavity, and the slight constriction seen externally in no way involved the heart.

Another possible suggestion is that during development the two primitive hearts had united lengthwise instead of side by side.



[*From the Proceedings of the Physiological Society*, Feb. 18, 1899.]

Preliminary account of the physiological action of choline and neurine. By F. W. Mott, M.D., F.R.S. and W. D. Halliburton, M.D., F.R.S.

(Third Communication.)

In the two communications which precede this[1], we have shown that cerebro-spinal fluid from cases of General Paralysis of the Insane contains choline, and that the fall of arterial blood pressure that takes place when the fluid is injected into animals is due to this substance. This base is absent from normal cerebro-spinal fluid, and is doubtless in the pathological fluid derived from the disintegration of lecithin in the cerebral tissue. The proof that the base is choline rests partly on its chemical identification in the fluid, and partly on the identical action which the fluid has with weak solutions (0·2 per cent.) of choline or choline hydrochloride.

The closely related and much more toxic base neurine is absent.

In the case of choline, the fall of blood pressure is produced by vascular dilatation especially in the intestinal area. Contrary to expectation the spleen does not participate in this dilatation, but is constricted; this constriction is followed by an increase of the normal splenic waves. It seems probable, that the material in extracts of brain which Schäfer and Moore[2] found to produce the same effect is choline. Neurine produces a much more intense constriction of the spleen, but no exaggeration of the splenic waves follows. The action of the base on the intestinal blood vessels is due to its action on the neuro-muscular mechanism of the blood vessels themselves. This was demonstrated by locally bathing the mesenteric vessels with solutions of choline; and by the fact that choline still continues to produce the usual fall of arterial pressure, (1) after the spinal cord has been divided high up; (2) after

[1] *Proc. Phys. Soc.* Feb. 13, 1897; Feb. 12, 1898.

[2] *Journ. of Physiology*, xx. p. 26. 1896.

the splanchnic nerves have been cut; and (3) after the animal has been poisoned with nicotine; the last method excludes any action of peripheral ganglia.

Neurine produces a fall of blood pressure (chiefly due to its action on the heart): this is followed by a rise of pressure due to constriction of peripheral vessels. Using the same methods as in the investigation of choline, this is not an action on the central nervous system. The constriction of the vessels is, however, probably due to the action of the base on the peripheral ganglia, for after nicotine poisoning it does not occur.

The animals used have been dogs, cats, and rabbits. These were always anæsthetised with ether, chloroform or A.C.E. mixture; in some cases they also had a subcutaneous injection of morphine. If, however, a small amount of atropine is mixed with the morphine, the effect of choline is always a rise of blood pressure; the lever of the intestinal oncometer also rises. We are not at present prepared to offer an explanation of this fact; it is not, however, without importance as showing how one poison may modify the action of another, and its bearing on General Paralysis will be pointed out in our full paper which we hope to publish shortly.

[*From the Proceedings of the Physiological Society*, Feb. 18, 1899.]

Chemistry of reticular tissue. By M. Christine Tebb.

The fibres of reticular or retiform tissue are anatomically continuous with, and histologically identical with, the white fibres of connective tissue. According to Siegfried they are however chemically different, for they yield not only gelatin, but also a new substance he has named reticulin.

On the supposition that Siegfried's statement is correct, the answer to the difficulty appeared to be, that reticulin is not specially characteristic of reticular fibres, but is present in all white connective tissue fibres. Accordingly search was made for reticulin in tendon; the method used was the same as that adopted by Siegfried in his investigation of the reticular tissue of the intestinal mucous membrane. The result was entirely negative.

The only other suppositions that remained were either that reticular fibres are chemically different from white connective tissue fibres, or that the new substance reticulin does not exist. So far as the research has at present proceeded, the second of these two suppositions appears to be the correct one. A careful examination of the reticular tissue of the intestinal mucous membrane has been made, and up to the present no reticulin has been obtained. Full details will be published later.

From the PROCEEDINGS OF THE ROYAL SOCIETY, VOL. 64.

OBSERVATIONS ON THE CEREBRO-SPINAL FLUID IN THE HUMAN SUBJECT.

BY

ST. CLAIR THOMSON, M.D.,

LEONARD HILL, M.B.,

AND

W. D. HALLIBURTON, M.D., F.R.S.

[*From the* Proceedings of the Royal Society, Vol. 64.]

"Observations on the Cerebro-Spinal Fluid in the Human Subject."
By StClair Thomson, M.D., Leonard Hill, M.B., and W. D. Halliburton, M.D., F.R.S. Received January 31,—Read February 16, 1899.

One of us (StC. T.) has had under his care for some years a young woman who has suffered from continuous dripping from the nose. The case has not been amenable to any treatment. At first it was thought to be one of nasal hydrorrhœa, but certain characters in the affection convinced the observer that this could not be so, and that the fluid, which dropped from one nostril only, was cerebro-spinal fluid. This was supported by the results of the chemical examination of the fluid. The escape of cerebro-spinal fluid from the nose has long been known to follow traumatic injury to the cribriform plate of the ethmoid bone, but the possibility of its spontaneous escape from the nose does not

appear to have been fully established before the present instance. However, considerable research into the literature of the subject has shown that there are several cases recorded in which, though no history of injury existed, the flow of fluid from the nose was of such a character that they must have been similar to the present case, although in the majority of instances the true nature of the fluid escaped observation.

Many of these patients exhibited cerebral symptoms in the course of the disease, and some ultimately died from inflammation of the cerebral meninges, which had probably spread from the nose through some opening in the bony lamina that normally separates the cranial and nasal cavities. The full clinical details of this rare case and of the similar ones just referred to are, however, reserved for publication elsewhere. The present paper is concerned only with the composition of the fluid and the variations it presents under different circumstances.

Characters of the Fluid.

Our opportunities for examining the fluid chemically have been fairly frequent. The fluid was always collected in sterilised glass vessels, and the examination made as soon as possible by one of us (W. D. H.) at King's College, London.

The fluid is perfectly clear and colourless, looking like water; its reaction is faintly alkaline; its specific gravity is about 1005. On microscopic examination it shows no cells or other deposit. It gives no precipitate with acetic acid. It contains a trace of proteid, coagulable by heat, but the quantity is too small to give more than an opalescence. In another portion of the fluid it was ascertained that this proteid is precipitable by saturation with magnesium sulphate; it is therefore a globulin. Albumin and other proteids are absent.

The fluid contains a substance which reduces Fehling's solution. A portion of the fluid was treated with excess of acidified alcohol; the proteid so precipitated was filtered off. The filtrate was evaporated to dryness over a water bath; the dry residue was taken up with alcohol, filtered, and again evaporated to dryness. Part was evaporated to dryness on a glass slide; the residue examined microscopically was seen to contain the needle-like crystals, occurring singly and in bundles, similar to those previously described and figured by one of us (W. D. H.)* as obtainable from cerebro-spinal fluid. The residue had also the characteristic pungent taste of pyrocatechin.

The remainder of the dry residue was dissolved in water and filtered. The filtrate reduces Fehling's solution well, but it does not ferment with yeast, nor does it give any osazone crystals on treatment with phenylhydrazine hydrochloride and sodium acetate. Control experiments with a weak solution of dextrose, which gave about the same

* 'Journ. of Physiol.,' vol. 10, p. 248.

amount of reduction with Fehling's solution, gave both these tests in a typical way.

The fluid was tested for creatinine with negative results.

The same results relative to the reducing substance have been obtained over and over again in various specimens of this fluid. They agree with the observations of nearly all writers on the cerebro-spinal fluid, but differ from those of Nawratski,* who in a recent paper has affirmed, principally from observations on the cerebro-spinal fluid of the calf, that the reducing substance present is dextrose.

The principal points to be noticed in the properties of the fluid which lead to the conclusion that it is cerebro-spinal fluid are the following:—

(1) Its clear, watery character.
(2) Its low specific gravity.
(3) The small amount of proteid in it and the absence of albumin.
(4) The presence in it of a substance which reduces Fehling's solution, but is not dextrose. It is possibly a substance related to pyrocatechin.

In comparison with this fluid, we examined also the secretion in some cases of true nasal hydrorrhœa. This fluid is opalescent, somewhat viscid, and on microscopic examination shows amorphous matter with mucous corpuscles. It gives with acetic acid a precipitate of mucinoid nature. It sometimes does and sometimes does not contain a reducing substance, and this substance when present is sugar.

A quantitative analysis of one of these nasal fluids showed that the percentage of solids, especially organic solids, is higher than in cerebro-spinal fluid. The results of the analysis are as follows:—

	Per cent.
Water	98·792
Total solids	1·208
Proteids (including mucin).....	0·260
Other organic substances	0·163
Inorganic substances	0·785

Our observations on the characters of the cerebro-spinal fluid were followed by others in which we sought to answer the following questions:—

The rate of flow.
The difference of composition at different times of the day.
The influence of straining, posture, and abdominal compression on the flow and composition of the fluid.
The effect on blood pressure of intra-venous injection of the fluid in animals.

* 'Zeits. f. Physiol. Chem.,' 1897, vol. 23, p. 532.

The Rate of Flow.

One portion, collected by the patient herself in the course of an hour, measured 4 c.c. Another portion, collected under the supervision of one of us (StC. T.) in ten minutes, measured 3·9 c.c.

If the first portion is taken as a measure of the rate of secretion, the amount formed in the day will be 96 c.c. Taking, however, the second observation as being more accurate, the amount formed in the twenty-four hours will be over half a litre (561·6 c.c.). It is possible that this estimate is too high, as doubtless the patient, being under the observation of a physician, would be somewhat excited, and the consequent alteration of the circulation would, as we shall immediately see, cause the flow to become more abundant.

Comparison of the Morning and Evening Fluid.

Cavazzani,* from experiments on dogs, found that the cerebro-spinal fluid collected in the morning was more alkaline than in the evening, and contained more solid residue. He considers that this is related to the activity of the nervous system, and that it confirms Obersteiner's theory of sleep. He obtained corresponding results in the case of a man with traumatic fistula of the frontal bone.

We considered it worth while to repeat this observation.

The qualitative examination of the fluid collected first thing on several mornings gave the same results as that of specimens collected the last thing in the evening. Both were distinctly alkaline, but no estimation of the relative alkalinity was made. The following table gives in percentages the results of the quantitative analyses :—

	Morning fluid.	Evening fluid.
Water	99·004	99·027
Solids	0·996	0·973
Organic solids	0·118	0·100
Inorganic solids ...	0·878	0·873

The evening fluid is thus slightly poorer in both classes of constituents than that of the morning; the difference is chiefly due to an alteration in the organic solids. This is just what we should expect, as the decreased capillary pressure during sleep would lessen the rate of exudation of water. Without committing ourselves to any theory on nervous activity or sleep, we may say that our experiments confirm those of Cavazzani.

* "Sul Liquido Cerebro-spinale," 'La Riforma Medica,' Anno VIII, 1892, vol. 2, p. 591.

The Influence of Straining and Posture on the Flow and Composition of the Fluid.

In a monograph on the cerebral circulation* one of us (L. H.) put forward the view that the rate of secretion of the cerebro-spinal fluid, when the cranio-vertebral cavity is opened, depends directly on the difference between the pressure in the cerebral capillaries and that of the atmosphere. At the same time it was shown that cerebral capillary pressure varies directly and absolutely with vena cava pressure. Thus the cerebral capillary pressure can be raised with great ease by any agency which causes a rise of pressure in the vena cava or cerebral veins. On the other hand, cerebral capillary pressure varies directly, but only proportionately, with aortic pressure, for between the aorta and the capillaries there lies the peripheral resistance.

It follows from the above that the easiest methods of raising the cerebral capillary pressure in man are :—

(*a*) By compression of the abdomen.
(*b*) By the assumption of the horizontal posture. In this position, however, the rise of venous pressure may be compensated by the fall of arterial pressure, which normally occurs when the body is at rest. This is, no doubt, the case during sleep.
(*c*) By straining or forced expiratory effort, with the glottis closed.

By all these methods the vena cava pressure is considerably raised ; and by the last method the venous inlets into the thorax may be completely blocked, and the pressure in the cerebral capillaries raised to something like aortic pressure.

It is true that by such a forced expiratory effort the aortic pressure is lowered. Nevertheless, the total effect on capillary pressure is a very great rise, for a fall of aortic pressure of 25 mm. of mercury produces a fall in cerebral capillary pressure of less than 5 mm. of mercury, while a rise of vena cava pressure of 25 mm. of mercury produces a rise of cerebral capillary pressure of 25 mm. Hg.

The present case gave us a unique opportunity of testing the correctness of these views on the living human subject, and our experiments entirely confirm them. As will be seen from the following figures, the flow of cerebro-spinal fluid is accelerated by all those circumstances which raise the cerebral capillary pressure. The increase in flow is, moreover, accompanied by a decrease in the percentage of solid matter.

The experiments were conducted under the supervision of two of us (StC. T. and L. H.); the chemical investigation of the fluid was performed, as before, by the third (W. D. H.).

* 'The Physiology and Pathology of the Cerebral Circulation,' by Leonard Hill, London, Messrs. Churchill, 1896.

1. Patient sitting quietly without straining. In five minutes 23 minims (1·357 c.c.) were collected.

2. Patient sitting and straining. In five minutes 35 minims (1·965 c.c.) were collected.

3. Patient sitting quietly. In five successive minutes the amounts collected were, respectively, 8, 7, 5, 5, 5 drops. The total measured 19 minims (1·021 c.c.).

4. Subsequent to this, five minutes were occupied by the patient in straining, and the amounts collected in consecutive minutes were 12, 10, 8, 9, and 10 drops respectively. The total measured 33 minims (1·947 c.c.).

5. Patient lying down and not straining. The drops fell as follows in five consecutive minutes—9, 6, 5, 5, and 5, and the total measured 27 minims (1·593 c.c.). Here the arterial pressure was probably not decreased owing to mental excitement, while the cerebral venous pressure was increased.

6. Patient lying flat on the stomach and head hanging over the end of a sofa. The drops fell as follows in five consecutive minutes, 8, 7, 6, 7, and 7. The total measured 28 minims (1·652 c.c.).

7. Finally, after the last experiment, the following was collected during quiet dropping, while the patient was sitting with the head forward. The drops fell as follows:—5, 4, 4, 4, and 4, in five successive minutes; and the total measured 15 minims (0·885 c.c.).

The following is the report on the chemical examination of the fluids:—

So far as the small quantities available admit of analysis, the fluids are the same qualitatively. The liquid which escaped passively, and that which passed under straining, both contained a small quantity of organic and inorganic solids. Among the organic substances present are the reducing substance and a trace of proteid. Judged by the amount of precipitate produced by alcohol in equal amounts of the two fluids, the proteid is less abundant in the fluid passed during straining, but the amount is too small to weigh.

Determination of the total solids gave the following results, expressed in percentages:—

A. The fluid passed passively, 1·1 per cent.

B. The fluid passed during straining, 0·43 per cent.

Even the higher of these numbers is less than in cases of cerebro-spinal fluid from meningocele and hydrocephalus, previously recorded by one of us (W. D. H.).*

In addition to the foregoing, two specimens were collected at home by the patient herself. Analysis of these gave the following results:—

A. Fluid collected while patient was sitting upright quietly. The percentage of solids was 1·11.

* 'Journ. of Physiol.,' vol. 10, p. 232.

B. Fluid collected while she was lying down. The percentage of solids was 1·03.

The effect of the horizontal posture is in the same direction, though not so marked as the effect of straining. This is what was to be expected, for the horizontal posture would not raise the venous, and thus the cerebral, capillary pressure so much as powerful expiratory efforts would. Moreover, the arterial pressure falls during quiet rest in the recumbent posture, as one of us has determined (L. H.).*

In order to note the effects of straining on the retinal circulation, Mr. Vernon Cargill was asked to examine the patient, and he kindly reported as follows:—"I noticed that when a straining effort was mads, a decided but transitory narrowing of the retinal arteries on and adjacent to the disc, and also a marked pulsation in the trunks of the retinal veins occurred."

The transitory narrowing of the arteries points to the temporary lowering of the aortic pressure, while the pulsation of the veins is a sign of the capillary engorgement due to venous congestion.

Experiments made with Abdominal Compression.

These experiments were made in order to complete and confirm those just recorded. The patient was seated, and the abdomen was compressed as firmly and evenly as possible by one of us (StC. T.), spreading both hands over the front of the abdomen. The number of drops per minute were counted as before, and periods of compression lasting five minutes were alternated with periods of the same duration, during which the patient was sitting quietly.

The following table gives the results succinctly:—

Condition of patient.	Drops in successive minutes.	Total collected.	
		Minims.	c.c.
A. Abdomen compressed.....	11, 9, 8, 7, 5	27	1·593
B. Sitting quietly.....	4, 5, 3, 4, 4	14	0·826
C. Abdomen compressed.....	11, 8, 8, 6, 6	24	1·416
D. Sitting quietly.....	6, 7, 8, 6, 6	Measurement omitted	

The fluids from experiments "A" and "C" were mixed together; also those from experiments "B" and "D." Determination of the total solids gave the following results:—

"A" and "C." Fluid collected during abdominal compression. Percentage of solids, 0·68.

"B" and "D." Fluid collected while the patient was sitting upright quietly. Percentage of solids, 1·14.

* 'Phys. Soc. Proc.,' January 15, 1898.

The experiments confirm those recorded in the preceding section. Abdominal compression raises the vena cava pressure, and so leads to increased cerebral capillary pressure, and in this way to increase in the volume of the cerebro-spinal fluid secreted. Increase of volume, as before, is accompanied with fall in the percentage of solids present.

Intra-vascular Injection of the Cerebro-spinal Fluid.

One of us (W. D. H.), in conjunction with Dr. Mott, F.R.S., has been for some time engaged in examining the results of injecting into animals cerebro-spinal fluid removed from cases of brain atrophy, especially from cases of general paralysis of the insane. This fluid contains a toxic substance, choline, doubtless derived from the disintegration of lecithin in the brain. Injection of such fluid into the jugular vein of animals (dogs, cats, rabbits), anæsthetised with ether, causes a marked lowering of arterial blood pressure, which is partly cardiac in origin, but principally due to the local action of the poison on the neuro-muscular apparatus of the peripheral vessels, especially in the splanchnic area.*

The fluid obtained from the present case was also injected in a similar way. Quantities varying from 7 to 10 c.c. were injected into the circulation in dogs, but with entirely negative results. Such a quantity in the case of fluid from a general paralytic would be quite sufficient to cause a marked fall of arterial pressure.

Similar negative results, both as regards blood pressure and respiration, were obtained with other specimens of normal cerebro-spinal fluid removed from other animals, or from cases of meningocele and hydrocephalus in children. In all such cases, also, choline was searched for chemically, but with negative results.

* 'Physiol. Soc. Proc.,' Feb., 1897, and Feb., 1898 ('Journ. of Physiol.,' vols. 21 and 22).

HARRISON AND SONS, PRINTERS IN ORDINARY TO HER MAJESTY, ST. MARTIN'S LANE.

From the PROCEEDINGS OF THE ROYAL SOCIETY, VOL. 64.

THE PHYSIOLOGICAL ACTION OF CHOLINE AND NEURINE.

BY

F. W. MOTT, M.D. F.R.S.,

AND

W. D. HALLIBURTON, M.D., F.R.S.

[*From the* PROCEEDINGS OF THE ROYAL SOCIETY, Vol. 65.]

"The Physiological Action of Choline and Neurine."

By F. W. MOTT, M.D., F.R.S., and W. D. HALLIBURTON, M.D., F.R.S.

Received March 13,—Read April 20, 1899.

(Abstract.)

The cerebro-spinal fluid removed from cases of brain atrophy, particularly from cases of General Paralysis of the Insane, produces when injected into the circulation of anæsthetised animals (dogs, cats, rabbits), a fall of arterial blood pressure, with little or no effect on respiration. This pathological fluid is richer in proteid matter than the normal fluid, and among the proteids, nucleo-proteid is present. The fall of blood pressure, is, however, due not to proteid, nor to inorganic constituents, but to an organic substance, which is soluble in alcohol. This substance is precipitable by phospho-tungstic acid, and by chemical methods was identified as choline. The crystals of the platinum double salt, which, when crystallised from 15 per cent. alcohol, are characteristic octahedra, form the most convenient test for the separation and identification of this base.

The nucleo-proteid and choline doubtless originate from the disintegration of the brain tissue, and their presence indicates that possibly some of the symptoms of General Paralysis may be due to auto-intoxication; these substances pass into the blood, for the cerebro-spinal fluid functions as the lymph of the central nervous system. We have identified choline in the blood removed by venesection from these patients during the convulsive seizures which form a prominent symptom in the disease.

Normal cerebro-spinal fluid does not contain nucleo-proteid or choline, or if these substances are present, their amount is so small that they cannot be identified. Normal cerebro-spinal fluid produces no effect on arterial pressure; neither does the alcoholic extract of normal blood or of ordinary dropsical effusions.

The presence of choline in the pathological fluids will not explain the symptoms of General Paralysis; for instance, it will not account for the fits just referred to. Its presence, however, is an indication that an acute disintegration of the cerebral tissues has occurred. If other poisonous substances are also present, they have still to be discovered.

Our proof that the toxic material we have specially worked with is choline, rests not only on chemical tests, but also on the evidence afforded by physiological experiments; the action of the cerebro-spinal substance exactly resembles that of choline. Neurine, an alkaloid closely related to choline, is not present in the fluid; its toxic action is much more powerful, and its effects differ considerably from those of choline.

Physiological Action of Choline.

The doses employed were from 1 to 10 c.c. of a 0·2 per cent. solution, either of choline or of its hydrochloride. These were injected intravenously.

The fall of blood pressure is in some measure due to its action on the heart, but is mainly produced by dilatation of the peripheral vessels, especially in the intestinal area. This was dmonstrated by the use of an intestinal oncometer. The limbs and kidneys are somewhat lessened in volume; this appears to be a passive effect, secondary to the fall in general blood pressure. The drug causes a marked contraction of the spleen, followed by an exaggeration of the normal curves, due to the alternate systole and diastole of that organ.

The action on the splanchnic vessels is due to the direct action of the base on the neuro-muscular mechanism of the blood vessels themselves; for after the influence of the central nervous system has been removed by section of the spinal cord, or of the splanchnic nerves, choline still causes the typical fall of blood pressure. The action of peripheral ganglia was in other experiments excluded by poisoning the animal previously with nicotine.

Section of the vagi produces no effect on the results of injecting choline.

We have obtained no evidence of any direct action of the base on the cerebral vessels.

Choline has little or no action on nerve trunks, as tested by their electrical response to stimulation. This aspect of the subject has been taken up by Dr. Waller and Miss Sowton, who will publish their results fully in a separate paper.

Choline has no effect on respiration.

The effect of choline soon passes off, and the blood pressure returns to its previous level. This is due partly to the great dilution of the substance injected by the whole volume of the blood, and may be partly due to the excretion of the alkaloid, or to its being broken up into simpler substances by metabolic processes. We could not find it in the urine.

If the animal has been previously anæsthetised with a mixture of morphine and atropine, the effect produced by choline is a rise of arterial pressure, accompanied by a rise of the lever of the intestinal oncometer. Other anæsthetics cause no change in the usual results. We consider this observation of some importance, for it shows how the action of one poison may be modified by the presence of another. This has some bearing on General Paralysis, for the arterial tension in that disease is usually high, not low, as it would be if choline were the only toxic agent at work.

Physiological Action of Neurine.

The doses employed varied from 1 to 5 c.c. of a 0·1 per cent. solution. These were injected intravenously.

Neurine produces a fall of arterial pressure, followed by a marked rise, and a subsequent fall to the normal level. Sometimes, especially with small doses, the preliminary fall may be absent. Sometimes, especially with large doses, by which presumably the heart is more profoundly affected, the rise is absent.

The effect of neurine on the heart of both frog and mammal is much more marked than is the case with choline; in the case of both choline and neurine, the action on the frog's heart is antagonised by atropine.

The slowing and weakening of the heart appear to account for the preliminary fall of blood pressure; in some cases this is apparently combined with a direct dilating influence on the peripheral vessels.

The rise of blood pressure which occurs after the fall, is due to the constriction of the peripheral vessels, evidence of which we have obtained by the use of oncometers for intestine, spleen, and kidney.

After the influence of the central nervous system has been removed by section of the spinal cord, or of the splanchnic nerves, neurine still produces its typical effects.

After, however, the action of peripheral ganglia has been cut off by the use of nicotine, neurine produces only a fall of blood pressure. It therefore appears that the constriction of the vessels is due to the action of the drug on the ganglia; in this, it would agree with nicotine, coniine, and piperidine.

Section of the vagi produces no influence on the results of injecting neurine.

In animals anæsthetised with morphine and atropine, injection of neurine causes only a rise of blood pressure, which is accompanied with constriction of peripheral vessels.

Neurine produces no direct results, so far as we could ascertain, on the cerebral blood vessels.

Neurine is intensely toxic to nerve-trunks (Dr. Waller and Miss Sowton).

It produces a marked effect on the respiration. This is first greatly increased, but with each successive dose the effect is less, and ultimately the respiration becomes weaker, and ceases altogether. The animal can still be kept alive by artificial respiration.

The exacerbation of respiratory movements will not account for the rise of arterial pressure; the two events are usually not synchronous, and an intense rise of arterial pressure (due, as previously stated, to contraction of peripheral blood vessels) may occur when there is little or no increase of respiratory activity or during artificial respiration.

As confirmatory of Cervello's statement that neurine acts like

curare on the nerve endings of voluntary muscle, and to which he attributes the cessation of respiration, we may mention that after an animal has been poisoned with neurine, asphyxiation causes little or none of the usual convulsions.

The full paper contains references to previous work on the subject, and complete details of the methods used, and the cases investigated; it is illustrated by reproductions of numerous tracings.

[*Note added April* 20, 1899.—It should be mentioned that in the cases of brain atrophy referred to, the cerebro-spinal fluid was removed soon after death. Since the foregoing abstract was written, we have, however, had the opportunity of examining two specimens removed during life by lumbar puncture, and the results of our experiments with these corroborate the conclusions previously arrived at.]

HARRISON AND SONS, Printers in Ordinary to Her Majesty, St. Martin's Lane.

Reprinted for the Authors from the BRITISH MEDICAL JOURNAL,
July 29th, 1899.

NOTE ON THE BLOOD IN A CASE OF BERI-BERI.

BY

F. W. MOTT, M.D., F.R.S. and W. D. HALLIBURTON, M.D., F.R.S.

(From the Physiological Laboratory, King's College, London.)

DURING the last few years we have been investigating the physiological action of choline and neurine, with special reference to the pathology of general paralysis of the insane. Our results will be fully published with illustrative tracings in the *Philosophical Transactions* of the Royal Society.[1] One conclusion we arrived at may be however briefly mentioned, namely, that the cerebro-spinal fluid of these patients produces when injected into the vascular system of animals (cats, dogs, rabbits) a considerable fall of blood pressure; and this is due to the presence of choline. This alkaloid doubtless originates from the lecithin of the disintegrating nervous tissues. The nearly related alkaloid neurine is not present; it is more toxic, and its most marked effect on the circulation is a preliminary fall of blood pressure (mainly cardiac in origin), followed by a great rise of pressure due to constriction of peripheral blood vessels. The fall of arterial pressure produced by choline is partly of cardiac origin, and partly due to the dilatation of the peripheral vessels, especially in the intestinal area. It may also be mentioned that the blood of these patients, removed by venesection during the epileptiform seizures, which form a prominent symptom of the disease, contains the same alkaloid; our proof that the toxic material both from blood and cerebro-spinal fluid is choline were partly chemical and partly physiological, that is, the effects of the material were exactly the same as those of minute doses of choline or of choline hydrochloride.

In the course of this investigation we had occasionally opportunities of testing the action of other fluids; thus we found that the alcoholic extracts[2] of normal blood, normal cerebro-spinal fluid, and ordinary serous effusions contain no choline and produce no results on blood pressure.

It is, however, to a specimen of blood from a case of beri-beri that we wish, in this note, to draw particular attention. We are indebted for the specimen to Dr. Patrick Manson. The blood was removed from the patient by venesection, undertaken with a view to relieve right heart distension, was immediately mixed with alcohol, and it was this mixture which Dr. Manson kindly forwarded to us.

Before we injected the material, the mixture was filtered; the filtrate was evaporated to dryness at 40° C., and the

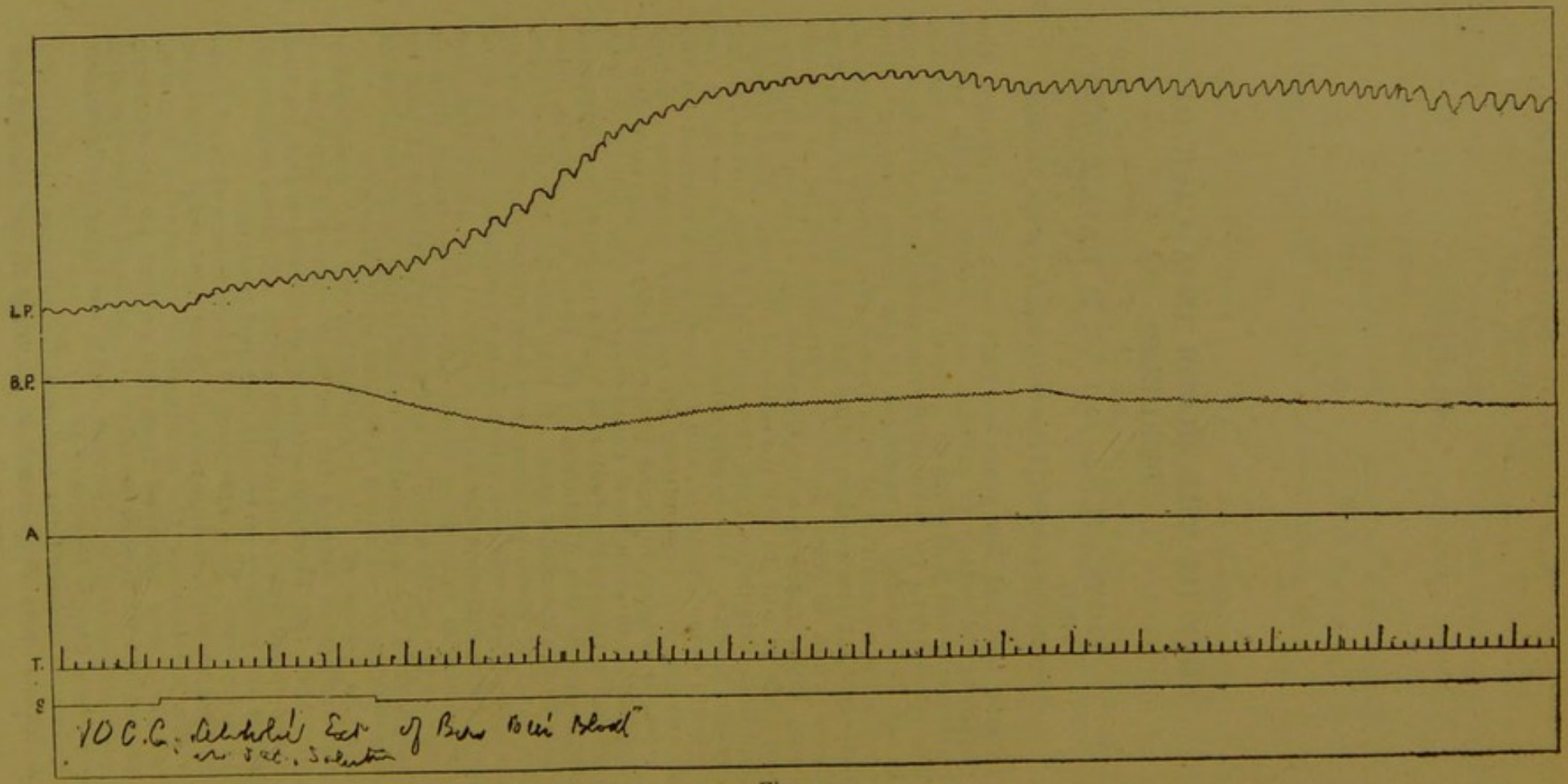

L.P.
B.P.
A
T.
S
10 C.C. Alcohol Ext of Brain to 100 Blood
in Sat. Solution

Fig. 1.

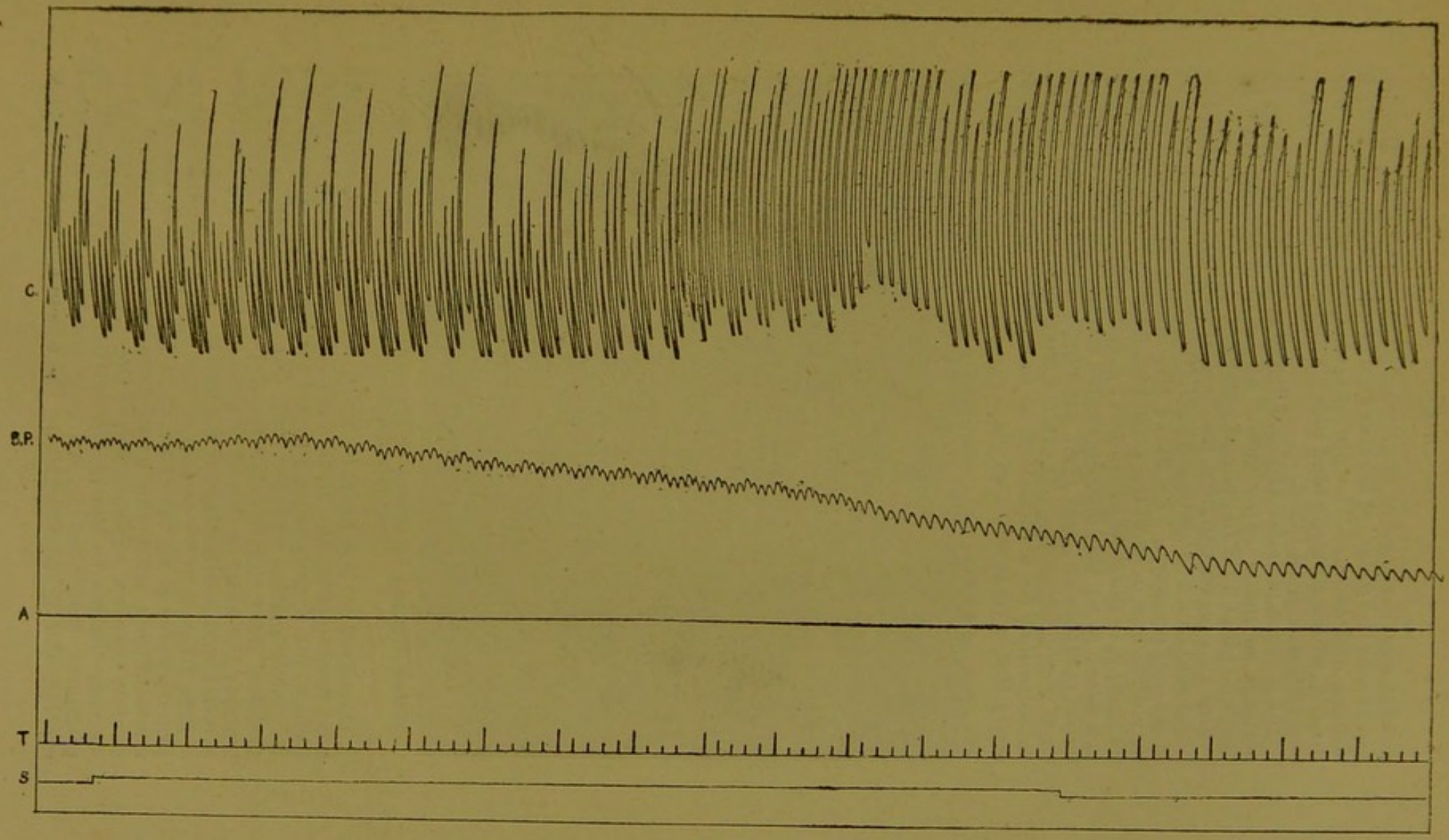
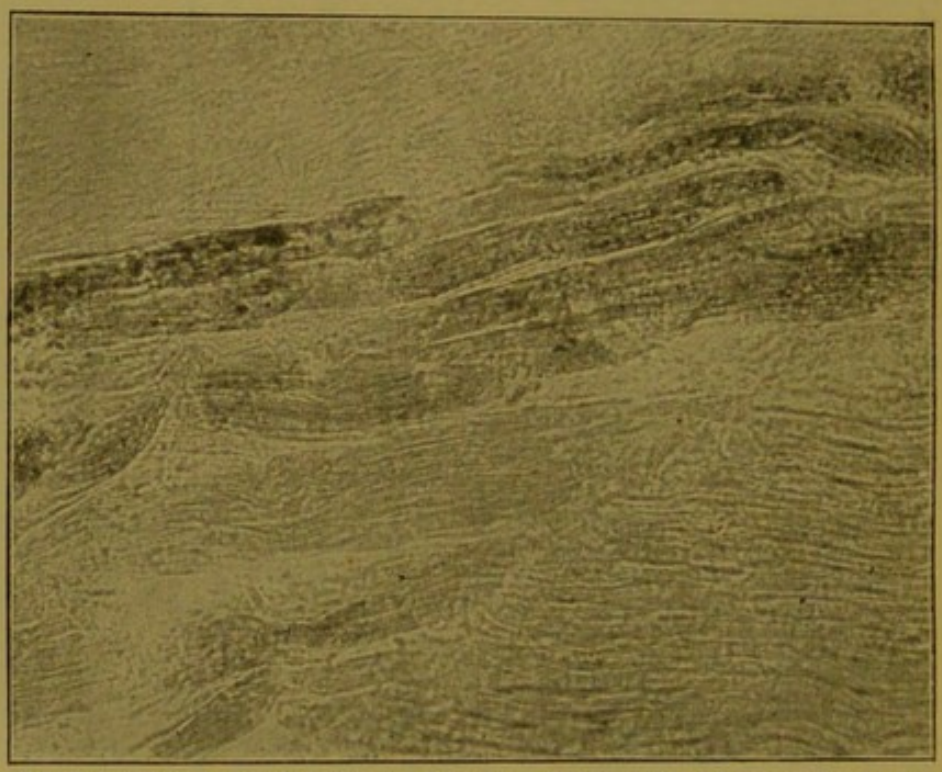


Fig. 2.

residue was dissolved in physiological saline solution, and injected into the external jugular vein of cats anæsthetised with alcohol and ether. The amount of material injected was 10 c.cm., which corresponded roughly to about twice that quantity of the original blood.

The amount of material at our disposal did not enable us to perform as many experiments as we were able to do with cerebro-spinal fluid in general paralysis.

The two accompanying tracings show, however, that the blood contains a substance which, like choline, produces a fall of arterial pressure. In Fig. 1 the lowermost line (s) shows by a difference of level the period when the injection was performed; T, is the time in seconds; A. is the abscissa of the blood pressure; B. P., the blood-pressure tracing taken with a mercurial manometer; and I. P., a tracing with Edmunds's intestinal plethysmograph. The fall of blood pressure is seen to be accompanied by a dilatation of the vessels of the intestinal area; the heart is also slowed.



Photomicrograph of section of heart after staining in Marchi fluid. Magnification 280 diameters.

The effect on the heart is much more clearly shown in the next tracing (Fig. 2). In this cat we used, in place of the intestinal plethysmograph, Barnard's cardiometer attached to a Hürthle's piston recorder (C); the downstrokes represent systole; here the fall of arterial pressure was greatly prolonged; the heart is slowed; its output is increased, and there is considerable dilatation of the heart.

It is difficult to be certain of the dose of toxic material injected; so far as we were able to judge, the effect is more pronounced than what one usually obtains with choline. We made a chemical examination of the blood, and attempted to identify choline by the characteristic crystalline form of its platinum double salt. We only obtained some ill-formed crystals of a light yellow tint. The amount of material at our

disposal did not enable us to do more than this. We thus obtained no positive chemical evidence of the presence of choline.

Specimens of beri-beri blood are, however, so rarely obtained, that we think it not altogether devoid of interest to record our imperfect observations; no doubt the subject will be taken up by others whose opportunities of seeing such patients are more extensive. The disease is a nervous one, accompanied by great cardiac failure, vascular depression, and œdema. Later on, in the present case, when the patient died, a *post-mortem* microscopical examination of the tissues performed by one of us (F. W. M.) showed extensive degeneration of peripheral nerves and fatty degeneration of the muscles, including the heart. It is therefore not to be wondered at that the blood should contain a toxic material producing vascular depression, nor (remembering the nerve degeneration) that the toxic substance should be similar in its action to choline.

The accompanying photomicrograph shows the fatty degeneration of the heart in beri-beri. This specimen was obtained from the heart of a Chinaman who died of the disease. At the *post-mortem* examination there was great venous congestion and distension of the right side of the heart with dark blood. The liver, stomach, and intestines were greatly congested, and numerous hæmorrhages throughout the substance of the liver were found microscopically.

NOTES.

[1] An abstract has already appeared in the *Proceedings* of the Society, 1899. [2] Naturally the alcohol was evaporated off, and the residue, dissolved in normal saline solution, was then injected.

KING'S COLLEGE, LONDON.

PHYSIOLOGICAL LABORATORY.

COLLECTED PAPERS.

No. V.

EDITED BY

W. D. HALLIBURTON, M.D., F.R.C.P., F.R.S.

PROFESSOR OF PHYSIOLOGY, KING'S COLLEGE, LONDON.

1903.

CONTENTS.

Reprinted from the BRITISH MEDICAL JOURNAL, September 15th, 1900.

THE FORMATION OF URIC ACID.

Being the Introduction to a Discussion in the Sectiou of Physiology at the Annual Meeting of the the British Medical Association held at Ipswich, July-August, 1900.

BY W. D. HALLIBURTON, M.D., F.R.S.,
Professor of Physiology, King's College, London ; President of the Section.

WHEN I accepted the presidential chair of this Section my gratification for the honour thus thrust upon me was considerably damped by the thought that it would be necessary to prepare a presidential address. For months I was perplexed with the question, What shall I talk about? My cogitations led to no definite result, until one morning all my difficulties were solved by the arrival of a circular letter stating that Presidents of Sections were not expected to deliver introductory addresses. I feel that the ruling powers of the Association are right in this decision. It is a wise course, when one considers how much a Section has to accomplish, that we should enter at once upon our business, and not curtail the already too short time we have to devote to the consideration of original communications.

Let me then thank those who will contribute to the success of our meeting by the reading of such papers, and welcome here all who propose to assist either by taking part in our discussions, or by listening to what others have to say.

The modern tendency of physiological teaching is to become more and more practical; this tendency is reflected in the mode in which examinations are now conducted, but it is especially seen in meetings such as those of the Physiological Society, where physiologists gather together to compare notes and interchange ideas. A demonstration rather than a communication is nowadays always preferred. I much regret that the absence of a physiological laboratory in Ipswich will limit, if not entirely prevent, any experimental demonstration of the facts of which we shall hear.

It is largely for this reason that the officers of the Section have resolved to adhere to the old custom of setting down for discussion certain questions of general interest; and I trust, in selecting uric acid and muscular tonus for these general discussions, that we have succeeded in choosing subjects which will interest not only the professional physiologist but also the practical physician.

The decree has gone forth that the task of opening the first of these two discussions shall rest with myself. At first I felt inclined to rebel, as the subject is one at which I have never done any original work, but, on second thoughts, this

appeared to be in one sense an advantage. I shall not be unduly biassed in favour of any one exclusive view, but I shall, I hope, be able to present the subject from the general standpoint, and leave it to the specialists who follow me to discuss the details.

Without further introductory or preliminary remarks, let us now apply ourselves to business, and proceed to consider the question of the origin of uric acid. The important pathological bearing of the uric acid question gives special interest to the theories which explain—or attempt to explain—the way in which it originates under normal circumstances. Previous to about ten years ago, I think I may safely say that all current theories left us entirely in the dark. The idea that deficient oxidation due to over-eating and sedentary habits would explain an abnormal increase in uric acid fitted in with experiments that showed that injection of urates into the blood stream was followed by a corresponding increase of urea in the urine if oxidation was active; the relatively large quantity of uric acid in fœtal urine was also compatible with this theory. But a fatal objection to the theory was at once seen when a wider survey of the animal kingdom was taken, and in particular the birds form an instance of animals in which the processes of oxidation are specially active, but in which also uric acid is the chief end product of nitrogenous metabolism. The oxidation theory, moreover, leaves us completely uninformed of the way in which the small amount of uric acid in normal urine is formed.

To say that the question of what is the most suitable vehicle for getting rid of waste nitrogen in the different classes of the animal world is a question of natural selection is no doubt true, but such a general statement is more of the nature of a platitude than an explanation. Again, in considering the question of the heredity of gout and allied disorders a hereditary predisposition to disordered nitrogenous metabolism is a high-sounding phrase which may comfort some minds, but is hardly calculated to satisfy the inquirer.

Physiologists and pathologists alike therefore welcomed with enthusiasm the theory that originated with Mares and Horbaczewski, for here at least there was a doctrine which rested on reasoning from experimental data. Mares showed that the greatest increase in uric acid excretion occurs a few hours after a meal, whereas general nitrogenous katabolism as indicated by the amount of urea excreted increases more slowly and does not reach a maximum until some hours later. Horbaczewski formulated his now familiar theory that this was due to a digestive leucocytosis and the consequent increased liberation of nuclein within the organism. He supported this view by pointing out the close chemical relationship between uric acid and the so-called alloxuric bases (adenine, xanthine, hypoxanthine, etc.) which are obtained by the decomposition of the nuclear material. He showed that the spleen pulp in a still living condition is able to oxidise these bases and so form uric acid, and he recorded numerous observations to demonstrate the connection between the number of leucocytes and the rise in the amount of uric acid excreted. This theory was widely accepted, and it certainly fitted in with such well-known pathological phenomena as the increase of uric acid in leucocythæmia.

I think we may fairly regard this work of Horbaczewski's as

epoch making. The details of the theory have perhaps not stood the test of ten years experimental criticism, but it has formed the basis of all subsequent work on the subject, and if it does not express the whole truth, it contains the essential germ of truth which I fancy nobody will question, namely, that we are to seek the origin of uric acid, in mammals at any rate, in the destructive changes that occur in nuclein. There may be other sources of uric acid which are still undetected, but the only one of which we have any certain knowledge is the one just alluded to.

I think that this important practical outcome of physiological investigation is a lesson to us that there is no such thing as useless research. The accusation is often levelled at physiologists that they work too much in the clouds, and devote too little attention to subjects of practica, interest. Truth, however, when found is always usefull whether it is discovered in the clouds, or on the dry land. Its application may not be always immediately forthcoming, and I fancy that when physiologists began to investigate such an out-of-the-way subject as the chemistry of cell nuclei, neither they nor their practical brethren imagined it would help towards the elucidation of the uric-acid problem. In working out the matter the labours of the pure chemists must not be neglected either. Fischer in particular who has, thoroughly worked out the question of uric-acid constitution, has shown that it and the alloxuric bases are all members of one family linked together by containing a common radicle which he has dubbed "purine." Physiologists and pathologists have too long devoted their attention to the most prominent member of the purine group, namely, uric acid, but in any general examination of the subject investigation would be incomplete if we did not also take into account the other members of the group.

The further researches started by Horbaczewski's work have been very numerous, and I do not propose to go fully into all of these. I may, however, select a few as typical examples of the rest.

It has been shown that a well-marked digestive leucocytosis may occur after a diet of egg-white or even after a non-nitrogenous meal, and yet there is no rise in the amount of uric acid excreted. The theory has therefore been modified by supposing that the acid arises from the breakdown of the nuclein of the food, independently of any intermediate leucocytosis; the striking increase of uric acid after a meal of sweetbread lends considerable support to this view. It was also shown that the amount of disintegration of the leucocytes might be measured by the output of phosphoric acid, for this substance is also a product of the disintegration of nuclein. The amounts of uric acid and phosphoric acid do not always run parallel, and in a case of leucocythæmia recorded by Milroy and Malcolm there was even a diminution of phosphates in the urine. One case, however, does not prove or disprove a rule, and the balance of present-day opinion is distinctly in favour of the view that nuclein is at any rate one of the sources of uric acid.

Other workers in the field have been Hopkins and Hope at Cambridge, and Smith Jerome at Oxford, and our two ancient universities are not at one on the question. Smith Jerome has shown by a new series of observations that feeding on nuclein and foods which contain nuclein distinctly raises the

uric-acid output. His most important point, however, is an answer to the most serious objection to the nuclein theory that Hopkins and Hope adduced. Hopkins and Hope pointed out that as nuclein resists digestion with gastric juice, it cannot be expected to supply the uric acid which is excreted a few hours after a meal, and the disproportion between the relatively small amount of nucleo-proteid in muscle and the large increase in uric ucid after a meal of flesh cannot be explained by the nuclein theory of its origin. Smith Jerome's answer to this is that any disproportion between the excretion of uric acid after flesh diet and the amount of nuclein in muscle is probably to be explained by the presence in such diet of purine bases, especially of hypoxanthine, in sufficient quantity to account for the rise produced; the presence of such bases in flesh diet would account also for the early rise in uric acid after a meal, and would further explain the increase found when pepsin-hydrochloric acid (that is, nuclein-free) extracts of thymus gland are taken. Smith Jerome is therefore strongly of opinion that uric acid may be formed from nucleo-proteid, nuclein, and one variety at least of purine base, and that there is at present no proof that it can arise in man independently of a substance containing a purine group.

Just as the onlooker in a game of chess often sees moves that are invisible to the actual players, so in experimental researches, the one who stands by may often detect fallacies or draw conclusions better than those actually engaged in the work. Though I do not pretend that what I have now to say really probes the matter to the bottom, I think that any suggestion from an impartial observer may be helpful in a complicated question of this kind.

In the first place my temperament is such as to lead me to fight shy of extremist views. The origin of uric acid from the nuclein of the food may be one factor; at the same time the origin of uric acid from the nuclein of the leucocytes is another; the two probably act together, and in one case the one, in another case the other, factor may be more predominant. I would further point out that there is a third factor, which never yet seems to have received any attention; this is that the katabolism of the nuclei of other animal cells, such as those of secreting glands, may contribute to the formation of the acid. Investigators hitherto seem to have got into the way of supposing that the leucocytes are the only cells whose nuclei need be troubled about. They are the cells which lend themselves best to investigation, but, after all, they constitute a mere fraction of the total cells of the body.

Another fallacy which appears to underlie a good deal of the work on the uric acid question is the habit of regarding the amount of uric acid in the urine as synonymous with the amount produced in the body. Recent experimental work points the other way. The uric acid produced in metabolism doubtless in great measure leaves the body by the urine, but all of it certainly does not; some may be retained within the body for a time; an exaggeration of this is seen in gouty concretions; some may be further oxidised and converted into urea and simpler products; some may enter into combination with other organic substances, lose its identity, and the nitrogen ultimately leave the organism not only as uric acid but in other forms also. This possibility receives emphasis when one considers the work which was published this year

by Kossel and Goto.[1] They showed that uric acid, like other purine substances, will form *in vitro* loose combinations with nucleic acid, and in this way uric acid may be held in solution. It is thus possible that the action of nucleic acid and its compounds in the body may be a factor in determining the solubility of uric acid there.

The work of Hugo Wiener[2] is also important from this point of view. He shows that the "surviving" liver of the ox, and extracts of the liver, kidney, and even of the muscles of some animals, have the power of destroying uric acid. The liver is able, therefore, not only to form uric acid from xanthine derivatives, but also to destroy it. As long as the relative intensities of the two processes are undetermined, the amount of uric acid in the urine is no measure of the amount actually produced in the body. These experiments have suggested to me a possible fallacy in connection with phosphoric acid. I have already mentioned the observation of Milroy and Malcolm that the phosphoric acid and uric acid are not present in parallel amounts, as they would be if both ultimately came from the same material, for what is true for uric acid may also be true for phosphoric acid; neither acid necessarily leaves the body at once, but may be further utilised in the organism before it is finally disposed of.

Another factor which is often neglected is the amount of purine substances that leaves the body in the fæces. This has been shown by Parker[3] to be by no means a negligeable quantity.

The last fault I feel inclined to find with many observations on uric acid formation is the narrow standpoint from which it is regarded; this I have already alluded to. Uric acid does not stand alone; it is a member of the great purine family, and in quantitative work all the members of the group must be considered.

One sees in the history of any physiological subject that when a great central idea is once started and research is directed towards working it out, how every worker profits by the errors of his predecessors. Mistakes in methods are remedied, fallacies in argument corrected, new ideas come into being. Each research improves on the one before, and gradually and slowly the links in the chain of truth are forged. The ink was hardly dry on the lines I had written when a paper was brought under my notice in which a well-conceived attempt to clear up the matter is made, and in which many of my criticisms are met. The paper is by Burian and Schur.[4] Though it is lengthy it will amply repay careful study, and I cannot do better than conclude my remarks by giving in outline the main ideas and results of the investigation. Every healthy adult excretes a certain characteristic amount of purine substances which is independent of his diet; this is the result of tissue metabolism and may be termed "endogenous urinary purine." Its amount may be directly estimated by examining the urine after a diet of substances which are practically free from purine compounds; such articles of diet are milk, white bread, potatoes, rice, green vegetables, etc. This method of examination is much better than that of analysing the urine during hunger. In inanition the upset

[1] *Sitzungst. Gesellsch. gesammt. Naturwissensch.*, Marburg, 1900, April 6th.
[2] *Arch. exp. Path. Pharm.*, 1899, xlii, 374-398.
[3] *American Journal of Physiology*, 1900, vol. iv, p. 83.
[4] *Pflüger's Archiv*, 1900, vol. lxxx, pp. 241-343.

produced in metabolism generally is sure to give untrustworthy results.

Diet.	Total Percentage of Purine Substances in Diet.	Percentage of Exogenous Urinary Purine.
Beef	0.06	0.030
Coffee	0.20	0.075
Calf's liver ...	0.12	0.060
Calf's spleen ...	0.16	0.080
Calf's thymus ...	0.40	0.100

But when a man takes his ordinary diet, which contains articles containing nuclein, or purine compounds, the amount of urinary purine is increased by a part of the purine derived from his diet, and this increase may be termed "exogenous urinary purine." The "nutrition putrine" does not pass wholly into the urine; a certain fraction remains in the organism, the purine double ring being broken down. The amount of the remainder (exogenous urinary purine) differs for different forms of food, and is but little affected by the individuality of the subject of the experiment. The table in the previous column gives some of the figures quoted.

By subtracting the exogenous from the total urinary purine, the endogenous urinary purine is obtained, and the numbers come out closely with the results obtained by direct estimation; it varies in the majority of people from 0.1 to 0.2 gram daily, but values both higher and lower than these were obtained.

Working on such lines as these it seems possible that in the future the whole metabolic history of the purine compounds will be unravelled, and then it will be the time to return to the one member of the group—namely, uric acid—to which the attention of so many up to now has been almost exclusively directed. Finally, when the normal life-history of uric acid is known its abnormal behaviour in diseased conditions will be a comparatively simple problem.

From what I have said, it will be seen that I have directed your attention principally to one side of the subject, the side with which I should be expected to be most familiar. I have not attempted to trespass on the domain of the pathologist nor of the therapeutist; the influence of age, of disease, of diet, of mineral waters, of drugs, and other remedial agents I have left all but untouched; still I hope that those who follow me in the discussion will not exhibit similar reticence. The debate will lose much of its value if we do not attempt to approach the subject from as many points of view as possible.

Reprinted from the BRITISH MEDICAL JOURNAL, July 7th, 1900.

REMARKS
ON
THE USE OF BORAX AND FORMALDEHYDE AS PRESERVATIVES OF FOOD.

BY W. D. HALLIBURTON, M.D., F.R.S.,
Professor of Physiology, King's College, London.

SOME weeks ago, when I was giving evidence before the Food Preservatives Committee of the Local Government Board, I urged that the use of foreign substances as preservatives of food stuffs should be abandoned, and, if possible, replaced by a more wholesale use of the method of cold transport and storage. I took this view partly on general grounds, which were the following: (1) An antiseptic is inimical to the life of the organisms that cause putrefaction; it cannot, therefore, be harmless to the vital processes in the higher animals. (2) Numerous clinical observations have been recorded which show that dyspeptic and other troubles follow the use of foods which have been treated with commonly employed preservatives like borax. (3) Even if, as in the case of boric acid and borax, the poison is not cumulative, the continuous passage of foreign substances through the kidney cannot be beneficial to those organs.

It is perfectly true that most of us are probably taking small doses of antiseptics in our foodstuffs without any obviously bad results; those who suffer are peculiarly susceptible to such drugs, but, as such cases are by no means uncommon, it appeared to me of sufficient importance to recommend legislation in the direction I have indicated. If, on the other hand, trade influences are too strong, at least articles mixed with drugs should be labelled as such, and not sold as fresh foods. In this way those people who are peculiarly susceptible to any particular antiseptic could avoid it.

In any question of this kind one has to balance the good and the bad, that is to say, whether more harm will result from the products of putrefaction or from the antiseptic used to hinder or prevent putrefaction. But if the method of cold storage were made compulsory no such question could arise, for putrefaction would be prevented without foreign admixture. I was particularly questioned whether on physiological grounds there was any reason why the sterilising of milk by heat could give rise to any toxic material. This I answered unhesitatingly in the negative. The milk proteids are rendered somewhat more difficult of digestion by heat, as

appeared to be in one sense an advantage. I shall not be unduly biassed in favour of any one exclusive view, but I shall, I hope, be able to present the subject from the general standpoint, and leave it to the specialists who follow me to discuss the details.

Without further introductory or preliminary remarks, let us now apply ourselves to business, and proceed to consider the question of the origin of uric acid. The important pathological bearing of the uric acid question gives special interest to the theories which explain—or attempt to explain—the way in which it originates under normal circumstances. Previous to about ten years ago, I think I may safely say that all current theories left us entirely in the dark. The idea that deficient oxidation due to over-eating and sedentary habits would explain an abnormal increase in uric acid fitted in with experiments that showed that injection of urates into the blood stream was followed by a corresponding increase of urea in the urine if oxidation was active; the relatively large quantity of uric acid in fœtal urine was also compatible with this theory. But a fatal objection to the theory was at once seen when a wider survey of the animal kingdom was taken, and in particular the birds form an instance of animals in which the processes of oxidation are specially active, but in which also uric acid is the chief end product of nitrogenous metabolism. The oxidation theory, moreover, leaves us completely uninformed of the way in which the small amount of uric acid in normal urine is formed.

To say that the question of what is the most suitable vehicle for getting rid of waste nitrogen in the different classes of the animal world is a question of natural selection is no dou bt true, but such a general statement is more of the nature of a platitude than an explanation. Again, in considering the question of the heredity of gout and allied disorders a hereditary predisposition to disordered nitrogenous metabolism is a high-sounding phrase which may comfort some minds, but is hardly calculated to satisfy the inquirer.

Physiologists and pathologists alike therefore welcomed with enthusiasm the theory that originated with Mares and Horbaczewski, for here at least there was a doctrine which rested on reasoning from experimental data. Mares showed that the greatest increase in uric acid excretion occurs a few hours after a meal, whereas general nitrogenous katabolism as indicated by the amount of urea excreted increases more slowly and does not reach a maximum until some hours later. Horbaczewski formulated his now familiar theory that this was due to a digestive leucocytosis and the consequent increased liberation of nuclein within the organism. He supported this view by pointing out the close chemical relationship between uric acid and the so-called alloxuric bases (adenine, xanthine, hypoxanthine, etc.) which are obtained by the decomposition of the nuclear material. He showed that the spleen pulp in a still living condition is able to oxidise these bases and so form uric acid, and he recorded numerous observations to demonstrate the connection between the number of leucocytes and the rise in the amount of uric acid excreted. This theory was widely accepted, and it certainly fitted in with such well-known pathological phenomena as the increase of uric acid in leucocythæmia.

I think we may fairly regard this work of Horbaczewski's as

epoch making. The details of the theory have perhaps not stood the test of ten years experimental criticism, but it has formed the basis of all subsequent work on the subject, and if it does not express the whole truth, it contains the essential germ of truth which I fancy nobody will question, namely, that we are to seek the origin of uric acid, in mammals at any rate, in the destructive changes that occur in nuclein. There may be other sources of uric acid which are still undetected, but the only one of which we have any certain knowledge is the one just alluded to.

I think that this important practical outcome of physiological investigation is a lesson to us that there is no such thing as useless research. The accusation is often levelled at physiologists that they work too much in the clouds, and devote too little attention to subjects of practica, interest. Truth, however, when found is always usefull whether it is discovered in the clouds, or on the dry land. Its application may not be always immediately forthcoming, and I fancy that when physiologists began to investigate such an out-of-the-way subject as the chemistry of cell nuclei, neither they nor their practical brethren imagined it would help towards the elucidation of the uric-acid problem. In working out the matter the labours of the pure chemists must not be neglected either. Fischer in particular who has, thoroughly worked out the question of uric-acid constitution, has shown that it and the alloxuric bases are all members of one family linked together by containing a common radicle which he has dubbed "purine." Physiologists and pathologists have too long devoted their attention to the most prominent member of the purine group, namely, uric acid, but in any general examination of the subject investigation would be incomplete if we did not also take into account the other members of the group.

The further researches started by Horbaczewski's work have been very numerous, and I do not propose to go fully into all of these. I may, however, select a few as typical examples of the rest.

It has been shown that a well-marked digestive leucocytosis may occur after a diet of egg-white or even after a non-nitrogenous meal, and yet there is no rise in the amount of uric acid excreted. The theory has therefore been modified by supposing that the acid arises from the breakdown of the nuclein of the food, independently of any intermediate leucocytosis; the striking increase of uric acid after a meal of sweetbread lends considerable support to this view. It was also shown that the amount of disintegration of the leucocytes might be measured by the output of phosphoric acid, for this substance is also a product of the disintegration of nuclein. The amounts of uric acid and phosphoric acid do not always run parallel, and in a case of leucocythæmia recorded by Milroy and Malcolm there was even a diminution of phosphates in the urine. One case, however, does not prove or disprove a rule, and the balance of present-day opinion is distinctly in favour of the view that nuclein is at any rate one of the sources of uric acid.

Other workers in the field have been Hopkins and Hope at Cambridge, and Smith Jerome at Oxford, and our two ancient universities are not at one on the question. Smith Jerome has shown by a new series of observations that feeding on nuclein and foods which contain nuclein distinctly raises the

uric-acid output. His most important point, however, is an answer to the most serious objection to the nuclein theory that Hopkins and Hope adduced. Hopkins and Hope pointed out that as nuclein resists digestion with gastric juice, it cannot be expected to supply the uric acid which is excreted a few hours after a meal, and the disproportion between the relatively small amount of nucleo-proteid in muscle and the large increase in uric ucid after a meal of flesh cannot be explained by the nuclein theory of its origin. Smith Jerome's answer to this is that any disproportion between the excretion of uric acid after flesh diet and the amount of nuclein in muscle is probably to be explained by the presence in such diet of purine bases, especially of hypoxanthine, in sufficient quantity to account for the rise produced; the presence of such bases in flesh diet would account also for the early rise in uric acid after a meal, and would further explain the increase found when pepsin-hydrochloric acid (that is, nuclein-free) extracts of thymus gland are taken. Smith Jerome is therefore strongly of opinion that uric acid may be formed from nucleo-proteid, nuclein, and one variety at least of purine base, and that there is at present no proof that it can arise in man independently of a substance containing a purine group.

Just as the onlooker in a game of chess often sees moves that are invisible to the actual players, so in experimental researches, the one who stands by may often detect fallacies or draw conclusions better than those actually engaged in the work. Though I do not pretend that what I have now to say really probes the matter to the bottom, I think that any suggestion from an impartial observer may be helpful in a complicated question of this kind.

In the first place my temperament is such as to lead me to fight shy of extremist views. The origin of uric acid from the nuclein of the food may be one factor; at the same time the origin of uric acid from the nuclein of the leucocytes is another; the two probably act together, and in one case the one, in another case the other, factor may be more predominant. I would further point out that there is a third factor, which never yet seems to have received any attention; this is that the katabolism of the nuclei of other animal cells, such as those of secreting glands, may contribute to the formation of the acid. Investigators hitherto seem to have got into the way of supposing that the leucocytes are the only cells whose nuclei need be troubled about. They are the cells which lend themselves best to investigation, but, after all, they constitute a mere fraction of the total cells of the body.

Another fallacy which appears to underlie a good deal of the work on the uric acid question is the habit of regarding the amount of uric acid in the urine as synonymous with the amount produced in the body. Recent experimental work points the other way. The uric acid produced in metabolism doubtless in great measure leaves the body by the urine, but all of it certainly does not; some may be retained within the body for a time; an exaggeration of this is seen in gouty concretions; some may be further oxidised and converted into urea and simpler products; some may enter into combination with other organic substances, lose its identity, and the nitrogen ultimately leave the organism not only as uric acid but in other forms also. This possibility receives emphasis when one considers the work which was published this year

by Kossel and Goto.[1] They showed that uric acid, like other purine substances, will form *in vitro* loose combinations with nucleic acid, and in this way uric acid may be held in solution. It is thus possible that the action of nucleic acid and its compounds in the body may be a factor in determining the solubility of uric acid there.

The work of Hugo Wiener[2] is also important from this point of view. He shows that the "surviving" liver of the ox, and extracts of the liver, kidney, and even of the muscles of some animals, have the power of destroying uric acid. The liver is able, therefore, not only to form uric acid from xanthine derivatives, but also to destroy it. As long as the relative intensities of the two processes are undetermined, the amount of uric acid in the urine is no measure of the amount actually produced in the body. These experiments have suggested to me a possible fallacy in connection with phosphoric acid. I have already mentioned the observation of Milroy and Malcolm that the phosphoric acid and uric acid are not present in parallel amounts, as they would be if both ultimately came from the same material, for what is true for uric acid may also be true for phosphoric acid; neither acid necessarily leaves the body at once, but may be further utilised in the organism before it is finally disposed of.

Another factor which is often neglected is the amount of purine substances that leaves the body in the fæces. This has been shown by Parker[3] to be by no means a negligeable quantity.

The last fault I feel inclined to find with many observations on uric acid formation is the narrow standpoint from which it is regarded; this I have already alluded to. Uric acid does not stand alone; it is a member of the great purine family, and in quantitative work all the members of the group must be considered.

One sees in the history of any physiological subject that when a great central idea is once started and research is directed towards working it out, how every worker profits by the errors of his predecessors. Mistakes in methods are remedied, fallacies in argument corrected, new ideas come into being. Each research improves on the one before, and gradually and slowly the links in the chain of truth are forged. The ink was hardly dry on the lines I had written when a paper was brought under my notice in which a well-conceived attempt to clear up the matter is made, and in which many of my criticisms are met. The paper is by Burian and Schur.[4] Though it is lengthy it will amply repay careful study, and I cannot do better than conclude my remarks by giving in outline the main ideas and results of the investigation. Every healthy adult excretes a certain characteristic amount of purine substances which is independent of his diet; this is the result of tissue metabolism and may be termed "endogenous urinary purine." Its amount may be directly estimated by examining the urine after a diet of substances which are practically free from purine compounds; such articles of diet are milk, white bread, potatoes, rice, green vegetables, etc. This method of examination is much better than that of analysing the urine during hunger. In inanition the upset

---

[1] *Sitzungst. Gesellsch. gesammt. Naturwissensch.*, Marburg, 1900, April 6th.
[2] *Arch. exp. Path. Pharm.*, 1899, xlii, 374-398.
[3] *American Journal of Physiology*, 1900, vol. iv, p. 83.
[4] *Pflüger's Archiv*, 1900, vol. lxxx, pp. 241-343.

produced in metabolism generally is sure to give untrustworthy results.

Diet.	Total Percentage of Purine Substances in Diet.	Percentage of Exogenous Urinary Purine.
Beef	0.06	0.030
Coffee	0.20	0.075
Calf's liver ...	0.12	0.060
Calf's spleen ...	0.16	0.080
Calf's thymus ...	0.40	0.100

But when a man takes his ordinary diet, which contains articles containing nuclein, or purine compounds, the amount of urinary purine is increased by a part of the purine derived from his diet, and this increase may be termed "exogenous urinary purine." The "nutrition putrine" does not pass wholly into the urine; a certain fraction remains in the organism, the purine double ring being broken down. The amount of the remainder (exogenous urinary purine) differs for different forms of food, and is but little affected by the individuality of the subject of the experiment. The table in the previous column gives some of the figures quoted.

By subtracting the exogenous from the total urinary purine, the endogenous urinary purine is obtained, and the numbers come out closely with the results obtained by direct estimation; it varies in the majority of people from 0.1 to 0.2 gram daily, but values both higher and lower than these were obtained.

Working on such lines as these it seems possible that in the future the whole metabolic history of the purine compounds will be unravelled, and then it will be the time to return to the one member of the group—namely, uric acid—to which the attention of so many up to now has been almost exclusively directed. Finally, when the normal life-history of uric acid is known its abnormal behaviour in diseased conditions will be a comparatively simple problem.

From what I have said, it will be seen that I have directed your attention principally to one side of the subject, the side with which I should be expected to be most familiar. I have not attempted to trespass on the domain of the pathologist nor of the therapeutist; the influence of age, of disease, of diet, of mineral waters, of drugs, and other remedial agents I have left all but untouched; still I hope that those who follow me in the discussion will not exhibit similar reticence. The debate will lose much of its value if we do not attempt to approach the subject from as many points of view as possible.

Reprinted from the BRITISH MEDICAL JOURNAL, July 7th, 1900.

REMARKS
ON
THE USE OF BORAX AND FORMALDEHYDE AS PRESERVATIVES OF FOOD.

BY W. D. HALLIBURTON, M.D., F.R.S.,
Professor of Physiology, King's College, London.

SOME weeks ago, when I was giving evidence before the Food Preservatives Committee of the Local Government Board, I urged that the use of foreign substances as preservatives of food stuffs should be abandoned, and, if possible, replaced by a more wholesale use of the method of cold transport and storage. I took this view partly on general grounds, which were the following: (1) An antiseptic is inimical to the life of the organisms that cause putrefaction; it cannot, therefore, be harmless to the vital processes in the higher animals. (2) Numerous clinical observations have been recorded which show that dyspeptic and other troubles follow the use of foods which have been treated with commonly employed preservatives like borax. (3) Even if, as in the case of boric acid and borax, the poison is not cumulative, the continuous passage of foreign substances through the kidney cannot be beneficial to those organs.

It is perfectly true that most of us are probably taking small doses of antiseptics in our foodstuffs without any obviously bad results; those who suffer are peculiarly susceptible to such drugs, but, as such cases are by no means uncommon, it appeared to me of sufficient importance to recommend legislation in the direction I have indicated. If, on the other hand, trade influences are too strong, at least articles mixed with drugs should be labelled as such, and not sold as fresh foods. In this way those people who are peculiarly susceptible to any particular antiseptic could avoid it.

In any question of this kind one has to balance the good and the bad, that is to say, whether more harm will result from the products of putrefaction or from the antiseptic used to hinder or prevent putrefaction. But if the method of cold storage were made compulsory no such question could arise, for putrefaction would be prevented without foreign admixture. I was particularly questioned whether on physiological grounds there was any reason why the sterilising of milk by heat could give rise to any toxic material. This I answered unhesitatingly in the negative. The milk proteids are rendered somewhat more difficult of digestion by heat, as

in the cooking of all albuminous foods. But most gastric juices are able to grapple with this difficulty. Of course, in cases of feeble digestion, raw or underdone meat and uncooked milk are preferable to foods which have been cooked too much, but there are no grounds for supposing that, in the case of milk, heating will split off from its proteid matter anything of the nature of a toxin.

The special ground on which I opposed the use of antiseptics arose from some experiments I had done with artificial digestive mixtures. These experiments were conducted to test the action of borax and formaldehyde on digestion. I selected these because they are the antiseptics most frequently employed as food preservatives. The experiments show that the antiseptics named act prejudicially on the digestive enzymes even in the doses which are employed as preservatives, and this furnishes an additional reason why their use should be prohibited. The following are the details of the experiments, the results of which I was briefly able to indicate before the Committee.

Boric Acid and Borax.

My attention was first called to the use of these substances in milk some years ago by Professor F. J. Allen (Birmingham). He noticed many cases of malnutrition in infants fed on such milk, particularly if borax was the adulterant. The condition passed off when pure milk was used. On examining the matter, Professor Allen found that borax prevents the normal action of rennet. The process of milk digestion in the stomach may be briefly explained as follows:—Milk is subjected to the action of two ferments in the gastric juice; one of these is called rennet the other pepsin. The preliminary breaking down of the molecules of caseinogen (the chief albuminous constituent of milk) by rennet is evidenced to the eye by the formation of a curd; this appears to be necessary before the subsequent peptonisation by pepsin can be properly carried out.

In repeating these experiments I have limited myself to observing the influence of borax and boric acid on the action of rennet. The following will serve as typical of numerous experiments I have made; all show analogous results:

Fresh cow's milk was divided into portions of 100 c.cm. each.
To No. 1 nothing was added.
To No. 2 0.05 gram of boric acid was added.
To No. 3 0.1 gram of boric acid was added.
To No. 4 0.15 gram of boric acid was added.
These are quantities comparable to those used generally to keep milk sweet.

After the lapse of an hour, during which time the milk was well shaken with the acid, much was found to be undissolved, even in specimen No. 2. One cannot therefore say that any more boric acid had passed into solution in those specimens to which more of the solid had been added. Boric acid is a comparatively insoluble substance.

From all four specimens samples were taken and treated with a few drops of rennet extract. A well-marked curd occurred in all in less than a minute at 40° C.

The small amount of boric acid which will dissolve in milk in an hour will therefore not prevent, or even markedly hinder, rennet activity.

I kept the remainder of the specimens, 2, 3, and 4, for a few hours longer in order to test the matter again when all the boric acid had dissolved, but I found that still there was no hindering of rennet action.

In spite of the boric acid the milk went sour in a little over a day.

These experiments do not prove very much. They show, what was previously well known—namely, that boric acid by itself is a very inefficient antiseptic, and has but little if any

inhibiting power over the unorganised ferment—rennet—selected for experiment. The cause of this feeble action both on organised ferments (micro-organisms) and unorganised ferments or enzymes is to be probably partly explained by its difficulty of solution.

I imagine it is for this reason that boric acid alone is seldom used as a preservative for milk; the preparation known as "glacialin" is what is usually employed. Glacialin is a mixture of boric acid and borax, and it can hardly be doubted that it is the borax which acts as the principal antiseptic. More interest and importance, therefore, must be attached to experiments with borax.

As before, the milk was divided into portions of 100 c.cm. each. At the end of two days the unadulterated milk had gone sour; the specimens to which borax was added were still sweet.

To No. 1 nothing was added.
To No. 2 0.05 gram of borax was added.
To No. 3 0.1 gram of borax was added.
To No. 4 0.15 gram of borax was added.

On testing samples of each with rennet at 40° C. half an hour after the borax had been added,

No. 1 coagulated in about 30 seconds.
No. 2 coagulated in about 2 minutes.
No. 3 and 4 did not coagulate at all.

These experiments show that 1 part of borax in 1,000 of milk completely inhibits rennet activity, and even smaller proportions will delay its action.

Formaldehyde.

This substance is most efficient as an antiseptic, but I cannot agree with Mr. A. H. Allen[1] that it is the least objectionable of the preservatives of food. Its very efficiency in counteracting ferment activities render it particularly objectionable. The aqueous solutions of formaldehyde are very irritating to the skin. I noticed this even with solutions containing only 5 parts in 10,000 of water; the skin is roughened after dipping the fingers into it for a few moments, and any little abrasions that may be present in the skin are rendered intensely painful. Cervello states that by painting the ear of a rabbit three times a day with it ulceration and gangrene are obtained within a week. From this it will be gathered that though formalin has but little taste, it can nevertheless not be harmless to the mucous membrane of the alimentary canal, which is far more easily injured than the external skin. Bruni has shown that in animals vomiting is produced by very dilute solutions.

If the drug is introduced into an animal by intravenous injection, the red corpuscles of the blood are disintegrated, respiration is disturbed and may stop, convulsions and coma may also supervene. Its power as an antiseptic is, however, so marvellous that the experiment has been tried of treating consumptive patients with air charged with its vapour. Physicians have not entirely relinquished this method of treatment, for it has been stated that by mixing the vapour with other substances the air may be rendered respirable; but it has been fully shown that an admixture of air with the vapour of formaldehyde only is so irritating, and after a short time so impossible, that more harm than good has been the result.

The experiments I have performed myself were to test the influence of formaldehyde in digestion. I could wish that they were more complete, but they at least show that sub-

stances treated with even weak solutions are rendered very indigestible. They may be divided into four categories: the influence of formaldehyde (1) on gastric digestion of proteid; (2) on the pancreatic digestion of proteid; (3) on the pancreatic digestion of starch; (4) on the curdling action of rennet. I will quote illustrative experiments under each of the four heads.

1. *Influence of Formaldehyde on the Gastric Digestion of Proteid.*—Artificial gastric juice was prepared by adding Benger's liquor pepticus to 0.2 per cent. hydrochloric acid in the proportion 1 to 5. Fibrin was taken as the proteid or albuminous matter to be digested. The fibrin was divided into a number of portions and placed in various strengths of formaldehyde for two or three days. Even the weakest solutions employed kept the fibrin perfectly free from decomposition, but in all cases it was rendered very hard. Equal quantities of each were subjected to the action of the same volumes of the artificial gastric juice in a warm oven at 40° C. The end of digestion was considered to be reached when all lumps had passed into solution. The following table gives the results:

Experiment A.

1.	Fresh fibrin.	Digestion complete in 45 minutes.
2.	Fibrin placed in 0.05 per cent. formaldehyde for 2 days	Digestion complete in 65 minutes.
3.	" " 0.10 " " "	
4.	" " 0.25 " " "	... In 3 hours.
5.	" " 0.50 " " "	... In 7 hours.
6.	" " 1.00 " " "	... In 24 hours.
7.	" " 2.00 " " "	Hardly any digestion at end of 24 hours.
8.	" " 4.00 " " "	

Experiment B.

1.	Fresh fibrin	Digested in 30 minutes.
2.	Fibrin placed in 0.05 per cent. formaldehyde for 3 days	... In 60 minutes.
3.	" " 0.01 " " "	... In 95 minutes.
4.	" " 0.25 " " "	... In 3 hours.
5.	" " 0.50 " " "	... In 5 hours.
6.	" " 0.10 " " "	... Had not digested at 6 hours, when the observation was stopped.

These experiments show that a percentage of 0.5 renders gastric digestion almost impossible; and that a percentage of over 0.05 considerably delays it.

2. *Influence of Formaldehyde on the Pancreatic Digestion of Proteid.*—These experiments were conducted in a corresponding way and led to a corresponding result. The fibrin, after a two-days' stay in various strengths of formaldehyde solution, was subjected to the action of artificial pancreatic juice at 40° C., and the time of digestion noted. The artificial pancreatic juice was made by mixing Benger's liquor pancreaticus with 1 per cent. solution of sodium carbonate in proportion of 1 to 4. The results are even more striking than in the case of gastric digestion. It will only be necessary to quote one illustrative experiment:

1.	Fresh fibrin	Digestion completed in 30 minutes.
2.	Fibrin previously placed in 0.05 per cent. formaldehyde	...In 95 minutes.
3.	" " " 0.10 " "	No trace of digestion at end of 24 hours.
4.	" " " 0.25 " "	
5.	" " " 0.50 " "	
6.	" " " 1.00 " "	
7.	" " " 2.00 " "	
8.	" " " 4.00 " "	

3. *Influence of Formaldehyde on the Pancreatic Digestion of Starch.*—A 1 per cent. solution of starch was prepared and divided into portions of 5 c.cm. each. To some of these were added 2 drops of formaldehyde of various strengths, and to all a few drops of Benger's liquor pancreaticus. All were placed in the warm bath at 40° C., and the time noted when the starch had disappeared—that is, owing to its conversion into dextrin and sugar, it ceased to give a blue colour with iodine. In the specimens to which no formaldehyde had been added the starch reaction was lost in one minute. In those to which formaldehyde had been added the time was prolonged to three to six minutes, according to the strength of the reagent added; even so weak an addition as 2 drops of a 0.1 solution of formaldehyde increased the digestion time to three minutes.

4. *The Action of Formaldehyde on the Rennet-curdling of Milk.*—The proportion in which formaldehyde is stated to be used in the milk trade is roughly 2 drops of formalin (40 per cent. formaldehyde) per fluid ounce.

In all cases such an addition to milk greatly delays rennet action. Various specimens of milk I have tested differ in the amount of delay. In some cases the amount added is sufficient to completely inhibit the action of the ferment; but in all cases the curd when it does form is very slow in making its appearance, and is never firm as it is in unaltered milk. Much smaller doses of formaldehyde have a similar though less marked effect; thus a few drops of a 0.2 per cent. solution of formaldehyde per ounce of milk raised the time of onset of curdling from 20 seconds to 1½ minute.

I have thought it desirable to publish my results as the question is now prominently before the public, and every additional piece of evidence has thus some value. Similar experiments have been published previously by others, for example, by Rideal and by Boyce, and although there are some differences of detail both of method and results between these experiments and my own, they all tend in the same general direction.

These experiments appear to me to prove conclusively the injurious effect produced by even minute quantities of certain preservatives on the activity of the enzymes concerned in ordinary digestion, and to furnish a cogent reason why the use of these substances should be prohibited for the preservation of food materials.

It will be noticed that all my experiments were performed *in vitro;* they require to be amplified by experiments *in vivo.* Artificial digestion experiments, however, as a rule form a very good guide to what occurs in natural digestion.

REFERENCE.

[1] *Commercial Organic Analysis*, vol. iv.

Reprinted from the BRITISH MEDICAL JOURNAL, April 12th, 1902.

THE COMPOSITION AND NUTRITIVE VALUE OF BILTONG.

BY W. D. HALLIBURTON, M.D., F.R.S.,
Professor of Physiology, King's College, London.

IN July, 1900, Major F. A. Saw, R.A.M.C., sent to the Editor of the BRITISH MEDICAL JOURNAL some samples of biltong from South Africa, accompanied by a letter from which the following are extracts:

> The biltong was made from some kind of buck, probably the hoodoo. It has not changed since I bought it about a year ago. The Boers scrape it with a knife. When grated on a nutmeg grater it is much more palatable than you would imagine from its appearance. I have never seen an analysis of biltong in any book, and I think if you can get one made it might be of interest to the profession generally.

Shortly after this the Editor sent on the specimens to me, but pressure of other work has prevented my attending to the analysis until recently. The material has kept quite well all this time; its appearance is certainly not appetizing; the strips of dried meat look very much like leather thongs. I have had it reduced to powder by means of a nutmeg grater, and the grated biltong has been kept in well-stoppered bottles. The powder from various pieces has been thoroughly mixed together, so that samples taken for analysis may give a more correct average. Although I have not actually tasted it, I can confirm Major Saw's statement that the grated material has not the unattractive appearance of the original strips.

In W. L. Sclater's *Fauna of South Africa*[1] the following account of the manufacture of biltong is given:

> The great trek-bokken, a periodical migration, is still to be witnessed in portions of Cape Colony. In 1896, for instance, on the borders of the Prieska and Hope Town divisions, the trekking spring-boks completely covered an immense extent of country, their numbers being estimated at the very lowest computation at 500,000 head. During the trek an enormous destruction takes place; all the neighbouring farmers and their people turn out and kill thousands, the skins are cured, and the flesh made into biltong, or sun-dried and cured meat for future consumption.

In carrying out the analysis I have received assistance from Miss M. Christine Tebb, Mr. C. H. Barber, B.A., and Mr. W. E. Blackall, B.A., whom I have to thank for their valuable aid. Messrs. Barber and Blackall carried out the preliminary grating and mixing of the material, the estimation of the proportion of water and solids, of inorganic and organic solids, and the examination of the ash. The estimations of nitrogen and of fat, and the experiments relating to artificial digestion, were executed by Miss Tebb.

It will not be necessary to enter fully into all the methods adopted which were the ordinary ones commonly in use. But before giving a statement of results, I purpose giving a brief

account of the analyses in a few cases. In all cases two, and sometimes three, analyses were made and the numbers given represent the average.

Proportion of Water and Solids.

Fresh meat roughly contains 75 per cent. of water. The small amount of water found in the biltong shows how very effective sun-drying is. When the total solids had been weighed after drying to constant weight at 110° C., the residue was incinerated, and the ash weighed; the organic solids are obtained by subtracting this from the total solids. The following are the figures obtained:—

Water	19.41 per cent.
Total solids	80.59 ,,
Inorganic solids	6.592 ,,
Organic solids	73.998 ,,

Constituents of the Ash.

The inorganic solids were examined qualitatively, but only a rough quantitative examination of them was made. Phosphates are the most abundant salts, then chlorides, while sulphates are present in quite small amounts. The metals present are potassium and sodium, and of these potassium is the more abundant. Calcium and magnesium are present in only small quantities, and of the two, magnesium is the more abundant; traces of iron are also found.

The organic solids are of more interest, and were examined in greater detail.

Nitrogen Determinations.

The fresh biltong was found to contain 12.445 per cent. of nitrogen; this corresponds to 15.445 per cent. of the dry material. Bernard Dyer's very simple modification of Kjeldahl's method was used.

Biltong after thorough extraction with alcohol contains less nitrogen; in the calculations this is taken to represent the proteid nitrogen[2]; the nitrogen which is present in the form of extractives soluble in alcohol is found by subtracting this from the total nitrogen. The following are the figures obtained:

	Parts Per Cent. in	
	Fresh Biltong.	Dry Material.
Nitrogen from proteids—Analysis 1	11.080	13.750
,, ,, Analysis 2	11.130	13.810
,, ,, Average	*11.105*	*13.780*
Total Nitrogen Analysis 1	12.440	15.440
,, Analysis 2	12.450	15.450
,, Average	*12.445*	*15.445*
Nitrogen in extractives—Average	*1.340*	*1.665*

The percentage of proteid calculated from the nitrogen is 65.866 for the fresh biltong, or 81.732 per cent. of the total solids

Estimation of the Carbohydrates.

The carbohydrates (sugar and glycogen) were estimated by making hot aqueous extracts of weighed quantities of the biltong. The successive extracts were mixed together and then concentrated. Sugar was estimated in this by Fehling's

solution. Another portion of the solution was then treated with sulphuric acid and another sugar estimation made. The increase of sugar was taken as the basis for calculating the amount of glycogen.[3] The fluid gave the iodine test for glycogen, though it was not markedly opalescent. The large proportion of glycogen present is an indication of the rapidity of the drying produced by the South African sun.

The mean figures in percentages of the fresh biltong are:

Sugar	0.090
Glycogen	0.133
Total carbohydrate	0.213

Estimation of the Fat.

The numbers given as fat represent the quantity of material extracted by ether in a Soxhlet's apparatus. After a first extraction in this way the remaining undissolved material was subjected to gastric digestion[4] and again extracted in a Soxhlet's apparatus. The numbers that follow represent the total amount of ethereal extract before and after gastric digestion. They are:

Analysis 1	5.26 of fat per cent.
,, 2	5.02 ,, ,,
Average	5.14 ,, ,,

This number (5.14) is the percentage of fat in the fresh material; it corresponds to 6.477 per cent. of the dry residue. This is a fairly low number; the Boers evidently select lean strips of meat for the preparation of the material; there is an absence of greasiness in the grated biltong.

Composition of Biltong.

Putting all these facts together we can now present the following table. The other organic substances which were not estimated are roughly grouped together as extractives, and their amount is obtained by subtracting the sum of the solids estimated from the total.

Water	19.410	per cent.
Solids	80.590	,,
Inorganic solids	6.592	,,
Organic solids	73.998	,,
Proteids	65.866	,,
Fat (ether extract)	5.140	,,
Glycogen	0.133	,,
Sugar	0.090	,,
Extractives (by difference)... ...	2.769	,,

Digestibility of Biltong.

The preceding figures show that chemically biltong is a valuable food; the loss of water by rapid drying renders it portable and concentrated. The large percentage of proteid points especially to its high nutritive value. Experience, moreover, has shown that it is not prone to undergo decomposition.

But mere chemical analysis is never an absolute criterion of the nutritive value of any food material, unless at the same time experiments to determine its digestibility are made also. There are many preparations which, from the chemical point of view, are highly nutritious, but which are really comparatively valueless, because their indigestibility renders it difficult for the body to utilize them.

The most satisfactory experiments from this standpoint are those performed *in vivo* upon animals, or, better still, upon man himself. Here the food taken in must be carefully

analysed, the excreta thoroughly examined, and the body weight, etc., observed. We are not aware that such experiments have ever been performed with biltong, and the small amount of material at our disposal prevented us from performing them. Still, although experiments of this nature made with scientific exactitude are lacking, an experiment on a vast scale *in vivo* is being made in South Africa, where the Boers, and to some extent our own troops, use biltong as their staple meat food. This experiment has shown that biltong at any rate possesses no marked disadvantages as an article of diet.

We have necessarily been limited to experiments made *in vitro* with artificial gastric and pancreatic juices. As a rule such experiments form a good guide to what occurs in natural digestion.

We found, as one would anticipate from *a priori* considerations that biltong is readily digestible in both varieties of artificial juice, and we can therefore safely say that it is a valuable and nutritious food.

Numerous observers have pointed out that various forms of cured meat are less easily digested than fresh flesh ; the same is true for cooked meat. But the natural digestive juices of healthy people are sufficiently powerful to overcome this disadvantage.

It was therefore a matter of interest to see quantitatively whether this is also the case for biltong.

We took the usual standard of comparison, namely, fibrin. This was first dried at 40°C. until it became something like biltong in appearance ; it was powdered and kept in stoppered bottles from which samples were taken for analysis (determination of water and solids), and for artificial digestion experiments.

Approximately equal weights (usually about 1 gram) of the fibrin and biltong were taken ; each was subjected to the action of the same amount of artificial juice ; at the end of a given time, the residue was collected, washed, dried and weighed. The numbers given are percentages of the dry residue in proportion to the dry material of the original substance.

Experiment 1.—Digestibility of fibrin and biltong in artificial gastric juice compared ; the digestion was stopped at the end of forty minutes, the temperature of the warm bath in which digestion took place was 38° C. At the end of this time 0.69 per cent. of the fibrin, and 7 91 per cent. of the biltong remained undigested.

Experiment 2.—Repetition of experiment 1 ; time of digestion thirty-five minutes—1.86 per cent. of the fibrin and 10.37 per cent. of the biltong remained undigested.

Experiment 3.—In this experiment artificial pancreatic fluid was employed instead of gastric juice. At the end of thirty-five minutes 2.76 per cent. of the fibrin and 7.40 per cent. of biltong remained undigested.

These experiments show that more residue occurred in all cases when biltong was used. The comparison, however, is not perfectly fair, because fibrin is a nearly pure proteid, and biltong contains a considerable proportion of solids which are not proteid in nature ; this will in part account for the comparatively large residue. The difference is not so marked in the case of pancreatic digestion ; this is what one would expect.

A further experiment was therefore made in which the digestibility of biltong was compared with that of another form of flesh. Miss Tebb had in stock some rabbit's muscle, which had been dried at a low temperature (30° C.) and subsequently powdered. This contained 16 per cent. of water.

Experiment 4.—Gastric digestion of dried rabbit's muscle compared with that of biltong. Time of digestion 45 minutes. At the end of this time 3.63 per cent. of the rabbit's muscle remained undigested and 13.30 per cent. of the biltong.

We here see that the digestibility of the biltong is less than that of rabbit's muscle; this probably indicates that the tropical sun had some coagulating influence on the proteids of buck's flesh.

After all, however, biltong is readily digestible, and a residue of even 13 per cent. is by no means large for a brief digestion in a flask. As stated before, digestion *in corpore* is more effective, and so our experiments, both from the chemical and physiological standpoints, show the valuable nutritive properties of the substance under investigation.

NOTES AND REFERENCES.

[1] London: R. H. Porter. 1900. [2] The number so obtained was used for calculating the amount of proteids on the basis of Chittenden's (*Zeitschr. f. Biol.*, vol. xxv, p. 358) analyses of myosin, which showed it to contain 16.86 per cent. of nitrogen. [3] Probably what is taken as glycogen includes also intermediate substances between glycogen and dextrose. [4] Dormeyer (*Pflüger's Archiv*, vol. lxi, p. 341, lxv, p. 90) has pointed out the importance of this precaution. If gastric digestion is omitted, all the fat is not extracted.

Reprinted from the BRITISH MEDICAL JOURNAL, June 29th, 1901.

AN EXPERIMENTAL INQUIRY INTO THE PATHOLOGY OF GASTRIC TETANY.*

BY

W. D. HALLIBURTON, M.D., F.R.C.P., F.R.S.,
Professor of Physiology, King's College, London.

and JOHN S. McKENDRICK, M.D., F.R.S.E.,
Extra Dispensary Physician to Glasgow Western Infirmary.

"GASTRIC TETANY," or tetany associated with dilatation of the stomach, has in recent years received considerable attention. This has been due not merely to the rarity of the complaint, but also to its high mortality, and to the vague nature of its etiology and pathology. The association of this nervous disorder with dilatation of the stomach was first described by Newman[1] in 1861, and shortly afterwards by Kussmaul,[2] and from that time up to the present frequent communications have been made on this subject. Most authors, however, have confined themselves to the minute description of cases, and few have made observations of an experimental nature so as to throw light on the causation of this obscure malady. We have recently had the opportunity of examining the gastric contents from a case of tetany with dilatation of the stomach, and of observing the results after their injection into animals.

We publish these results as we believe them to be of pathological importance, not because we desire to draw positive conclusions from a single case, but rather to indicate some of the lines of research which should be followed in the future.

I.

We have to acknowledge our indebtedness to Dr. Finlayson, of Glasgow, for giving one of us the opportunity of seeing this case, and for allowing us to experiment with the vomited matter. We regret, however, that we were unable to obtain the vomited material during the tetanoid seizure. The contents were procured during a partial convalescence.

* In the following paper Dr. Halliburton is only responsible for the experiments recorded.

The patient was a gentleman about middle life who had suffered from "his stomach" for fully fifteen years. Within the last few years he had experienced severe pain after food, which was, as a rule, relieved by the vomiting of a large quantity of sour-smelling material. He had consulted various physicians of note, and from their reports it was found that hydrochloric acid was seldom present in excess. On one examination it was absent, but it was generally normal in amount. Sarcinæ were usually present, but in various samples they were absent. He had gastric ulcer when a young man. For the last three years he has used the stomach tube two or three times a day.

The onset of his present illness was sudden, and apart from a severe frontal headache and a tingling and numbness of his fingers, he was in his usual health except for some undue constipation. He had been taking no liberties with his diet, nor had he indulged in any way in alcoholic beverages. He is totally unaware of the cause of this recent illness.

When first seen he was semi-conscious, but soon he passed into a state of coma which lasted for fully thirty-six hours. By the use of drastic medicines and injections high up into the bowel an evacuation was obtained which quickly caused the nerve symptoms to abate. The hands and fingers were in a state of contracture, forming the usual and characteristic appearance of the "accoucheur's hand." The hands were equally affected on the two sides, but the feet were free. There were no spasms of the hands and fingers (as is so often the case), but a long continual cramp or contracture which lasted during the whole period of unconsciousness. After the contracture abated the cardinal signs of Erb, Trousseau, and Chvostek could not be elicited. The heart and lungs were normal. The stomach was markedly dilated, the greater curvature passing well down below the umbilicus. The peristaltic wave was readily obtained. The urine during the tetanoid seizure contained a trace of albumin and also acetone. After the cessation of the spasm, these substances passed away and gave place to a trace of sugar. The specific gravity averaged 1028. The vomit contained acetone, hydrochloric, acetic and butyric acids, but no sarcinæ.

The following note we have from Dr. Finlayson:

> After recovering sufficiently to return to his home in England the patient was operated upon by-and-by, apparently with great success; his appearance and his report in February, 1901 (a few months after his illness), when he showed himself to Dr. Finlayson, was very satisfactory in every respect.

II.

This case presents several interesting features:

(*a*) The dilatation of the stomach resulted most probably from the cicatrisation of an old-standing gastric ulcer causing pyloric stenosis. Hydrochloric acid was found in excess in only one previous examination. It was once absent, but as a rule it was present in almost normal proportion. Sarcinæ were not always present in the contents, although they were frequently found. The chemical condition of the gastric contents may in part be accounted for by the prolonged use of the stomach tube. The patient remembers having had tingling and creeping sensations along the arms and down to the finger-tips, associated with headache and often a feeling of nausea. We believe that these were the premonitory symptoms of tetany, which were prevented from increasing

in severity by gastric lavage. We have frequently observed cases of dilated stomach, or even cases of distension of the stomach associated with hyperacidity due to hydrochloric acid, where these premonitory symptoms were present, and we believe that in all such cases gastric lavage should at once be performed.

(*b*) Associated with a strong clonic contracture of the arms and hands there was complete loss of consciousness for thirty-six hours. Although the leg muscles were not affected, we think that this case might well be classed with those of the third degree of Bouveret and Devic,[3] where symptoms resembling an epileptic attack with unconsciousness and coma are present, without, however, any convulsive phenomena. This form is not so grave as that of the second degree, when the tetanic contractures are more or less general, often without loss of consciousness, but where the muscles of the thorax become involved in the contracture, and death from asphyxia results.

(*c*) Albumin was present in the urine during the tetanoid seizure, while it completely disappeared after its cessation. Albuminuria has been noted by many observers in gastric tetany. In some cases a chronic nephritis was discovered *post mortem* (Trevelyan),[4] while in others the kidneys were healthy (Dreyfus Brissac,[5] Muller,[6] Loeb[7]). We consider that the presence of albumin in the urine during the tetanoid seizure, with its disappearance after the attack has abated, is an indication of the severity of the storm, the albuminuria being caused by an irritation of the parenchyma of the kidney by the poison circulating at the time in the blood. We are not disposed to entertain the idea of uræmia being the cause of the tetanoid seizure, although Jurgensen[8] has recently described such a case. Acetone was present in small quantity in this patient's urine. Biscaldi[9] describes a case of tetany where acetone was found in the urine, and which was considered by him to be the cause of the disease.

Numerous investigations have been made on the urine in such cases. Fenwick[10] discovered sugar in the urine, while Ewald and Jacobson,[11] and later Albu found an alkaloidal substance, which they described as a picrin salt, and to which they attributed the cause of tetany. Oddo and Sarles[12] found indican, while Gumprecht[13], after a series of elaborate investigations, found that the urotoxic co-efficient was increased in certain cases of tetany. He was unable, however, to separate the poison in such cases from the urine, so that his results were for the most part negative.

(*d*) In our patient the tetanoid seizure passed off, and during the interval of convalescence he underwent an abdominal operation. Since the operation he has been well, and he has had no return of the tetanic symptoms. Operations in such cases were clearly put before us a few years ago by May Robson[14]. After a series of operations he was able to state definitely that an immediate operation of the nature of a pylorectomy or a gastro-enterostomy relieved the symptoms, and prevented the recurrence of this grave disorder. We believe that such treatment, by preventing the retention of food stuffs in the stomach and consequently preventing their absorption into the blood, will be a radical means of curing this malady, and thus allow tetany to become a curable disease.

We would advocate, however, such operative intervention before the disease has manifested itself in the form of a

nervous disorder, provided that those nerve symptoms do not abate under gastric lavage. Moreover, it appears to us that all cases of gastric dilatation with a history of gastric ulcer should be operated on without delay, when the possibility of such a complication is imminent.

III.

The contents of the stomach from this case of gastric tetany were prepared in the following manner: The gastric contents were emptied into a beaker, and to them was added six times their volume of rectified spirit. The mixture was allowed to stand for twenty-four hours and then filtered; the filtrate was evaporated on a water bath to dryness. The residue was taken up with alcohol, filtered, and the filtrate a second time evaporated to dryness. This process was repeated twice more. The fluid was still deeply coloured brown, but gave no spectroscopic bands. This fluid was evaporated once more to dryness, but on this occasion the residue was taken up with normal saline solution. After filtration the solution was used for injection experiments. The original tetany fluid had a strong chloroform smell, was extremely acid, and the substance or substances which we investigated were odourless and soluble both in alcohol and physiological saline solution. We did no experiments with the insoluble residues or volatile materials.

IV.

This tetany fluid then, prepared as above, was injected into an animal.

Experiment A.

A cat was anæsthetised with A.C.E. mixture, and 5 c.cm. of the saline solution were injected into the external jugular vein; this was equal to 20 c.cm. of the original gastric fluid. No spasms or convulsions were produced; whether these would have occurred had not the animal been anæsthetised it is impossible to say. We are inclined to believe, however, that the alcoholic extract was weak, owing to its having been prepared from gastric fluid after the cessation of the tetanoid seizure during the convalescence of the patient. As will be seen from the tracing (Fig. 1) there was considerable heart slowing and a large fall of arterial pressure (taken from the carotid with mercury manometer).

The pressure slowly recovered its original height, but the heart beat faster than before the injection. After the blood pressure had regained its original force, the vagi were cut, and soon after 5 c.cm. of the tetany fluid were again injected. The result showed an almost negative response (see Fig. 2).

The main fall of blood pressure was therefore due to an action on the cardio-inhibitory centre, though whether the action on the centre was direct or reflex we cannot say. The fluid was acid, giving the colour tests for hydrochloric, but not for lactic acid. The amount of total acidity reckoned as hydrochloric acid, estimated by titration with standard alkaline solution was = 0.115 per 100 c.cm. of original gastric contents.

Experiment B.

In view of the question whether the acid substance or substances might cause the fall in blood pressure, the original tetany fluid was injected as before into the external jugular vein, after neutralisation (Fig. 3).

The injection only produced a slight fall of pressure, which may be due to the fact that potash was used for neutralisation instead of soda. After section of the vagi there was a similar slight effect as a result of the injection. Some acid substance, then, we take it, appears to be responsible for the marked fall of pressure in the first tracing.

V.

Bouveret and Devic,[15] in their inquiries, injected solutions

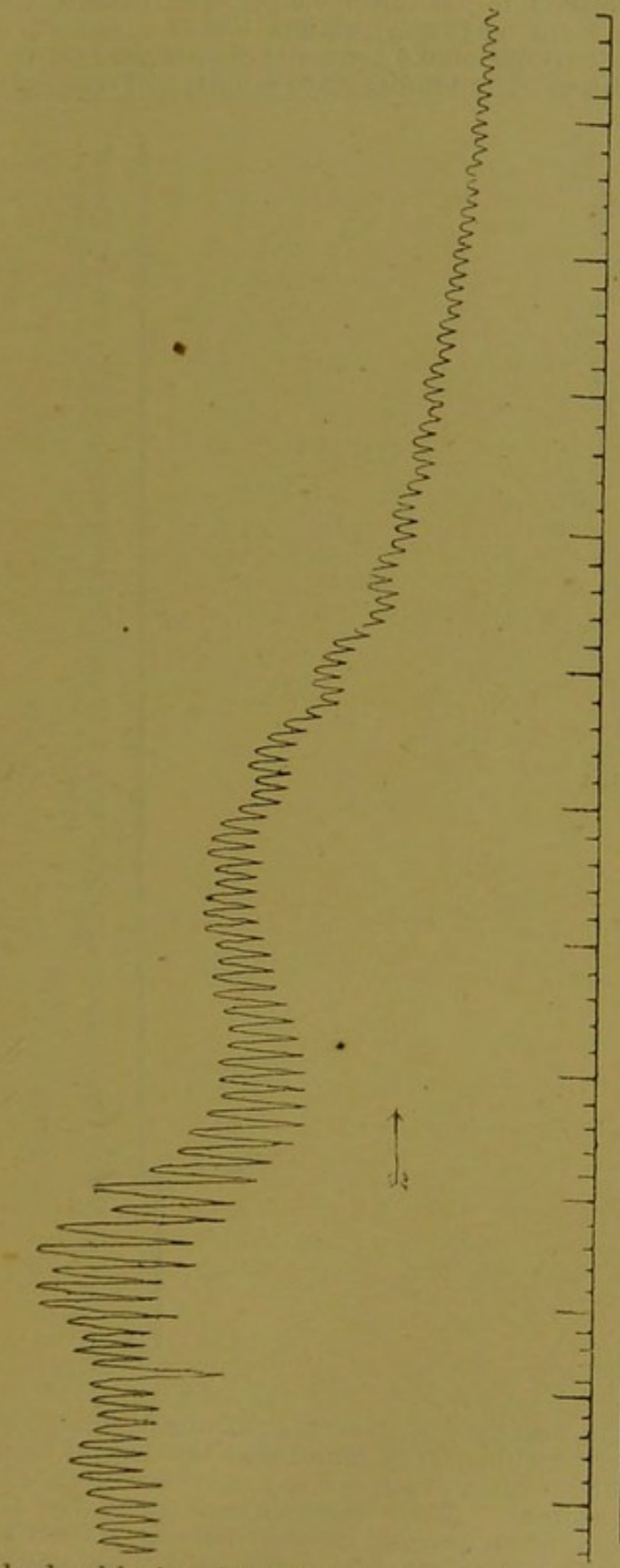


Fig. 1.—Fall of arterial blood pressure and slowing of heart in cat produced by injection of fluid from a case of tetany prepared as described in the text. The time tracing indicates seconds. In subsequent figures where the time tracing is omitted, the rate of movement of the blackened paper was the same as in this one. The raising of the abscissa (lowermost line) indicates the period during which the injection took place. This and all subsequent tracings read from left to right.

of hydrochloric acid into animals. They commenced with a solution of 0.1 per cent., but made observations with solu-

tions up to 0.9 per cent. Solution 0.1 per cent. caused a fall of temperature from 39.3° to 38.6°, but the animal was little affected by the injection. Solution 0.6 per cent. caused a fall of temperature from 39.3° to 37.8°, but likewise caused no grave symptoms. Solution 0.9 per cent. caused general convulsions and death. The temperature rapidly fell from 38.6°

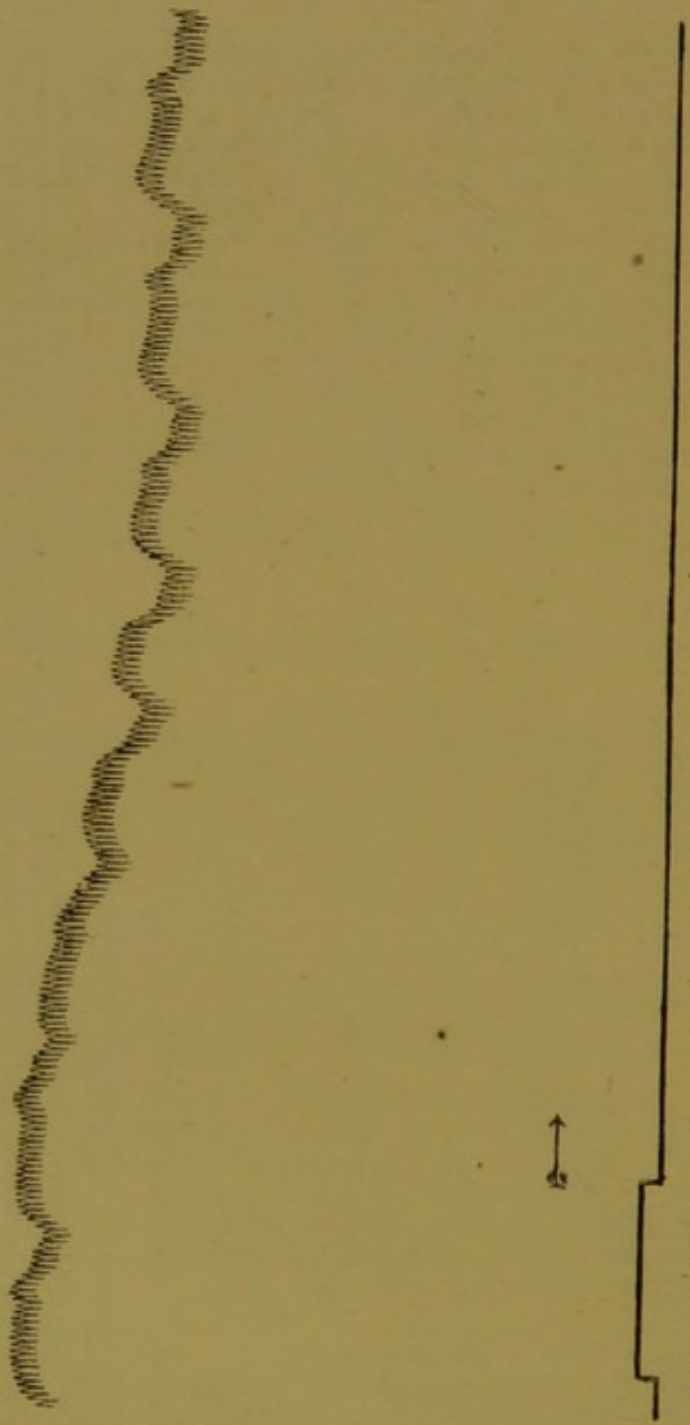


Fig. 2.—Injection of the same amount of the same fluid after the two pneumogastric nerves of the cat had been cut.

to 37.6°. At the necropsy a sero-sanguineous discharge was found in the peritoneum. They found that the dose of hydrochloric acid which causes death is 0.40 per kilogramme of the weight of the animal. Their researches then proved that hydrochloric acid, when present in fairly normal proportions (0.2 to 0.3 per cent.), if injected into the vein of an animal, caused little disturbance.

We made experiments to see the effects of the injections of free hydrochloric acid of the same strength as that of the tetany fluid:

Experiment C.

The animal was anæsthetised as before, and 2.5 to 10 c.cm. of HCl. of strength 0.2 to 0.4 were injected into the external jugular vein (Fig. 4). There is an insignificant fall of pressure, which is not abolished by section of the vagi.

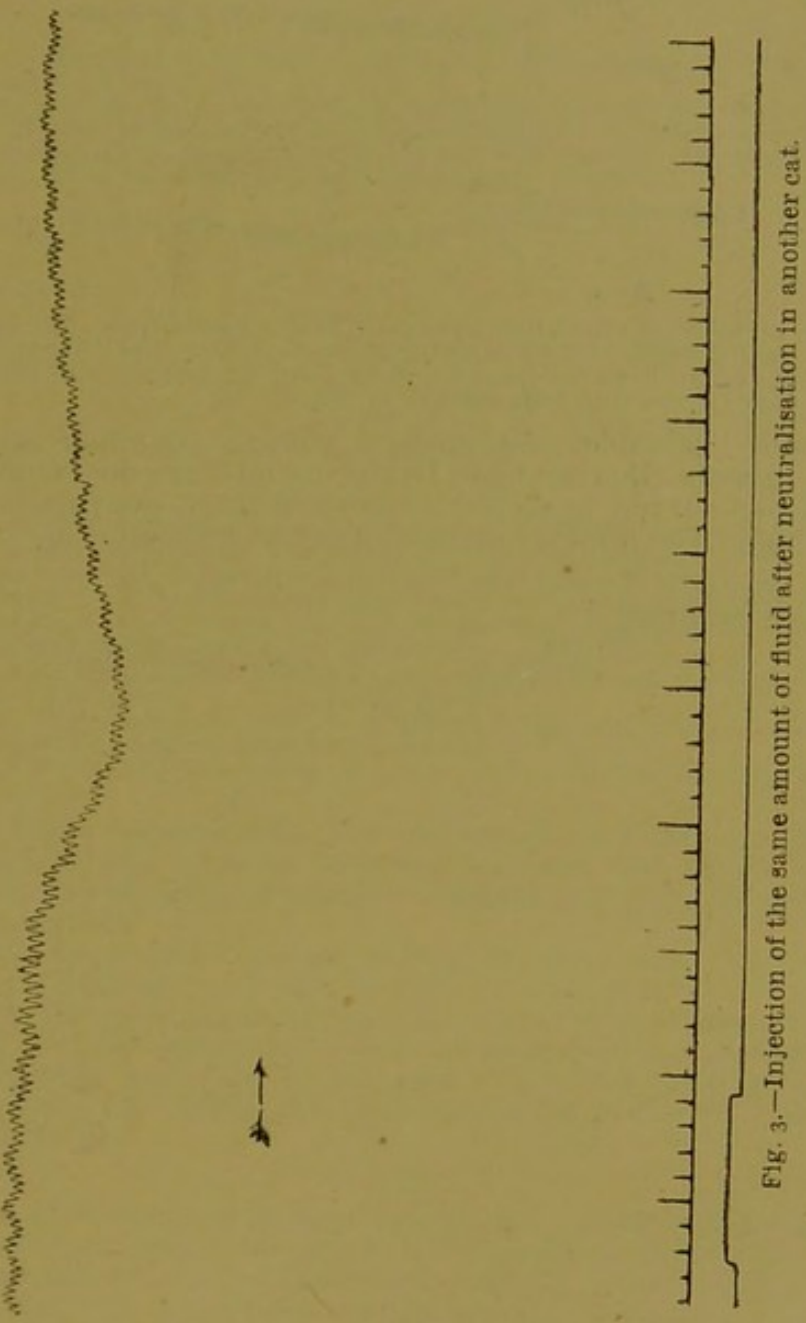


Fig. 3.—Injection of the same amount of fluid after neutralisation in another cat.

It therefore appears to us to be clear from the results of these three experiments that the tetany fluid contained a substance much more poisonous than the hydrochloric acid itself, but which was distinctly acid in reaction.

VI.

The results obtained by the injections of pepsin and peptones into the blood have been studied by various physiologists,[16] but these substances do not produce convulsions or

tetanoid contractions, except when injected in very large doses. We did not repeat these experiments, but we are satisfied that the symptoms produced by these substances are different from those produced by the tetany fluid. Certainly no peptone or pepsin was present in the fluid we injected, because it had been so repeatedly treated with alcohol.

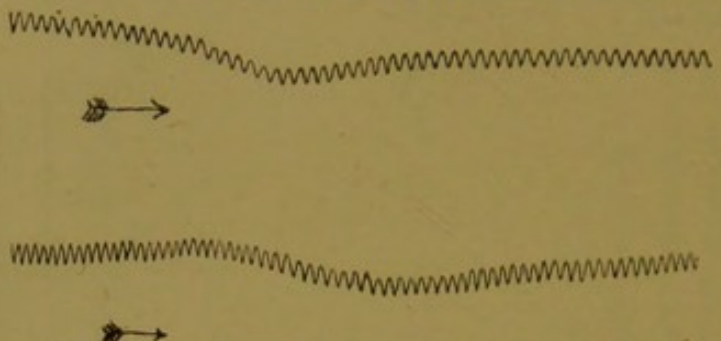


Fig. 4.—Effect of injecting 0.2 per cent. hydrochloric acid. The upper tracing was the result of an injection of 2.5 c.cm., the lower tracing of 5 c.cm. The abscissa is not shown, but the height of the tracing above it was about the same as in Fig. 5.

Alcohol itself does not produce convulsions when injected into animals. Bouveret and Devic[17] found that a dose sufficient to kill an animal produced narcosis without convulsive phenomena. The fluid we injected was free from alcohol.

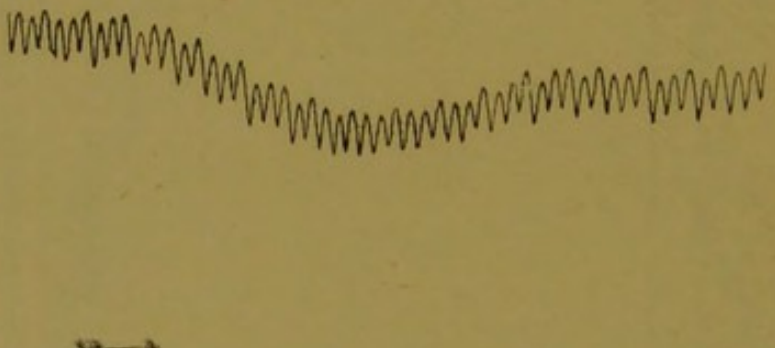


Fig. 5.—Effect of injecting the same amount of fluid from a case of mild dyspepsia.

To conclude our research we injected into animals the gastric contents obtained from patients in health or suffering only from mild dyspepsia. (Experiments D and E.) The gastric contents were prepared in exactly the same manner as above, so that 5 c.cm. of the solution injected corresponded to 20 c.cm. of the original gastric fluid.

Experiment D.

The acidity of the vomit was 0.06 free HCl. The animal was anæsthetised as before. Injections were made before and after sections of the vagi. (See Fig. 5.)

The effect is an insignificant fall of arterial pressure (Fig. 5) whether the vagi were cut or not.

Experiment E.

The acidity of the vomit was 0.13 free HCl. Four experiments were performed in this case.

1.5 c.cm of the fluid (= 20 c.cm. of the original normal gastric contents) were injected into the animal, with the result that there was a slight fall of blood pressure, accompanied by the expansion of the intestinal blood vessels. (See rise of intestinal plethysmogram. Fig. 6.)

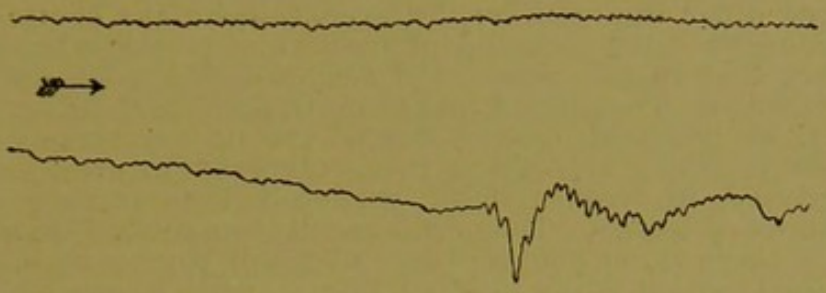


Fig. 6.—Effect of injecting the same amount of fluid from another case of mild dyspepsia. The slight fall noticed here and in Fig. 5 is about equal to that produced by dilute hydrochloric acid. The upper line is the intestinal plethysmogram; expansion of vessels is seen; dilute hydrochloric acid causes the same vascular dilatation. The abscissa of the blood pressure is omitted.

2. 5 c.cm. of 0.2 per cent. HCl were injected, giving a result almost similar to the above.

3. Vagi were cut, and 5 c.cm. of 0.2 per cent. HCl was injected, causing a rather more pronounced fall in blood pressure than in 1.

4. After vagi were cut, 5 c.cm. of gastric fluid were injected, with result similar to 3.

In these experiments the intestinal plethysmograph of Edmunds[18] was used to ascertain whether the fall of blood pressure in these cases was due to dilatation of the splanchnic blood vessels.

This apparatus has the advantage over the plethysmograph of Bayliss[19] and that of Hallion and François Frank,[20] in that the oncometric medium is air instead of oil or salt solution. In these experiments there was a rise of the plethysmograph with each fall of pressure which indicated vascular dilatation, the result, we have no doubt, of the acid in the gastric fluid.

VII.

The pathology of gastric tetany has given rise to much speculation. Some observers have confined their attention to the alterations in the cerebro-spinal system, but their investigations have been for the most part negative. Slight alterations of brain structure have been observed, with thickenings of lining membranes and inflammatory exudations, but the changes in themselves cannot account for the nervous symptoms of tetany. The theory of dehydration of the tissues generally, from the constant bouts of vomiting and from the permanent hypersecretion of the gastric juice into the stomach, has now been discarded. The reflex theory likewise receives little support from recent investigations.

We are convinced that the only plausible theory is that of autointoxication. A poisonous substance is formed in the

stomach, which, when absorbed into the blood stream in sufficient amount, gives rise to the tetanoid contractures and other concomitant symptoms which are well known in this disease.

Bouveret and Devic[21] uphold this view, and although their results have not been confirmed by some other observers, we think that their elaborate investigations have done much to clear up the uncertain prevailing ideas on this affection. Their results are briefly as follows: they associate this form of tetany to that condition of the stomach in which there is a constant hypersecretion of gastric juice. Tetany, they say, results from an autointoxication, the poisonous substance or substances being produced by a chemical change in the stomach in presence of excess of hydrochloric acid.

They differ from Brieger,[22] who considers that a peptotoxin is produced in the normal peptonisation of proteids, but they believe that in the presence of alcohol such a substance can be obtained. They found that the introduction of this peptotoxin, so prepared, caused violent tetanic convulsions of a nature precisely similar to those observed in man, and if introduced in weaker doses produced the ordinary contractures of tetany. They believe that in a dilated stomach, where there is an excess of free acid, this peptotoxin can be obtained, which when injected into animals causes convulsions. They proved this on several occasions. They do not know the nature of this peptotoxin, but they do not consider it to be either an ethylamine hydrochlorate or an ethylenediamine. They believe it to be a form of syntonin to which new properties have been added by the action of alcohol.

Gumprecht[23] declines to believe that autointoxication is the underlying cause in such cases, as he has failed to produce convulsions in animals by intravenous injection, although he has been able to separate an albumose which had toxic properties, but which he could not discover in the urine.

Ewald[24] has recently described a case where there was an entire absence of hydrochloric acid; and Sievers,[25] after making a survey of 27 cases, believes that hyperchlorhydria is not essential for the causation of gastric tetany.

We have found that a toxic substance existed in the stomach in the case under consideration, which when injected into an animal produced a marked fall of blood pressure and slowing of the heart beat. This substance was not present in the normal gastric contents of health; and, moreover, after neutralisation of the tetany fluid, practically no fall of pressure was obtained. We do not know what this toxic substance is; it has a marked acid reaction. Whether the brown colour and chloroform-like smell of the alcoholic solution are due to this material we cannot say. It is soluble in alcohol and in normal saline solution. By its injection into animals it causes either direct or reflex excitation of the cardio-inhibitory centre. It is, therefore, by no means improbable that other centres in the brain or cord are similarly excited.

As we mentioned before, the gastric contents were obtained after the patient was in a state of partial convalescence, and it is possible that had the fluid been obtained during the period of coma with contracture, this poisonous substance would have been present in greater amount, and would, when injected into the veins of an animal, have produced convulsions and death.

VIII.

We append a bibliography in chronological order. This is necessarily limited, and is confined almost entirely to reports on cases of gastric tetany. Special attention has been given to the more recent works on this interesting subject.

Bibliography.

1831—Dance, *Arch. Gén. de Méd.* 1852—Corvisart gave the name "Tetanie." 1861—Newman, *Deutsche Klinik.* 1868—Trousseau, *Clinical Medicine* (Bazire), vol. i, p. 370. 1869—Kussmaul, *Deut. Arch. f. klin. Med.*, Bd. vi. 1878—Gassner, *Thèse de Strassbourg.* 1879—Leven, *Traité des Maladies de l'Estomac*, p. 222. 1883—Galliard, *Gaz. des Hôp.* 1884—Renvers, *Berl. klin. Woch.*, p. 74; Dujardin-Beaumetz, *Thèse de Laprévotte*, Paris. 1885—Brisac, *Gaz. Hebdom.* 1887—Brieger, *Microbes. Ptomaines, et Maladies*, Traduct. française de MM. Roussy et Winter; Cheadle, *Lancet*, May; Martin, *Lancet*, January 8th. 1888—Gerhardt, *Berl. klin. Woch.* 1889—Stewart, *Amer. Journ. Med. Sci.*, 549; De Baurmann, *Bull. Soc. Méd. des Hôp.*, p. 166; Müller, *Charité-Annalen*, xiii, p. 309. 1890—Stewart, Braithwaite's *Retrospect of Medicine*, p. 180; Von Jaksch, Klin. Berträge zur Kenntniss der Tetanie, *Zeit. f. klin. Med.*, xvii; Loeb, *Deut. Arch. f. klin. Med.*; Merlin, *La Loire Médicale.* 1891—Chvostek, Ueber das Verhalten der Sensiblen Nerven, und der Hörnerven bei Tetanie, *Zeit. f. klin. Med.*, 489; Frank-Hochwart, *Tetanie*, Wien; Collier, *Lancet*, June 6th. 1892—Bouveret et Devic, Recherches Cliniques et Expérimentales sur la Tétanie d'Origine Gastrique, *Rev. de Méd.*; Biscaldi, *Rev. des Sci. Méd.*, 1, xli, pp. 147; Ewald, Hufelandsche Gesellschaft; Bamberger, *Centralbl. f. klin. Med.*, No. 47; Fenwick, *Practitioner.* 1893—Ewald, Ueber Tetanie, *Wien. med. Presse*, 858; Gowers, *Diseases of the Nervous System*; Jacobson and Ewald, *Verhandlungen des Congress für innere Medicin.* 1894—Berlizheimer, *Berl. klin. Woch*; Blazicek, *Wien. klin. Woch.*; Oddo and Sarles, *Marseille Médical*, October; Carpenter, *Journ. of Amer. Med. Assoc.*, 182; Heim, Tetanie bei Gastraktasie, *Schmidt's Jahrbuch*, 249; Schlesinger, *Wien. klin. Woch.*, 165. 1895—Fenwick, Fatal Form of Tetany, *Trans. of Clin. Soc.*, London, 13 and 38. 1896—Lothrop, *Bost. Med. and Surg. Journ.*, vol. cxxxv, No. 20. 1897—Gumprecht, *Centralbl. f. inn. Med.*; Berlizheimer, *Berl. klin. Woch.*, No. 36. 1898—Sievers, *Berl. klin. Woch.*, also *Arch. f. Verdauungs*, Berlin, Bd. iv., s. 387; Trevelyan, *Lancet*, September 24th; Souttar McKendrick, *Lancet*, September 24th; Mayo Robson, *Lancet*, November 26th; Kuckein, *Berl. klin. Woch.*, November; Peiper, *Deut. med. Woch*; Jürgensen, *Deut. Arch. f. klin. Med.*, Leipzig, Bd. lx., s. 327. 1900—Christian Simpson, *Practitioner*, London, September; Fenwick (Samuel and Soltau), *Ulcer of the Stomach and Duodenum and its Consequences*; Ury, *Deut. med. Woch.*, Leipzig, v., Berl. xxvi., 471-473 and 481-483; Montagnon, Tétanie-Guérison par les Injectiones de Sérum Artificiel, *Echo Méd. Toulouse*, 2, S. xiv., 169-172, 193-201; Grousinoff, *Klin. J. Mosk*, No. 2, 132-153.

References.

[1] *Deut. Klin.*, 1861. [2] *Deut. Arch. f. klin. Med.*, Bd. vi., 1869. [3] *Rev. de Méd.*, 1892. [4] *Lancet*, September 24th, 1898. [5] *Gaz. Heb.*, 1885. [6] *Charité-Annalen*, 1889, xiii., p. 309. [7] *Deut. Arch. f. klin. Med.*, 1890. [8] *Deut. Arch. f. klin. Med.*, Leipzig, Bd. lx, S. 327, 1898. [9] *Rev. des Sci. Méd.*, 1, xli, p. 147, 1892. [10] *Practitioner*, 1892. [11] *Verhand. des Congress f. inn. Med.*, 1893. [12] *Marseille Méd.*, October, 1894. [13] *Centralbl. f. inn. Med.*, 1897. [14] *Lancet*, 1898, November 26th. [15] *Loc. cit.* [16] Hildebrand, *Virchow's Arch.*, Bd. cxxi, 1890; W. H. Thompson, *Brit. Assoc. Reports*, 1897. [17] *Loc. cit.* [18] *Journ. of Phys.*, vol. 22, p. 380, [19] *Journ. of Phys.*, vol. xjv., p. 303. [20] *Arch. de Phys.*, 1896, (5) viii, 2. [21] *Loc. cit.* [22] *Microbes, Ptomaines, et Maladies*, Traduction Française de MM. Roussy et Winter, 1887. [23] *Loc. cit.* [24] Hufelandsche Gesselschaft. October, 1892. [25] *Berl. klin. Woch.*, 1898.

Reprinted from the BRITISH MEDICAL JOURNAL, June 15th & 22nd, 1901.

THE CROONIAN LECTURES
ON
THE CHEMICAL SIDE OF NERVOUS ACTIVITY.

[ABSTRACT.]

Delivered at the Royal College of Physicians, London, June, 1901.

BY W. D. HALLIBURTON, M.D., F.R.S., F.R.C.P.,
Professor of Physiology, King's College, London.

LECTURE I.

AFTER thanking the College for the honour of the lectureship, Dr. Halliburton alluded to the large number of times the nervous system had been selected as the subject of the Croonian lectures, either from the histological, experimental, or clinical side. The chemical side had, however, been in main neglected. Researches on the brain, the organ of mind, had been most fruitful in yielding results which are useful to those who treat disease but had not unearthed the ultimate meaning of mental processes. The scalpel and the microscope have not been successful in discovering the mind; one would also not expect to find it in the test tubes of the chemist. Nevertheless, there are a large number of interesting chemical facts in connection with the nervous system which are daily becoming more numerous, and are not devoid of pathological importance. These the lecturer wished to lay before the College, and stated that he intended especially to dwell upon those researches which had been done either by himself or by workers in his laboratory during the last few years. He did so, not because he considered them of greater importance than work which had issued from other laboratories, but because he was limited by time, and, further, because he understood that one object of the Croonian lectureship was to give an investigator the opportunity of presenting his own original work in a connected and orderly way before the medical public. He expressed his thanks to those who had worked with him, particularly to Dr. Mott and Dr. Brodie. Much of what he had to say would supplement

and extend the chemical work which Dr. Mott laid before the College in his Croonian lectures last year. The subject of his first lecture would be

The General Composition of Nervous Structures.

He first dwelt on the large quantity of water in these tissues; this is highest in the grey matter (80 to 90 per cent.), less in white matter (70 per cent.), and least in nerves (61 to 65 per cent.). These facts were illustrated by a number of analytical tables.

The solid materials of nervous tissues are extremely numerous, and a list of them was given. The most important and most abundant, and those of which we have most accurate knowledge, are the proteids and the phosphorised fats.

The percentage of proteids is especially high, and here again the lecturer had recourse to analytical tables compiled from his own observations. In grey matter the amount of proteid comprises over 50 per cent. of the solids; this is what would be expected from the fact that protoplasmic structures are most abundant there. In any nervous structure the amount of grey matter, of water, and of proteid vary directly the one with the others.

The proteids present are three in number; the most abundant is nucleo-proteid (containing 0.5 per cent. of phosphorus). There is not much of this, however, in the white matter. The other two proteids are globulins, one of which coagulates at 47° C. and the other at 70° C. The neuroglobulin which coagulates at the lower temperature is analogous to the cell globulin obtained from most protoplasmic structures, and in view of hyperpyrexia, a subject the lecturer intended to discuss at greater length later, particular attention was drawn to the low temperature at which t is converted into coagulated proteid.

The other important substance in the nerve tissues is protagon, the phosphorised fat. On decomposition it yields two substances; one of these is lecithin, the other is cerebrin or cerebrins. The cerebrins are sometimes called cerebrosides; they are nitrogenous glucosides, the sugar obtainable having been identified as galactose. The composition of lecithin was described in some detail, but the most important fact in relation to it is its difference from an ordinary fat. An ordinary neutral fat on hydrolysis yields a fatty acid and glycerine. Lecithin on hydrolysis yields not only fatty acid and glycerine, but phosphoric acid and choline in addition. The phosphoric acid contains all the phosphorus of the original lecithin; and the choline all its nitrogen. Choline is chemically an alkaloid, trimethyl-oxyethyl-ammonium hydroxide. It is somewhat toxic, and its importance lies in the fact that it is a substance which can be easily recognised by both chemical and physiological tests, and its presence indicates that lecithin, and therefore nervous tissues, have undergone disintegration.

The lecture concluded with a brief account of the cerebro-spinal fluid. This fluid differs from ordinary lymph in many particulars, but it apparently plays the part of the lymph of the central nervous system. It is characterised by its low specific gravity, its clear watery character, its paucity in proteid, and the presence in it of an unknown reducing substance which is not sugar. A number of analyses were given, and special

attention was directed to Dr. StClair Thomson's well-known case in which the fluid dripped from a patient's nostril, and which furnished a unique opportunity where the absolutely normal fluid could be examined. The observations and experiments made with this fluid by Dr. Thomson in conjunction with Dr. Leonard Hill and the lecturer were given at some length.

The importance of cerebro-spinal fluid from the pathological standpoint is that here one would naturally look for the products of brain katabolism. The normal fluid, for instance, contains the merest traces of choline and of proteid, but in pathological conditions in which the disintegrative side of metabolism preponderates over the assimilative, these materials, the chemical evidence of nervous breakdown, are much increased in quantity.

LECTURE II.

Metabolism in Nerve Tissues.

The evidence that nervous material is during activity in that state of unstable equilibrium which is designated by the term "metabolism" is rather circumstantial than direct. Nervous action stands apart from the action of most other tissues (for example, muscle) in the extraordinary economy of wear and tear which is noticeable. This is accounted for either by the fact that a very small amount of chemical change is necessary for the manifestation of activity, or else the anabolic side of metabolic phenomena is very evenly and rapidly balanced against the katabolic or disintegrative side.

The signs of action which do exist are to be found mainly at the beginnings and endings of the fibres ; (that is, the cells in brain and cord, and the end-organs in muscle and other peripheral structures), and hardly at all in the more passive transmitters of energy, the fibres themselves. The statement is usually made that nerve fibres are not capable of exhaustion.

These facts coincide with the arrangements of the vascular supply ; the centres are richly supplied with blood vessels, but in a nerve these are comparatively insignificant. The necessity for an abundant supply of oxygen to the brain is seen from the rapidly fatal effects of cerebral anæmia ; and Dr. L. Hill's experiments with methylene blue strikingly illustrate how oxygen is used up on cerebral activity. If an anæsthetised animal receives methylene blue by intravenous injection, any part of the brain thrown into action by electrical stimulation loses its blue tint, due to the formation of a colourless reduction product.

A number of observations on the reaction of nervous tissue were then described ; the normal reaction is alkaline ; on death it becomes acid ; but on activity there is little or no evidence that acid is formed. This was carefully tested in some experiments performed in conjunction with Dr. Brodie on non-medullated nerves. These nerves were selected, as the possibly masking effect of a large mass of myelin would then be absent. As a result of excessive stimulation for many hours the nerves in question never became acid. In nerve centres there is, however, a formation of acid products, especially where nerve cells are situated.

The lecturer next described Dr. Waller's experiments which have led him to the conclusion that carbonic acid is produced during the activity of nerve, and discussed his interesting theory that the medullary sheath and axis cylinder are related nutritionally, so that the absence of fatigue is due to the reintegrating action of the medullary sheath. This view is supported by the experiments of Miss Sowton on non-medullated nerves in which the gradual weakening of the electrical response on repeated stimulation indicates that fatigue can be demonstrated in nerves which do not possess a medullary sheath. As some doubt has been expressed in regard to the trustworthiness of the galvanometric response, the lecturer had in conjunction with Dr. Brodie investigated the same problem by another method. The non-medullated nerves selected were those of the spleen, and the muscular contraction of the spleen was recorded plethysmographically. The general plan of the experiments resembled that adopted by Bowditch and others in the investigation of fatigue in medullated nerves; curare is not applicable as a blocking agent, and the constant current, even when weak, produces of itself so much depression, and this lasts so long, that it also was given up. The best block ultimately found was cold. The experiments showed that fatigue does not occur, or cannot be demonstrated, in the splenic nerves.

Evidence of fatigue products can be found in the central nervous system by examining cerebro-spinal fluid. The word metabolism is so often associated with the activity of the proteid constituents of protoplasm, that one is apt sometimes to forget that other materials frequently exhibit a similar alternate or simultaneous series of anabolic and katabolic phases. In nervous structures this is particularly true for the complex phosphorised molecules of protagon. The trace of choline found in normal cerebro-spinal fluid represents the small balance on the wrong (katabolic) side of the account. Further, physiological saline solution—the most harmless of all reagents—will extract choline from perfectly fresh nervous tissues, especially from grey matter, the most active portion of nervous material. All observers do not at present agree with the lecturer that choline is the most important substance which goes into solution in such extracts, but all are agreed on its presence, which is the main point. It is also the only organic substance which, up to the present, has been satisfactorily identified. The fact that it is obtained by such simple means indicates that the protagon or lecithin from which it is derived is in a labile condition.

The importance of microchemical methods was next insisted on. The most useful of these are those associated with the names of Golgi and Nissl.

Golgi's silver-chrome method is one that has led to important results. It is on this method that the adherents of the neuron theory mainly rely. Much doubt has, however, been expressed as to its absolute trustworthiness, and certainly it is not nearly so sensitive and delicate a method as that of Nissl for demonstrating changes in nerve cells.

The significance of Nissl's granules, which are demonstrable by methylene blue, formed the subject of the next section of the lecture. Though it is doubtful whether chromatolysis occurs during ordinary fatigue, there is no doubt that excessive activity and numerous other pathological processes are

characterised by the disintegration and disappearance of Nissl's granules. Chemically the granules are composed of nucleo-proteid, and no doubt their presence is an indication of a store of energy-producing substance.

The lecture concluded by a reference to sleep, Nature's great restorer for exhausted "nerves."

Chemical theories which explain sleep by the production of soporific substances (leucomaines, reducing substances, etc.) during waking hours, depend upon the slenderest of evidence which will not stand rigid inquiry. Loeb's recent researches on rhythmical action in contractile tissues have shown that the stimulus to alternate periods of rest and action consists of certain ions. In order that rhythmic action may be maintained, a nicely balanced admixture of ions must be present. It is just within the bounds of possibility that nerve cells may be brought within the same category, and that the phenomenon of alternate periods of wakefulness and sleep may in the future be found explicable by the comparatively simple action of inorganic substances or of their constituent ions.

Some observers have sought for the meaning of sleep by examining the brains of animals which have been more or less deeply narcotised. The monilliform swellings which can then be seen on the dendrites have been taken by Demoor and others to be evidence that the dendrons have retracted, and so connections with other dendrons are interrupted. Lugaro, on the other hand, has failed to find these varicosities, and has formulated another bio-physical theory of sleep which is the exact contrary of Demoor's; he maintains that the interlacing of dendrites is much more intimate during sleep than during consciousness, and he explains sleep by supposing that the definite and limited relationships between neurons no longer exists, and these are lost or rendered ineffective by the universality of the connecting paths. Such a divergence of views is easy to understand, for they depend upon observations made by a single method (Golgi's) and that not a very trustworthy one.

A more satisfactory investigation of the effect of anæsthetics on nerve cells has been carried out by Dr. Hamilton Wright mainly in the Physiological Laboratory at King's College. The research is important, because methods other than Golgi's, especially that of Nissl, have been employed as well. In rabbits and dogs the monilliform enlargements certainly are always seen. Lugaro failed to find them because the dogs he used were not anæsthetised for a sufficient length of time; dogs are more resistant than rabbits to ether and chloroform, and changes in the nerve cells are not noticeable until the narcosis has lasted at least four hours.

Owing to the chemical action of the drug on the cells the Nissl bodies have no longer an affinity for methylene blue, and the cells consequently present what Wright calls a rarefied appearance; when this is pronounced the cells appear like skeletons of healthy cells. In extreme cases the cells look as though they had undergone a degenerative change, and after eight or nine hours anæsthesia in dogs even the nucleus and nucleolus lose their affinity for basic dyes. The change, however, is not a real degeneration, and passes off soon after the disappearance of the drug from the circulation. Even after nine hours' deep anæsthesia there is not a cell

which is permanently deformed; the branches no longer present varicosities, and all the axis cylinders as tested by the Marchi method are healthy. The action of chloroform and ether is no doubt bio-chemical; the chemical action of the anæsthetic interferes with the normal metabolic activity of the cell bodies, and this produces effects on the branches of the cell. The monilliform enlargements seen during the temporary pseudo-degenerative effects produced by the drug are exactly comparable to similar swellings of the axis cylinder produced by hydration or some chemical change of that nature, which occurs during the early stages of Wallerian degeneration. The enlargements are therefore not the primary cause of loss of consciousness, but merely secondary results of changes in the cell body.

Dogs and rabbits differ in their suceptibility to the influence of anæsthetics, and this corresponds to the readiness with which the histological changes just described are produced. The effects are probably the same in kind for all animals, man included, though they differ in degree.

One should, however, be very chary in concluding that the artificial sleep of a deeply-narcotised animal is any criterion of what occurs during normal sleep. The sleep of anæsthesia is a pathological condition due to the action of a poison; the drug reduces the chemico-vital activities of the cells, and unconsciousness is in a sense dependent on an increasing condition of exhaustion, which may culminate in death. The sleep of health, on the other hand, is not produced by a poison; it is rather the normal manifestation of one stage in the rhythmical activity of the nerve cells, and though it may be preceded by fatigue or exhaustion, it is accompanied by repair, the constructive side of metabolic action.

LECTURE III.

The lecturer stated that in his two concluding lectures he proposed to deal with the more strictly pathological side of the subject. Chemical pathology is a comparatively new branch of science, and has a great future before it. He stated that the credit of opening up a new area of research, namely, the application of chemical methods to the investigation of nervous diseases, should be largely given to his colleague, Dr. Mott. The three researches with which he proposed to deal were:

1. The Chemical Pathology of Hyperpyrexia.
2. The Chemical Pathology of General Paralysis of the Insane.
3. The Chemistry of Wallerian Degeneration.

The Chemical Pathology of Hyperpyrexia.

The experiments on this subject were described in detail, but they may be summed up by saying that they fully confirm the hypothesis that the physico-chemical cause of death from hyperpyrexia is heat coagulation of cell globulin. When

this constituent of cell protoplasm is coagulated, the vitality of protoplasm is destroyed, just as muscle loses its irritability when the corresponding proteid in that tissue is coagulated. The temperature at which such coagulation is most readily produced is 47° C. Such a temperature in animals is almost instantaneously fatal; but 47° C. (117° F.) is unknown in man. Nevertheless, proteids will coagulate at temperatures below their normal coagulating point provided the heating is continued long enough. In the case of cell globulin, coagulation can be produced by a temperature as low as 42° C. (108° F.). This chemical change in the brain substance can be demonstrated by experiments with saline extracts of that tissue, or with the "surviving" brain of animals just killed. They are coincident with the histological (chromatolytic) changes in the nerve cells which can be rendered evident by the use of the methylene blue process. The expression "coagulation necrosis" employed by Marinesco for this appearance is therefore justifiable. Marinesco and others who have employed exclusively histological methods of research have naturally failed to grasp the chemical meaning of their observations, and have consequently missed the connection of the temperature necessary to produce these changes with that of the coagulation temperature of cell globulin. Although the nerve cells are those which lend themselves most readily to the histological part of the research, it is by no means improbable (looking at the wide distribution of cell globulin) that many other cells of the body are affected by high temperatures in a corresponding manner; some varieties of what is called "cloudy swelling" are without doubt instances of coagulation necrosis.

Chemical Pathology of General Paralysis of the Insane.

The lecturer stated that his work (in connection with Dr. Mott) on this subject, had been previously published; he therefore would only describe it in general terms. He did so, in order to lead up to the consideration of the third pathological subject he proposed to deal with, and which he reserved for his final lecture.

The cerebro-spinal fluid removed from cases of general paralysis of the insane is much increased in quantity, and takes the place of the atrophied brain material. It produces when injected into the circulation of anæsthetised animals (dogs, cats, rabbits), a fall of arterial blood pressure, with little or no effect on respiration. This pathological fluid is richer in proteid matter than the normal fluid, and among the proteids nucleo-proteid is present. The fall of blood pressure is, however, due not to proteid nor to inorganic constituents, but to an organic substance, which is soluble in alcohol. This substance is precipitable by phospho-tungstic acid, and by chemical methods was identified as choline. The crystals of the platinum double salt, which, when crystallised from 15 per cent. alcohol, are characteristic octahedra, form the most convenient test for the separation and identification of this base.

The nucleo-proteid and choline doubtless originate from the disintegration of the brain tissue, and their presence

indicates that possibly some of the symptoms of general paralysis may be due to autointoxication ; these substances pass into the blood, for the cerebro-spinal fluid acts as the lymph of the central nervous system. Choline can be detected in the blood removed by venesection from those patients during the convulsive seizures which form a prominent symptom in the disease.

Normal cerebro-spinal fluid does not contain nucleo-proteid and the amount of choline is so small that it cannot be readily identified. Normal cerebro-spinal fluid produces no effect on arterial pressure; neither does the alcoholic extract of normal blood or of ordinary dropsical effusions.

The presence of choline in the pathological cerebro-spinal fluid and blood will not explain all the symptoms of general paralysis; for instance, it will not account for the fits just referred to. Its presence, however, is an indication that an acute disintegration of the cerebral tissues has occurred. If other poisonous substances are also present, they have still to be discovered.

The proof that the toxic material is choline rests not only on chemical tests, but also on the evidence afforded by physiological experiments; the action of the cerebro-spinal substance exactly resembles that of choline. Neurine, an alkaloid closely related to choline, is not present in the fluid ; its toxic action is much more powerful, and its effects differ considerably from those of choline.

Physiological Action of Choline.—The doses employed were from 1 to 10 c.cm. of a 0.2 per cent. solution, either of choline or of its hydrochloride. These were injected intravenously in anæsthetised animals. The fall of blood pressure is in some measure due to its action on the heart, but is mainly produced by dilatation of the peripheral vessels, especially in the intestinal area. This was demonstrated by the use of an intestinal oncometer. The limbs and kidneys are somewhat lessened in volume; this appears to be a passive effect, secondary to the fall in general blood pressure. The drug causes a marked contraction of the spleen, followed by an exaggeration of the normal curves, due to the alternate systole and diastole of that organ.

The action on the splanchnic vessels is due to the direct action of the base on the neuro-muscular mechanism of the blood vessels themselves ; for after the influence of the central nervous system has been removed by section of the spinal cord or of the splanchnic nerves, choline still causes the typical fall of blood pressure. The action of peripheral ganglia was in other experiments excluded by poisoning the animal previously with nicotine.

Section of the vagi produces no effect on the results of injecting choline. There was no evidence of any direct action of the base on the cerebral vessels. Choline has no effect on respiration.

The effect of choline soon passes off, and the blood pressure returns to its previous level. This is due partly to the great dilution of the substance injected by the whole volume of the blood, and may be partly due to the excretion of the alkaloid, or to its being broken up into simpler substances by metabolic processes. It does not pass as such into the urine.

If the animal has previously received an injection of atropine, the effect produced by choline is a rise of arterial pressure, accompanied by a rise of the lever of the intestinal oncometer. This observation appears to be of some importance, for it shows how the action of one poison may be modified by the presence of another. This has some bearing on general paralysis, for the arterial tension in the early stages of that disease is usually high, not low, as it would be if choline were the only toxic agent at work. After a succession of fits the tension sinks, and this may be explained as a result of choline action.

Physiological Action of Neurine.—The doses employed varied from 1 to 5 c.cm. of a 0.1 per cent. solution. These were injected intravenously. Neurine produces a fall of arterial pressure, followed by a marked rise, and a subsequent fall to the normal level. The effect of neurine on the heart of both frog and mammal is much more marked than is the case with choline; in the case of both choline and neurine, the action on the heart is antagonised by atropine. The slowing and weakening of the heart account for the preliminary fall of blood pressure. The rise of blood pressure which occurs after the fall is due to the constriction of the peripheral vessels, evidence of which was obtained by the use of oncometers for intestine, spleen, and kidney. It produces a marked effect on the respiration. This is first greatly increased, but with each successive dose the effect is less, and ultimately the respiration becomes weaker, and ceases altogether. The animal can still be kept alive by artificial repiration. In large doses neurine acts like curare on the nerve endings of voluntary muscle.

It should be mentioned that in the cases of brain atrophy referred to, the cerebro-spinal fluid was removed as a rule soon after death. Specimens removed during life by lumbar puncture give, however, the same results.

The lecture was illustrated by a number of lantern slides to show the histological changes in nerve cells referred to; and also the graphic records of the experiments on blood pressure, etc., in connection with choline, neurine, and cerebro-spinal fluid.

LECTURE IV.

The Chemistry of Nerve Degeneration.

The concluding lecture dealt with the chemistry of Wallerian degeneration, the third of the pathological questions to which the lecturer had alluded at the commencement of his previous lecture.

It has been now shown that in the disease, general paralysis of the insane, the marked degeneration that occurs in the brain is accompanied by the passing of the products of degeneration into the cerebro-spinal fluid. Of these, nucleo-proteid and choline are those which can be most readily detected. Choline can also be found in the blood. On continuing work in this direction, it was found that this is not peculiar to the disease just mentioned, but that in various other degenerative nervous diseases (combined sclerosis, disseminated sclerosis, alcoholic neuritis, and beri-beri) choline can also be

detected in the blood. The tests employed to detect choline are mainly two: (1) A chemical test, namely, the obtaining of the characteristic octahedral crystals of the platinochloride from the alcoholic extract of the blood; and (2) a physiological test, namely, the lowering of blood pressure (partly cardiac in origin, and partly due to dilatation of peripheral vessels), which a saline solution of the residue of the alcoholic extract produces; this fall is abolished or even replaced by a rise of arterial pressure if the animal has been atropinised. It is possible that such tests may be of diagnostic value in the distinction between organic and so-called functional diseases of the nervous system. The chemical test can frequently be obtained with 10 c.cm. of blood.

A similar condition of the blood was produced artificially in cats by a division of both sciatic nerves, and is most marked in those animals in which the degeneration process is at its height, as tested histologically by the Marchi reaction.

The Marchi reaction is the black staining produced by Marchi's fluid, a mixture of Muller's fluid and osmic acid, after the tissue has been previously hardened in Muller's fluid. Osmic acid by itself stains the medullary sheath of fresh healthy nerve fibres black; but if the healthy nerve fibres have been previously treated with a chromic solution like Muller's fluid, they take on a greenish-grey colour with Marchi's fluid. Neutral fat, on the other hand, such as in ordinary adipose tissue, is stained black by Marchi's fluid under all conditions; and the degenerated fat which appears in Wallerian degeneration resembles the fat of adipose tissue in this respect. The chemical explanation of the Marchi reaction is the replacement of phosphorised by non-phosphorised or neutral fat. When the Marchi reaction disappears in the later stages of degeneration, the non-phosphorised fat has been absorbed. These facts were established by chemical analysis of the nerves.

The axis cylinder participates in the fatty degeneration; and the multiplication of the nuclei of the primitive sheath is first noticed about the eighth day, and is possibly due to the irritation set up by the presence of the products of disintegration.

The main facts regarding the experiments in animals may be summarised as follows:

A series of 18 cats was taken, both sciatic nerves were divided, and the animals were subsequently killed at intervals varying from 1 to 106 days. The nerves remain practically normal as long as they remain irritable; that is, up to three days after the operation. They then show a progressive increase in the percentage of water, and a progressive decrease in the percentage of phosphorus until degeneration is complete. When regeneration occurs the nerves return approximately to their previous chemical condition. The absorption of the fatty products occurs earlier in the peripheral nerves than in the central nervous system.

This confirms previous observations by Mott and Barratt on human spinal cords, in which unilateral degeneration of the pyramidal tract by brain lesions produced an increase of water and a diminution of phosphorus in the degenerated side of the cord, which stained by the Marchi reaction.

The main results of the experiments on animals are given in the following table:

Cats' Sciatic Nerves.

Days After Section.	Water.	Solids.	Percentage of Phosphorus in Solids.	Condition of Blood.	Condition of Nerves.
Normal	65.1	34.9	1.1	Minimal traces of choline present	Nerves irritable, and histologically healthy.
1—3	64.5	35.5	0.9		
4—6	69.3	30.7	0.9	Choline more abundant	Irritability lost; degeneration beginning.
8	68.2	31.8	0.5	Choline abundant	Degeneration well shown by Marchi reaction.
10	70.7	29.3	0.3		
13	71.3	28.7	0.2		
25—27	72.1	27.9	Traces	Choline much less	Marchi reaction still seen, but absorption of degenerated fat has set in.
29	72.5	27.5	0.0		
44	72.6	27.4	0.0	Choline almost disappeared	Absorption of fat practically complete.
100—106	66.2	33.8	0.9		Return of function; nerves regenerated.

Our chemical knowledge of the process of Wallerian degeneration is thus mainly limited to what occurs in lecithin, the main constituent of myelin. This substance is broken up into its constituents, and each of these is in time removed by absorption. The products of disintegration are four in number:

1. Choline. This is removed first, and can be detected for a time in the blood.

2. Phosphoric acid. This disappears next.

3 and 4. Fatty acid and glycerine. These are the elements of a neutral fat, and the fat so formed resembles other neutral fats by giving the black Marchi reaction. In time, however, this is removed also. In the removal of the fat, certain cells, probably the hypertrophied cells of the neurilemma can be seen microscopically to play a phagocytic *rôle*.

Noll has shown another interesting fact in connection with "disuse atrophy;" that is, the change which occurs in the central end of a divided nerve before regeneration has occurred. Here there is also some diminution in the amount of protagon, though not nearly so great as in the peripheral end of the nerve.

The lecture was illustrated by a number of lantern slides, to show the relationship between the histological and chemical changes which occur in divided nerves.

Other slides were reproductions of the tracings showing the effects upon arterial pressure of the choline separated out from the blood of the cases of nervous disease mentioned, and from the blood of the cats operated on.

These and others will be reproduced, and full details of all the experiments given when the lecturer publishes these lectures in a separate form, which he hopes to be able to do shortly.

[*From the* PROCEEDINGS OF THE ROYAL SOCIETY, VOL. 68.]

"The Chemistry of Nerve-degeneration." By F. W. MOTT, M.D., F.R.S., and W. D. HALLIBURTON, M.D., F.R.S. Received March 1,—Read March 14, 1901.

(Abstract.)

We have previously shown that in the disease, General Paralysis of the Insane, the marked degeneration that occurs in the brain is accompanied by the passing of the products of degeneration into the cerebro-spinal fluid. Of these, nucleo-proteid and choline are those which can be most readily detected. Choline can also be found in the blood.

We have continued our work, and we find that this is not peculiar to the disease just mentioned, but that in various other degenerative nervous diseases (combined sclerosis, disseminated sclerosis, alcoholic neuritis, beri-beri) choline can also be detected in the blood. The tests we have employed to detect choline are mainly two: (1) a chemical test, namely, the obtaining of the characteristic octahedral crystals of the platinum double salt from the alcoholic extract of the blood; (2) a physiological test, namely, the lowering of blood pressure (partly cardiac in origin, and partly due to dilatation of peripheral vessels) which a saline solution of the residue of the alcoholic extract produces; this fall is abolished, or even replaced by a rise of arterial pressure, if the animal has been atropinised. It is possible that such tests may be of diagnostic value in the distinction between organic and so-called functional diseases of the nervous system. The chemical test can frequently be obtained with 10 c.c. of blood.

A similar condition was produced artificially in cats by a division of both sciatic nerves, and is most marked in those animals in which the degenerative process is at its height, as tested histologically by the Marchi reaction. A chemical analysis of the nerves themselves was also made. A series of eighteen cats was taken, both sciatic nerves divided, and the animals subsequently killed at intervals varying from 1 to 106 days. The nerves remain practically normal as long as they remain irritable, that is, up to three days after the operation. They then show a progressive increase in the percentage of water, and a progressive decrease in the percentage of phosphorus, until degeneration is complete. When regeneration occurs, the nerves return approximately to their previous chemical condition. The chemical explanation of the Marchi reaction appears to be the replacement of phosphorised by non-phosphorised fat. When the Marchi reaction disappears in the later stages of degeneration, the non-phosphorised fat has been absorbed. This absorption occurs earlier in the peripheral nerves than in the central nervous system.

This confirms previous observations by one of us (M.) in the spinal cord in which unilateral degeneration of the pyramidal tract by brain lesions produced an increase of water and a diminution of phosphorus in the degenerated side of the cord, which stained by the Marchi reaction.

The full paper is illustrated by tracings of the effects on arterial pressure of the choline separated out from the blood of the cases of nervous disease mentioned, and from the blood of the cats operated on.

Tables are also given of the analyses of the nerves, and drawings and photo-micrographs from histological specimens of the nerves.

A summary giving the main results of the experiments on animals is shown in the following table:—

Days after section.	Cats' sciatic nerves.			Condition of blood.	Condition of nerves.
	Water.	Solids.	Percentage of phosphorus in solids.		
Normal ..	65·1	34·9	1·1	Minimal traces of choline present.	Nerves irritable and histologically healthy.
1—3	64·5	35·5	0·9		
4—6	69·3	30·7	0·9	Choline more abundant.	Irritability lost; degeneration beginning.
8	68·2	31·8	0·5	Choline abundant.	Degeneration well shown by Marchi reaction.
10	70·7	29·3	0·3		
13	71·3	28·7	0·2		
25—27 ..	72·1	27·9	traces	Choline much less.	Marchi reaction still seen, but absorption of degenerated fat has set in.
29	72·5	27·5	0·0		
44	72·6	27·4	0·0	Choline almost disappeared.	Absorption of fat practically complete.
100—106..	66·2	33·8	0·9		Return of function; nerves regenerated.

Harrison & Sons, Printers in Ordinary to Her late Majesty, St. Martin's Lane.

British Association for the Advancement of Science.

BELFAST, 1902.

ADDRESS

TO THE

PHYSIOLOGICAL SECTION

BY

W. D. HALLIBURTON, M.D., F.R.S.,
Professor of Physiology in King's College, London,

PRESIDENT OF THE SECTION.

The Present Position of Chemical Physiology.

An engineer who desires to thoroughly understand how a machine works must necessarily know its construction. If the machine becomes erratic in its action, and he wishes to put it into proper working order, a preliminary acquaintance with its normal structure and function is an obvious necessity.

If we apply this to the more delicate machinery of the animal body we at once see how a knowledge of function (physiology and pathology) is impossible without a preliminary acquaintance with structure or anatomy.

It is therefore not surprising, it is indeed in the nature of things, that physiology originated with the great anatomists of the past. It was not until Vesalius and Harvey by tedious dissections laid bare the broad facts of structure that any theorising concerning the uses of the constituent organs of the body had any firm foundation.

Important and essential as the knowledge is that can be revealed by the scalpel, the introduction and use of the microscope furnished physiologists with a still more valuable instrument. By it much that was before unseen came into view, and microscopic anatomy and physiology grew in stature and knowledge simultaneously.

The weapons in the armoury of the modern physiologist are multitudinous in number and complex in construction, and enable him in the experimental investigation of his subject to accurately measure and record the workings of the different parts of the machinery he has to study. But pre-eminent among these instruments stands the test-tube and the chemical operations typified by that simple piece of glass.

Herein one sees at once a striking distinction between the mechanism of a living animal and that of a machine like a steam engine or a watch. It is quite possible to be an excellent watchmaker or to drive a steam engine intelligently without any chemical knowledge of the various metals that enter into its composition. In order to set the mechanism right if it goes wrong all the preliminary knowledge which is necessary is of an anatomical nature. The parts of which an engine is composed are stable; the oil that lubricates it and the fuel that feeds it never become integral parts of the machinery. But with the living engine all this is different. The parts of which it is made take up the nutriment or fuel and assimilate it, thus building up new living substance to replace that which is destroyed in the wear and tear associated with activity. This condition of unstable chemical equilibrium is usually designated metabolism, and metabolism is the great and essential attribute of a living as compared with a non-living thing.

It seems childish at the present day, and before such an audience as this, to point out how essential it is to know the chemical structure as well as the anatomical structure of the component parts of the body. But the early anatomists to whom I have alluded had no conception of the connection of the two sciences. Speaking of Vesalius, Sir Michael Foster says: 'The great anatomist would no doubt have made use of his bitterest sarcasms had someone assured him that the fantastic school which was busy with occult secrets and had hopes of turning dross into gold would one day join hands in the investigation of the problems of life with the exact and clear anatomy so dear to him.' Nor did Harvey, any more than Vesalius, pay heed to chemical learning. The scientific men of his time ignored and despised the beginning of that chemical knowledge which in later years was to become one of the foundations of physiology and the mainstay of the art of medicine.

The earliest to recognise this important connection was one whose name is usually associated more with charlatanry than with truth, namely, Paracelsus, and fifty years after the death of that remarkable and curious personality his doctrines were extended and developed by van Helmont. In spite, however, of van Helmont's remarkable insight into the processes of digestion and fermentation, his work was marred by the mysticism of the day which called in the aid of supernatural agencies to explain what could not otherwise be fully comprehended.

In the two hundred and fifty years that have intervened between the death of van Helmont and the present day alchemy became a more and more exact science, and changed its name to chemistry, and a few striking names stand out of men who were able to take the new facts of chemistry and apply them to physiological uses. Of these one may mention Mayow, Lower, Boerhaave, Réaumur, Borelli, Spallanzani, and Lavoisier. Mulder in Holland and Liebig in Germany bring us almost to the present time, and I think they may be said to share the honour of being regarded as the father of modern chemical physiology. This branch of science was first placed on a firm basis by Wöhler when he showed that organic compounds can be built out of their elements in the laboratory, and his first successful experiments in connection with the comparatively simple substance urea have been followed by numberless others, which have made organic chemistry the vast subject it is to-day.

Sir Michael Foster's book on the History of Physiology, from which I have already quoted, treats of the older workers who laid the foundations of our science, and whose names I have not done much more than barely mention. Those interested in the giants of the past should consult it. But what I propose to take up this morning is the work of those who have during more recent days been engaged in the later stages of the building. The edifice is far from completion even now. It is one of the charms of physiological endeavour that as the older areas yield their secrets to the explorers new ones are opened out which require equally careful investigation.

If even a superficial survey of modern physiological literature is taken, one is at once struck with the great preponderance of papers and books which have a chemical bearing. In this the physiological journals of to-day contrast very markedly with those of thirty, twenty, or even ten years ago. The sister science of chemical pathology is making similar rapid strides. In some universities the importance of biological chemistry is recognised by the foundation of chairs which deal with that subject alone; and though in the United Kingdom, owing mainly to lack of funds, this aspect of the advance of science is not very evident, there are signs that the date cannot be far distant when every well-equipped university or university college will follow the example set us at many seats of learning on the Continent and at Liverpool.

With these introductory remarks let me now proceed to describe what appear to me to be the main features of chemical physiology at the present time.

The first point to which I shall direct your attention is the rapid way in which chemical physiology is becoming an exact science. Though it is less than twenty years since I began to teach physiology, I can remember perfectly well a time when those who devoted their work to the chemical side of the science might

almost be counted on the fingers of one hand, and when chemists looked with scarcely veiled contempt on what was at that time called physiological chemistry: they stated that physiologists dealt with messes or impure materials, and therefore anything in the nature of correct knowledge was not possible. There was a good deal of truth in these statements, and if physiologists to-day cannot quite say that they have changed all that, they can at any rate assert with truth that they are changing it. This is due to a growing *rapprochement* between chemists and physiologists. Many of our younger physiologists now go through a thorough preliminary chemical training; and on the other hand there is a growing number of chemists—of whom Emil Fischer may be taken as a type—who are beginning to recognise the importance of a systematic study of substances of physiological interest. A very striking instance of this is seen in the progress of our knowledge of the carbohydrates, which has culminated in the actual synthesis of several members of the sugar group. Another instance is seen in the accurate information we now possess of the constitution of uric acid. When Miescher began his work on the chemical composition of the nuclei of cells, and separated from them the material he called nuclein, he little foresaw the wide practical application of his work. We now know that it is in the metabolism of cell-nuclei that we have to look for the oxidative formation of uric acid and other substances of the purine family. Already the chemical relationships of uric acid and nuclein have taught practical physicians some of the secrets that underlie the occurrence of gout and allied disorders.

With the time at my disposal, it would be impossible to discuss all the chemico-vital problems which the physiologists of the present day are attempting to solve, but there is one subject at which many of them are labouring which seems to me to be of supreme importance—I mean the chemical constitution of proteid or albuminous substances. Proteids are produced only in the living laboratory of plants and animals; proteid metabolism is the main chemical attribute of a living thing; proteid matter is the all-important material present in protoplasm. But in spite of the overwhelming importance of the subject chemists and physiologists alike have far too long fought shy of attempting to unravel the constitution of the proteid molecule. This molecule is the most complex that is known: it always contains five, and often six, or even seven elements. The task of thoroughly understanding its composition is necessarily vast, and advance slow. But little by little the puzzle is being solved, and this final conquest of organic chemistry, when it does arrive, will furnish physiologists with new light on many of the dark places of physiological science.

The revival of the vitalistic conception in physiological work appears to me a retrograde step. To explain anything we are not fully able to understand in the light of physics and chemistry by labelling it as vital or something we can never hope to understand is a confession of ignorance, and, what is still more harmful, a bar to progress. It may be that there is a special force in living things that distinguishes them from the inorganic world. If this is so, the laws that regulate this force must be discovered and measured, and I have no doubt that those laws when discovered will be found to be as immutable and regular as the force of gravitation. I am, however, hopeful that the scientific workers of the future will discover that this so-called vital force is due to certain physical or chemical properties of living matter which have not yet been brought into line with the known chemical and physical laws that operate in the inorganic world, but which as our knowledge of chemistry and physics increases will ultimately be found to be subservient to such laws.

Let me take as an example the subject of osmosis. The laws which regulate this phenomenon through dead membranes are fairly well known and can be experimentally verified; but in the living body there is some other manifestation of force which operates in such a way as to neutralise the known force of osmosis. Is it necessary to suppose that this force is a new one? May it not rather be that our much vaunted knowledge of osmosis is not yet complete? It is quite easy to understand why a dead and a living membrane should behave differently in relation to substances that are passing through them. The

molecules of the dead membrane are, comparatively speaking, passive and stable; the molecules in a membrane made of living cells are in a constant state of chemical integration and disintegration; they are the most unstable molecules we know. Is it to be expected that such molecules would allow water, or substances dissolved in water, to pass between them and remain entirely inactive? The probability appears to me to be all the other way; the substances passing, or attempting to pass, between the molecules will be called upon to participate in the chemical activities of the molecules themselves, and in the building up and breaking down of the compounds so formed there will be a transformation of chemical energy and a liberation of what looks like a new force. Before a physicist decides that his knowledge of osmosis is final, let him attempt to make a membrane of some material which is in a state of unstable chemical equilibrium, a state in some way comparable to what is called metabolism in living protoplasm. I cannot conceive that such a task is insuperable, and when accomplished, and the behaviour of such a membrane in an osmometer or dialyser is studied, I am convinced that we shall find that the laws of osmosis as formulated for such dead substances as we have hitherto used will be found to require revision.

Such an attitude in reference to vital problems appears to be infinitely preferable to that which too many adopt of passive content, saying the phenomenon is vital and there is an end of it.

When a scientific man says this, or that vital phenomenon cannot be explained by the laws of chemistry and physics, and therefore must be regulated by laws of some other nature, he most unjustifiably assumes that the laws of chemistry and physics have all been discovered. He forgets, for instance, that such an important detail as the constitution of the proteid molecule has still to be made out.

The recent history of science gives an emphatic denial to such a supposition. All my listeners have within the last few years seen the discovery of the Röntgen rays and the modern development of wireless telegraphy. On the chemical side we have witnessed the discovery of new elements in the atmosphere and the introduction of an entirely new branch of chemistry called physical chemistry. With such examples ready to our hands, who can say what further discoveries will not shortly be made, even in such well-worked fields as chemistry and physics?

The mention of physical chemistry brings me to what I may term the second head of my discourse, the second striking characteristic of modern chemical physiology: this is the increasing importance which physiologists recognise in a study of inorganic chemistry. The materials of which our bodies are composed are mainly organic compounds, among which the proteids stand out as pre-eminently important; but everyone knows there are many substances of the mineral or inorganic kingdom present in addition. I need hardly mention the importance of water, of the oxygen of the air, and of salts like sodium chloride and calcium phosphate.

The new branch of inorganic chemistry called physical chemistry has given us entirely new ideas of the nature of solutions, and the fact that electrolytes in solution are broken up into their constituent ions is one of fundamental importance. One of the many physiological aspects of this subject is seen in a study of the action of mineral salts in solution on living organisms and parts of organisms. Many years ago Dr. Ringer showed that contractile tissues (heart, cilia, &c.) continue to manifest their activity in certain saline solutions. Howell goes so far as to say, and probably correctly say, that the cause of the rhythmical action of the heart is the presence of these inorganic substances in the blood or lymph which usually bathes it. The subject has more recently been taken up by Loeb and his colleagues at Chicago: they confirm Ringer's original statements, but interpret them now as ionic action. Contractile tissues will not contract in pure solutions of non-electrolytes like sugar or albumin. But different contractile tissues differ in the nature of the ions which are their most favourable stimuli. An optimum salt solution is one in which stimulating ions, like those of sodium, are mixed with a certain small amount of those which like calcium restrain activity. Loeb considers that the ions act because they affect either the physical condition of the colloidal substances (proteid, &c.) in protoplasm or the rapidity of chemical processes.

Amœboid movement, ciliary movement, the contraction of muscle, cell division, and karyokinesis all fall into the same category as being mainly dependent on the stimulating action of ions.

Loeb has even gone so far as to consider that the process of fertilisation is mainly ionic action; he denies that the nuclein of the male cell is essential, but asserts that all it does is to act as the stimulus in the due adjustment of the proportions of the surrounding ions, and supports this view by numerous experiments on ova in which without the presence of spermatozoa he has produced larvæ by merely altering the saline constituents and so the osmotic pressure of the fluid that surrounds them. Whether such a sweeping and almost revolutionary notion will stand the test of further verification must be left to the future; so also must the equally important idea that nervous impulses are to be mainly explained on an electrolytic basis. But whether or not all the details of such work will stand the test of time, the experiments I have briefly alluded to are sufficient to show the importance of physical chemistry to the physiologist, and they also form a useful commentary on what I was saying just now about vitalism. Such eminently vital phenomena as movement and fertilisation are to be explained in whole or in part as due to the physical action of inorganic substances. Are not such suggestions indications of the undesirability of postulating the existence of any special mystic vital force?

I have spoken up to this point of physical chemistry as a branch of inorganic chemistry; there are already indications of its importance also in relation to organic chemistry. Many eminent chemists consider that the future advance of organic chemistry will be on the new physical lines. It is impossible to forecast where this will lead us; suffice it to say that not only physiology, but also pathology, pharmacology, and even therapeutics, will receive new accessions to knowledge the importance of which will be enormous.

I have now briefly sketched what appear to me to be the two main features of the chemical physiology of to-day, and the two lines, organic and inorganic, along which I believe it will progress in the future.

Let me now press upon you the importance in physiology, as in all experimental sciences, of the necessity first of bold experimentation, and secondly of bold theorising from experimental data. Without experiment all theorising is futile; the discovery of gravitation would never have seen the light if laborious years of work had not convinced Newton that it could be deduced from his observations. The Darwinian theory was similarly based upon data, and experiments which occupied the greater part of its author's lifetime to collect and perform. Pasteur in France and Virchow in Germany supply other instances of the same devotion to work which was followed by the promulgation of wide-sweeping generalisations.

And after all it is the general law which is the main object of research; isolated facts may be interesting and are often of value, but it is not until facts are correlated and the discoverers ascertain their interrelationships that anything of epoch-making importance is given to the world.

It is, however, frequently the case that a thinker with keen insight can see the general law even before the facts upon which it rests are fully worked out. Often such bold theorisers are right, but even if they ultimately turn out to be wrong, or only partly right, they have given to their fellows some general idea on which to work; if the general idea is incorrect, it is important to prove it to be so in order to discover what is right later on. No one has ever seen an atom or a molecule, yet who can doubt that the atomic theory is the sheet anchor of chemistry? Mendeleeff formulated his periodic law before many of the elements were discovered; yet the accuracy of this great generalisation has been such that it has actually led to the discovery of some of the missing elements.

I purpose to illustrate these general remarks by a brief allusion to two typical sets of researches carried out during recent years in the region of chemical physiology. I do not pretend that either of them has the same overwhelming importance as the great discoveries I have alluded to, but I am inclined to think that one of them comes very near to that standard. The investigations in question are those of Ehrlich and of Pawlow. The work of Ehrlich mainly illustrates the useful

part played by bold theorising, the work of Pawlow that played by the introduction of new and bold methods of experiment.

I will take Pawlow first. This energetic and original Russian physiologist has by his new methods succeeded in throwing an entirely new light on the processes of digestion. Ingeniously devised surgical operations have enabled him to obtain the various digestive juices in a state of absolute purity and in large quantity. Their composition and their actions on the various foodstuffs have thus been ascertained in a manner never before accomplished; an apparently unfailing resourcefulness in devising and adapting experimental methods has enabled him and his fellow workers to discover the paths of the various nerve impulses by which secretion in the alimentary canal is regulated and controlled. The importance of the psychical element in the process of digestion has been experimentally verified. If I were asked to point out what I considered to be the most important outcome of all this painstaking work, I should begin my answer by a number of negatives, and would say, not the discovery of the secretory nerves of the stomach or pancreas; not the correct analysis of the gastric juice, nor the fact that the intestinal juice has most useful digestive functions; all of these are discoveries of which anyone might have been rightly proud; but after all they are more or less isolated facts. The main thing that Pawlow has shown is that digestion is not a succession of isolated acts, but each one is related to its predecessor and to that which follows it; the process of digestion is thus a continuous whole; for example, the acidity of the gastric juice provides for a delivery of pancreatic juice in proper quantity into the intestine; the intestinal juice acts upon the pancreatic, and so enables the latter to perform its powerful actions. I am afraid this example, as I have tersely stated it, presents the subject rather inadequately, but it will serve to show what I mean. Further, the composition of the various juices is admirably adjusted to the needs of the organism; when there is much proteid to be digested, the proteolytic activity of the juices secreted is correspondingly high, and the same is true for the other constituents of the food. It is such general conclusions as these, the correlation of isolated facts leading to the formulation of the law that the digestive process is continuous in the sense I have indicated and adapted to the needs of the work to be done, that constitute the great value of the work from the Russian laboratory. Work of this sort is sure to stimulate others to fill in the gaps and complete the picture, and already has borne fruit in this direction. It has, for instance, in Starling's hands led to the discovery of a chemical stimulus to pancreatic secretion. This is formed in the intestine as the result of the action of the gastric acid, and taken by the blood-stream to the pancreas. Whether this *secretin* as it is called may be one of a group of similar chemical stimuli which operate in other parts of the body has still to be found out.

The other series of researches to which I referred are those of Ehrlich and his colleagues and followers on the subject of immunity. This subject is one of such importance to every one of us that I am inclined to place the discovery on a level with those great discoveries of natural laws to which I alluded at the outset of this portion of my Address. I hesitate to do so yet because many of the details of the theory still await verification. But up to the present all is working in that direction, and Ehrlich's ideas illustrate the value of bold theorising in the hands of clear-sighted and far-seeing individuals.

But when I say that the doctrine is bold, I do not mean to infer that the experimental facts are scanty; they are just the reverse. But in the same way that a chemist has never seen an atom, and yet he believes atoms exist, so no one has yet ever seen a toxin or antitoxin in a state of purity, and yet we know they exist, and this knowledge promises to be of incalculable benefit to suffering humanity.

It may not be uninteresting to state briefly, for the benefit of those to whom the subject is new, the main facts and an outline of the theory which is based upon them.

We are all aware that one attack of many infective maladies protects us against another attack of the same disease. The person is said to be *immune* either partially or completely against that disease. Vaccination produces in a

patient an attack of cowpox or vaccinia. This disease is related to smallpox, and some still hold that it is smallpox modified and rendered less malignant by passing through the body of a calf. At any rate an attack of vaccinia renders a person immune to smallpox, or variola, for a certain number of years. Vaccination is an instance of what is called *protective inoculation*, which is now practised with more or less success in reference to other diseases like plague and typhoid fever. The study of immunity has also rendered possible what may be called *curative inoculation*, or the injection of antitoxic material as a cure for diphtheria, tetanus, snake poisoning, &c.

The power the blood possesses of slaying bacteria was first discovered when the effort was made to grow various kinds of bacteria in it; it was looked upon as probable that blood would prove a suitable soil or medium for this purpose. It was found in some instances to have exactly the opposite effect. The chemical characters of the substances which kill the bacteria are not fully known; indeed, the same is true for most of the substances we have to speak of in this connection. Absence of knowledge on this particular point has not, however, prevented important discoveries from being made.

So far as is known at present, the substances in question are proteid in nature. The bactericidal powers of blood are destroyed by heating it for an hour to 56° C. Whether the substances are enzymes is a disputed point. So also is the question whether they are derived from the leucocytes; the balance of evidence appears to me to be in favour of this view in many cases at any rate, and phagocytosis becomes more intelligible if this view is accepted. The substances, whatever be their source or their chemical nature, are sometimes called alexins, but the more usual name now applied to them is that of *bacterio-lysins*.

Closely allied to the bactericidal power of blood, or blood-serum, is its globulicidal power. By this one means that the blood-serum of one animal has the power of dissolving the red blood-corpuscles of another species. If the serum of one animal is injected into the blood-stream of an animal of another species, the result is a destruction of its red corpuscles, which may be so excessive as to lead to the passing of the liberated hæmoglobin into the urine (hæmoglobinuria). The substance or substances in the serum that possess this property are called *hæmolysins*, and though there is some doubt whether bacterio-lysins and hæmolysins are absolutely identical, there is no doubt that they are closely related substances.

Another interesting chemical point in this connection is the fact that the bactericidal power of the blood is closely related to its alkalinity. Increase of alkalinity means increase of bactericidal power. Venous blood contains more diffusible alkali than arterial blood and is more bactericidal; dropsical effusions are more alkaline than normal lymph and kill bacteria more easily. In a condition like diabetes, when the blood is less alkaline than it should be, the susceptibility to infectious diseases is increased. Alkalinity is probably beneficial because it favours those oxidative processes in the cells of the body which are so essential for the maintenance of healthy life.

Normal blood possesses a certain amount of substances which are inimical to the life of our bacterial foes. But suppose a person gets run down; everyone knows he is then liable to 'catch anything.' This coincides with a diminution in the bactericidal power of his blood. But even a perfectly healthy person has not an unlimited supply of bacterio-lysin, and if the bacteria are sufficiently numerous he will fall a victim to the disease they produce. Here, however, comes in the remarkable part of the defence. In the struggle he will produce more and more bacterio-lysin, and if he gets well it means that the bacteria are finally vanquished, and his blood remains rich in the particular bacterio-lysin he has produced, and so will render him immune to further attacks from that particular species of bacterium. Every bacterium seems to cause the development of a specific bacterio-lysin.

Immunity can more conveniently be produced gradually in animals, and this applies, not only to the bacteria, but also to the toxins they form. If, for instance, the bacilli which produce diphtheria are grown in a suitable medium, they produce the diphtheria poison, or toxin, much in the same way that yeast-cells will produce

alcohol when grown in a solution of sugar. Diphtheria toxin is associated with a proteose, as is also the case with the poison of snake venom. If a certain small dose called a 'lethal dose' is injected into a guinea-pig the result is death. But if the guinea-pig receives a smaller dose it will recover; a few days after it will stand a rather larger dose; and this may be continued until after many successive gradually increasing doses it will finally stand an amount equal to many lethal doses without any ill effects. The gradual introduction of the toxin has called forth the production of an antitoxin. If this is done in the horse instead of the guinea-pig the production of antitoxin is still more marked, and the serum obtained from the blood of an immunised horse may be used for injecting into human beings suffering from diphtheria, and rapidly cures the disease. The two actions of the blood, antitoxic and antibacterial, are frequently associated, but may be entirely distinct.

The antitoxin is also a proteid probably of the nature of a globulin; at any rate it is a proteid of larger molecular weight than a proteose. This suggests a practical point. In the case of snake-bite the poison gets into the blood rapidly owing to the comparative ease with which it diffuses, and so it is quickly carried all over the body. In treatment with the antitoxin or antivenin, speed is everything if life is to be saved; injection of this material under the skin is not much good, for the diffusion into the blood is too slow. It should be injected straight away into a blood-vessel.

There is no doubt that in these cases the antitoxin neutralises the toxin much in the same way that an acid neutralises an alkali. If the toxin and antitoxin are mixed in a test-tube, and time allowed for the interaction to occur, the result is an innocuous mixture. The toxin, however, is merely neutralised, not destroyed; for if the mixture in the test-tube is heated to 68° C. the antitoxin is coagulated and destroyed and the toxin remains as poisonous as ever.

Immunity is distinguished into *active* and *passive*. Active immunity is produced by the development of protective substances in the body; passive immunity by the injection of a protective serum. Of the two the former is the more permanent.

Ricin, the poisonous proteid of castor-oil seeds, and *abrin*, that of the Jequirity bean, also produce when gradually given to animals an immunity, due to the production of antiricin and antiabrin respectively.

Ehrlich's hypothesis to explain such facts is usually spoken of as the *side-chain theory* of immunity. He considers that the toxins are capable of uniting with the protoplasm of living cells by possessing groups of atoms like those by which nutritive proteids are united to cells during normal assimilation. He terms these *haptophor* groups, and the groups to which these are attached in the cells he terms *receptor* groups. The introduction of a toxin stimulates an excessive production of receptors, which are finally thrown out into the circulation, and the free circulating receptors constitute the antitoxin. The comparison of the process to assimilation is justified by the fact that non-toxic substances like milk introduced gradually by successive doses into the blood-stream cause the formation of anti-substances capable of coagulating them.

Up to this point I have spoken only of the blood, but month by month workers are bringing forward evidence to show that other cells of the body may by similar measures be rendered capable of producing a corresponding protective mechanism.

One further development of the theory I must mention. At least two different substances are necessary to render a serum bactericidal or globulicidal. The bacterio-lysin or hæmolysin consists of these two substances. One of these is called the *immune body*, the other the *complement*. We may illustrate the use of these terms by an example. The repeated injection of the blood of one animal (*e.g.*, the goat) into the blood of another animal (*e.g.*, a sheep) after a time renders the latter animal immune to further injections, and at the same time causes the production of a serum which dissolves readily the red blood-corpuscles of the first animal. The sheep's serum is thus hæmolytic towards goat's blood-corpuscles. This power is destroyed by heating to 56° C. for half an hour, but returns when fresh goat's serum is added. The specific immunising substance formed in the

sheep is called the immune body; the ferment-like substance destroyed by heat is the complement. The latter is not specific, since it is furnished by the blood of non-immunised animals, but it is nevertheless essential for hæmolysis. Ehrlich believes that the immune body has two side groups—one which connects with the receptor of the red corpuscles and one which unites with the haptophor group of the complement, and thus renders possible the ferment-like action of the complement on the red corpuscles. Various antibacterial serums which have not been the success in treating disease they were expected to be are probably too poor in complement, though they may contain plenty of the immune body.

Quite distinct from the bactericidal, globulicidal, and antitoxic properties of blood is its agglutinating action. This is another result of infection with many kinds of bacteria or their toxins. The blood acquires the property of rendering immobile and clumping together the specific bacteria used in the infection. The test applied to the blood in cases of typhoid fever, and generally called Widal's reaction, depends on this fact.

The substances that produce this effect are called *agglutinins*. They also are probably proteid-like in nature, but are more resistant to heat than the lysins. Prolonged heating to over 60° C. is necessary to destroy their activity.

Lastly, we come to a question which more directly appeals to the physiologist than the preceding, because experiments in relation to immunity have furnished us with what has hitherto been lacking, a means of distinguishing human blood from the blood of other animals.

The discovery was made by Tchistovitch (1899), and his original experiment was as follows:—Rabbits, dogs, goats, and guinea-pigs were inoculated with eel-serum, which is toxic: he thereby obtained from these animals an antitoxic serum. But the serum was not only antitoxic, but produced a precipitate when added to eel-serum, but not when added to the serum of any other animal. In other words, not only has a specific antitoxin been produced, but also a specific *precipitin*. Numerous observers have since found that this is a general rule throughout the animal kingdom, including man. If, for instance, a rabbit is treated with human blood, the serum ultimately obtained from the rabbit contains a specific precipitin for human blood; that is to say, a precipitate is formed on adding such a rabbit's serum to human blood, but not when added to the blood of any other animal.[1] The great value of the test is its delicacy: it will detect the specific blood when it is greatly diluted, after it has been dried for weeks, or even when it is mixed with the blood of other animals.

I have entered into this subject at some length because it so admirably illustrates the kind of research which is now in progress; it is also of interest to others than mere physiologists. I have not by any means exhausted the subject, but for fear I may exhaust my audience let me hasten to a conclusion. I began by eulogising the progress of the branch of science on which I have elected to speak to you. Let me conclude with a word of warning on the danger of over-specialisation. The ultra-specialist is apt to become narrow, to confine himself so closely to his own groove that he forgets to notice what is occurring in the parallel and intercrossing grooves of others. But those who devote themselves to the chemical side of physiology run but little danger of this evil. The subject cannot be studied apart from other branches of physiology, so closely are both branches and roots intertwined. As an illustration of this may I be permitted to speak of some of my own work? During the past few years the energies of my laboratory have been devoted to investigations on the chemical side of nervous activity, and I have had the advantage of co-operating to this end with a number of investigators, of whom I may particularly mention Dr. Mott and Dr. T. G. Brodie. But we soon found that any narrow investigation of the chemical properties of nervous matter and the changes this undergoes during life and after death was impossible. Our work extended in a pathological direction so as to investigate the matter in the brains of those suffering from nervous disease; it

[1] There may be a slight reaction with the blood of allied animals; for instance, with monkey's blood in the case of man.

extended in a histological direction so as to determine the chemical meaning of various staining reactions presented by normal and abnormal structures in the brain and spinal cord; it extended in an experimental direction in the elucidation of the phenomena of fatigue, and to ascertain whether there was any difference in medullated and non-medullated nerve fibres in this respect; it extended into what one may call a pharmacological direction in the investigation of the action of the poisonous products of the breakdown of nervous tissues. I think I have said enough to show you how intimate are the connections of the chemical with the other aspects of physiology, and although I have given you but one instance, that which is freshest to my mind, the same could be said for almost any other well-planned piece of research work of a bio-chemical nature.

We have now before us the real work of the Section, the reading, hearing, and seeing the researches which will be brought forward by members of the Association, and I must, in thanking you for your attention, apologise for the length of time I have kept you from these more important matters.

PRINTED BY
SPOTTISWOODE AND CO. LTD., NEW-STREET SQUARE
LONDON

Regeneration of Nerves.

By W. D. Halliburton, *M.D., F.R.S., and* F. W. Mott, *M.D., F.R.S.*

Some experiments which we performed on the nerves of cats, and which had for their object the study of the process of degeneration in nerve-fibres, have led us during the past year to take up the related question of regeneration.

From the microscopic study of the distal portions of divided nerve-trunks we arrived at the conclusion that the activity of the neurilemmal cells has some relation to the development of the new nerve-fibres. These cells elongate and become connected end to end, and thus lead to the formation of what look like embryonic nerve-fibres.

Those who have worked at the regeneration of nerve-fibres may be divided into two schools: those who believe that the new fibres sprout out from the central stump of the divided nerve, and those who consider that the new fibres have a peripheral origin. Those who hold the latter view rely almost exclusively on histological evidence; a strand that looks like a nerve-fibre to the microscope cannot be a nerve-fibre unless it is shown experimentally by stimulation to be both excitable and capable of conducting nerve-impulses. Among recent writers, Howell and Huber, who have used both histological and experimental methods, have arrived at the conclusion that although the peripheral structures are active in preparing the scaffolding, the axis cylinder, the essential portion of a nerve-fibre, has an exclusively central origin. Our experiments, which have been made on monkeys, are at present incomplete, and this communication must therefore be regarded as only of a preliminary nature. But so far as we have gone at present our conclusions tend to confirm those of Howell and Huber.

One experiment which we have done was suggested to us by Professor Gotch, and the result was very striking. A large nerve was divided and the ends sutured together. After a sufficient length of time had passed, restoration of function led us to suppose that regeneration had occurred. The nerve was exposed; the union of the two ends was found to have been accomplished, and the nerve was excitable below and above the junction. A piece of the nerve was then excised a little distance below the junction, and on histological examination of this, new nerve-fibres were discovered in it. After this second operation the wound was closed up and the animal allowed to live for ten days longer. It was then killed, and the nerve both above and below the second cut was then examined; no degeneration was found in the nerve-fibres above the lesion, but there was distinct evidence of the degenerative process in the fibres of the peripheral end, which was quite inexcitable. This shows us that the degeneration process which follows the direction of growth had occurred in a peripheral direction only, and is a strong piece of evidence that growth had not started from the periphery centralwards, or at any rate that the direction of nutritive control is from the centre towards the periphery.

Other experiments which we have done illustrate the important influence of stimulus in the regenerative process. A monkey's arm was rendered immobile by the division of a number of the upper posterior roots. The anterior cornual cells from which the motor-fibres originate are thus not subjected to stimuli from the periphery, and the arm is as much paralysed as if the anterior roots had been cut. Warrington has already shown that under these circumstances the anterior horn cells undergo the chromatolytic change which is associated with inactivity. A large nerve in the arm (ulnar or median) was then divided, and the same nerve was divided on the non-paralysed side as a control experiment. The animal was finally killed; the interval between the operation and death varied in different experiments. We have already found that if sufficient time had elapsed, union of

the divided ends occurred on both sides of the body, but on the side corresponding to that on which the posterior roots had been divided the nerve was either inexcitable or required very strong Faradic stimulation to make it respond; histologically the nerve showed a much looser texture, and new nerve-fibres though present were less numerous than on the control side, where the microscope revealed that regeneration had occurred in the usual way, and the new nerve-fibres responded to stimuli readily. This tends to show the importance of stimulus to the reparative process.

PRINTED BY
SPOTTISWOODE AND CO. LTD., NEW-STREET SQUARE
LONDON

A DISCUSSION ON THE PROTEIDS WHICH MAY OCCUR IN URINE.

Introduction by W. D. Halliburton, F.R.S.

It was with considerable hesitation that I consented to open here to-night a discussion on the subject of albuminuria. I beg to thank the Society for the honour they have done physiology in selecting me for such a responsible position. I, however, feel that one who is more in touch with the clinical aspect of the subject would have been altogether more suitable. Division of labour is nowadays necessary in science as well as in the arts and crafts, but specialisation, though it has obvious advantages, is always attended with disadvantages. The clinical observer has, as a rule, neither the time nor the laboratory in which to carry out chemical and experimental researches; on the other hand, the physiological investigator has seldom the opportunity or the energy to carry on simultaneously bedside observations. The new departures which the Pathological Society is now making with such marked success will do much, I am convinced, to reduce this disadvantage to a minimum; and a free interchange of opinions and intercourse between the two sections of workers is the only way in which medicine and the institutes of medicine may mutually benefit.

It was some such feeling as this that prompted me to accept the invitation so kindly proffered to me. The Society must, however, at the outset understand that all I shall attempt to do is to open the discussion, to set the ball a-rolling in the hope that if anything valuable does arise, it will do so in the later phases of the debate, for I fear that I cannot bring before you anything startling, or, indeed, anything which is new.

The whole subject of the proteids is one which always possessed for me a singular fascination; the feeling must resemble

that of an explorer in an unknown and mysterious new country. When the country is well explored, accurately mapped, and its mysteries discovered to be mere matters of fact, travel there is still pleasant, it is in fact easier, and may lead to more useful and lasting results. So, no doubt, it will be in time when chemists and physiologists have settled all the vexed questions concerning those compounds which we are discussing to-night, compounds which lie at the root of protoplasm, and the behaviour of which is the fundamental character of every vital action. At present, however, we are all groping in the dark, and so thick does the blackness sometimes appear that one would be tempted to retire from the field, were it not for the remembrance that the darkest hours are often those that precede the dawn. The many glimmers of light that are now continually breaking out from far and near laboratories do indeed indicate that the final conquest of organic chemistry cannot be very far distant. A glance at the past will show us that some advance has been made, and our knowledge now, compared with what it was say twenty years ago, may be seen by a glance at the text-books of the two periods. During this time the properties and distinctive characters of the albumoses or proteoses have been discovered; the nucleo-proteids have been born, and are growing into important units of the proteid fraternity; the histones, too, and the protamines have been more lately included in the same family.

There is hardly a single member of this large and growing tribe that has not at one time or another been found straying into foreign lands, and the presence of these strangers, who then may become active and dangerous foes, attracts particular attention and interest when they appear within the urine, owing to the breaking down of the first line of defence in the close array of the stalwart renal epithelium.

The proteids which most frequently break through the barrier are those which occupy the frontier country, the blood; and of these the twin brethren, serum albumin and serum globulin, are the commonest invaders. But in other cases the proteids came from more distant regions, such as those which are characteristic of digestive proteolysis, the proteoses and peptones; and if we study the literature of the subject we find cases recorded where even histones, an imperfectly-known race, and other rare specimens have been detected.

It is obvious that in an address of about thirty minutes duration one cannot do justice to all branches and aspects of so wide a subject, and so I propose to restrict what I have yet to say, first, to those proteids which most frequently occur in the urine, and secondly, to a brief allusion to a few of the more common of the rarer species. I shall also exclude from consideration, the necessary proteid contamination of the urine which occurs when the blood, owing to hæmorrhage, appears there, or when pus or semen are present. Although hæmoglobin is a proteid in the wider sense, I would suggest that the subject of hæmoglobinuria and the appearance in the urine of derivatives of the blood pigment should be excluded from the discussion.

We must, however, still devote a few more remarks of a preliminary nature to the question, Is any proteid present in normal urine? The problem has passed through as many vicissitudes as the corresponding question relating to presence of sugar in the normal secretion. Both have finally received an affirmative answer, but in both cases the quantity is so small that for all practical purposes normal urine may be considered to be free from both sugar and albumin. The most recent work on this subject has been performed by K. A. H. Mörner, of Stockholm. In order to obtain a quantity of the proteid sufficient to work with subsequently, it was necessary to use many hundred litres of normal human urine. One always suspects a source of fallacy when one hears of such vast quantities of urine; they must have been collected from numerous people, and although Mörner doubtless selected his subjects with the greatest care, there is always the possibility that one or more of them may have had, if not undetected kidney disease, at least a condition of slight temporary or functional albuminuria. If this was the case, of course the whole experiment was vitiated. While we bear this in mind, one cannot help feeling that Mörner's work is the only really thorough investigation yet published, and we are bound to accept his results until they are demonstrated to be incorrect. The amount of proteid is very small, averaging 0·003 per cent. The larger quantity of this is present in suspension in the ordinary mucous cloud or nubecula; from it he separated a specific number of the mucin group, which he calls urine-mucoid. The remainder of the proteid is in solution, and consists of traces of serum-albumin, together with a small quantity of a proteid pre-

cipitable by acetic acid. This consists of nucleo-proteid, and precipitated with it was a small quantity of chondroitin-sulphuric acid, a substance which up to that time had been found exclusively in cartilage. He does not adduce any proof as to how much of the proteid leaves the body by the kidney and how much by the urinary bladder and ducts. The presumption is that the greater part, including naturally the whole of the nubecula, originates from the mucous membrane of the urinary passages. In this connection we should also mention the substance which Bondzynski and Gottlieb call oxy-proteic acid. This they find to be a small but constant constituent of human and dogs' urine, and though it is not at present a very well characterised substance, it is doubtless an immediate proteid derivative, possibly an oxidation product.

From normal urine one passes naturally next to briefly consider what is called "physiological or functional albuminuria." I always feel inclined to object to the adjective physiological, for one must regard the conditions under which it occurs as being really pathological, even though the morbid condition is not a very serious one. The most marked condition in which this occurs is after prolonged muscular exercise. It is probable that vigorous muscular work in a certain proportion of people, variously given from 3 to 16 per cent. of those examined—it may be in those whose kidneys are below par,—will produce the albuminuria by causing a temporary congestion of the kidneys; experimentally we know that interference with the circulation through the kidneys lowers the activity of the kidney-cells, and so produces a leakage of albumin. A somewhat similar condition occurs in some people after the application of cold to the body, as after a cold bath; the blood is driven into the interior of the body from the skin, and the renal vessels are thus overfilled. In some cases, again, derangements of the nervous system (which interfere with the vaso-motor regulation of the kidney vessels) and derangements of digestion and anæmia (which alter temporarily the composition of the blood or lower the vitality of the renal cells) may lead to a similar albuminuric state. The pressure of tumours or of the pregnant uterus on the renal veins will cause albuminuria, as in experiments on animals in which the renal veins are compressed; venous congestion in heart disease will act in the same way. Various poisons introduced from with-

out, like phosphorus, or morphine, or manufactured within the body, as in typhoid fever, diphtheria, and other acute specific diseases, will lower the vitality of the renal epithelium, and so allow the serum-albumin of the blood to escape. In all these milder forms of albuminuria it is not without interest to note that the proteid which appears in greatest abundance, and sometimes exclusively, is the albumin and not the globulin of the blood.

When, however, we pass to real and serious cases of kidney disease, to truly pathological cases of albuminuria, we all but invariably find (in confirmation of Senator's original statement) that the so-called albumin is in reality a mixture of serum albumin with a varying proportion of serum globulin. The amount of the two proteids together rarely exceeds 1 per cent., though it may rise as high as 4 per cent. The relation of albumin to globulin has been a good deal investigated, but so far as we know at present the matter is mainly one of theoretical interest. The practical point a physician wishes to ascertain is whether or not there is in the urine a proteid which is coagulable by heat; both albumin and globulin are precipitated on heating the urine, and the size of the coagulum gives a rough indication of the amount of proteid present.

When I say that this question of the proportion of albumin to globulin has no practical interest, I hope I may not be misunderstood. For I believe that when the subject is more fully investigated, it will be found that there are definite laws that regulate the proportion, but the observations must be very numerous before these laws are discovered. At present the experiments which have been performed are in part vitiated by erroneous methods of analysis; and in part, where correct methods have been employed, the number of experiments has been too small. For these reasons different observers have contradicted each other, and the practical physician onlookers have exclaimed, "A plague on both your proteids, let us reckon them as one." Thus some have said that a high proportion of globulin indicates a waxy kidney, while others find that this is not invariably the case. One finds in the albuminuria that occurs in diabetes the proportion of albumin to globulin is a certain fraction, while subsequent observers' figures do not come anywhere near this; some have found that the albuminuria of heart disease is charac-

terised by a small, others by a large, proteid quotient. The term proteid quotient, it may be explained in passing, means the proportion of albumin to globulin.

Still, in spite of all these contradictions, certain facts do stand out as fairly certain, and though there are exceptions even to these rules, those cases can hardly be on all fours with the average cases of Bright's disease, but on seeking, probably some very exceptional condition either of the state of the kidney, the composition of the blood, the diet of the patient, or the method of the analysis, would be discoverable. The rules that may be laid down are the following:

1. That in the great majority of cases albumin is more abundant, often much more abundant, than globulin.

2. That this is much more marked in slight cases, and in cases of so-called physiological albuminuria than in severe cases.

3. That a high proportion of globulin usually indicates severe disease of the kidney.

4. That where both proteids are present there is usually found a rough correspondence between the proteid quotient of the blood and that of the urine.

5. That the proteid quotient of the blood is a very variable quantity. It varies in different animals, in the same animal or persons at different times, and such differences are not proved to be related to differences in the kind of nutriment ingested.

6. That the proteid quotient in the urine is still more variable than that in the blood, and that these variations do not necessarily correspond to variations in the proteid quotient of the blood.

These can hardly be called rules, but even to these there are exceptions; thus one will sometimes find, even in an apparently mild case, that the amount of globulin exceeds that of albumin, and occasionally one may find that albumin is absent, and globulin the only proteid present. This was so in one case out of forty examined by Hammarsten. It was also so in the well-known case published by Byrom Bramwell and Noël Paton, in which a globulin was curiously enough separated in a crystalline form. Albumins have been frequently crystallised, but this is the only hitherto recorded instance in which a globulin has been

obtained either spontaneously or artificially in a crystalline condition.

But if we consider the general rule rather than the exceptions, how do we account for the fact that albumin is so much more abundant in the urine than globulin? In answering such a question two obvious courses are open to one; one may say that it is due to the vital action of the kidney cells. This explanation is no explanation at all; it is merely a confession that we do not understand it, and can never hope to understand it, but we will shelter ourselves beneath a mystic expression. The other course is to attempt to find an explanation on chemico-physical grounds. From this standpoint we must regard the leakage of proteid from the blood or lymph into the urine as due to physical factors —first, the pressure exercised by the blood on the renal epithelium; secondly, the permeability of the renal cells; and thirdly, the size of the molecules that pass through. When the kidney cells are in a healthy state they are practically impermeable to the large molecules of the blood proteids. When they are damaged, especially if the blood-pressure is high, they will let the proteid molecules through. But they will let through in larger quantity the smaller proteid molecules in preference to the larger ones. It has been shown by some very careful experiments by Gottwalt, which were confirmed by Noël Paton, that serum albumin passes under pressure through membranes more readily than serum globulin does. It is, therefore, not at all surprising to find that in normal urine the only blood-proteid present, and that in mere traces, is serum albumin; that in cases of mild or so-called physiological albuminuria the amount of globulin is almost negligible; and that it is not until the kidney cells are so injured by chronic disease, or by a severe acute attack, that the amount of globulin becomes considerable.

If we pursue the same line of argument we may next proceed to an examination of a new question, which is this:—In the blood-plasma the proteids in solution fall into two categories—(1) the serum albumin (or serum albumins, for there are probably several) comprises the proteids soluble in water, coagulable by heat, and non-precipitable by saturation with such a neutral salt as magnesium sulphate: (2) the globulins; these are also coagulable by heat, but are insoluble in water, and readily precipitable by excess of various neutral salts. They are two in

number; one of them, present in largest quantity, is the serum globulin we have just been talking about; the other is the mother substance of fibrin, and is called fibrinogen. The question to which we have been leading up is, Why does fibrinogen hardly ever pass into the urine? I imagine different people would answer this question in very different ways. Some might say that there is such a little fibrinogen in the blood (only 0·3 out of a total proteid percentage of 8) that if it passed into the urine it might escape detection, being masked by the relatively large amount of other proteids present. In answer to this I would reply that when fibrinogen is present, especially if it becomes converted into fibrin in the urine, its existence is unmistakable. Others might deny my premises altogether, and say that fibrinogen is often present; they would point to work by Lauder Brunton and D'Arcy Power, by Führy-Snethlage, by Gerhardt and by Pohl, who found in many specimens of albuminous urine a proteid coagulated by heat at the low temperature of 56° C. In answer to this I would point out that the mere coagulation temperature of a proteid is a most untrustworthy guide to its identity. I think I may speak with some authority on this subject. In its proper place, and in combination with other reactions, coagulation temperature has its proper value, but it varies greatly for the same proteid under varying conditions. The boiling point of water is 100° C.; this is a fairly fixed quantity; but take the water up a mountain and it no longer boils at 100° C., the conditions of the observation having been changed. In the case of proteids, no doubt their coagulation temperature is a fixed point under the same conditions; the difficulty is to get the conditions the same, for slight variations in the composition and concentration of the fluid in which they are dissolved will shift the coagulating point tremendously.

I of course know that cases of fibrinuria do occur sometimes, and there is, also, but little doubt that the substratum of a urinary cast is a slow formation of fibrin from fibrinogen, which exudes slowly through the walls of the damaged tubules; but the main contention with which I started appears to be indisputable, that fibrinogen is generally absent in the urine in cases where the other blood-proteids are present.

I should be inclined to explain this by an extension of the theory concerning molecular size. Though the size of the

fibrinogen molecule has not been accurately gauged, I gather from *a priori* considerations that it must exceed that of the other blood-proteids. When some does pass through, the renal epithelium is in a very bad way and we get urinary casts; when more passes through, so as to cause a clot of fibrin throughout the urine, the damage to the kidney is even more severe; thus we see it sometimes in chyluria, where the kidney or the urinary passages are pervious not only to big molecules of proteid, but even to microscopically visible particles of fat. We get it after the administration of cantharides, when the kidney bleeds; we get it in certain growths of the bladder, which naturally give rise to a lymph-like exudation from their surface; and we get it sometimes, as in a case recorded by Dr. D. M. Greig, of Dundee, where there is an exudation of liquor sanguinis in the kidney itself.

I should lay it down as a general rule, that in proteid substances the molecular size varies directly as its readiness of precipitation by neutral salts or by alcohol, and indirectly as its temperature of heat coagulation. If we contrast the proteids with the carbohydrates, we there find much the same state of things; the colloid carbohydrates, starch and glycogen, are substances with heavy molecules, and they are readily precipitable by neutral salts and by alcohol; the crystalline carbohydrates, like the sugars, have small molecules and are not precipitable by either group of reagents; the dextrins have molecules of intermediate size, and their precipitability is also intermediate in degree.

I know it is always risky to prophesy about anything in physiology; I hope some day to have time to put my ideas to the test of experiment, and so confirm or correct them. At present it is merely a working hypothesis to consider that the low coagulation temperature of fibrinogen, the readiness with which it can be salted out from solutions, and its tendency to pass into an insoluble condition (fibrin) with slight provocation, are all indications of its extreme colloidal nature; and that the comparative rarity or scarcity of its appearance in the urine, even when the other blood-proteids can traverse the renal cells, is readily explicable on the ground of the huge size of its molecules.

Now let us pass with a leap from the heaviest to the lightest

proteids, and briefly consider the principal known facts in relation to the appearance in the urine of the proteoses and peptones.

It is well known that although these substances are formed in the alimentary canal, they do not pass as such into the blood. Even during the active digestion of the most succulent of beef-steaks, not a trace of proteose or peptone is found either in the portal blood or in the lymph, still less in the urine. The columnar epithelium that lines the alimentary canal is the agent by which the harmful peptone or proteose is regenerated or synthesised into the useful albumin and globulin. In diseases of the alimentary canal in which this lining membrane is disintegrated or diseased, it seems possible that some of the morbid symptoms might be accounted for by supposing that the poisonous products of proteolysis pass through into the blood. If this is so, they should leave the body by the urine, for if in animals "peptone" is injected into the circulation it is not wholly utilised, but is in great measure cast out as a foreign substance by the kidneys. I have long been on the look out, both in urines submitted to me and in current literature, but have never yet come across a well-attested case of this kind; even in cholera and other severe forms of intestinal trouble, peptonuria does not appear to have been noticed. It is possible in such cases where the epithelium has vanished, the lymph cells beneath it may form a second line of defence, and carry out what they do not appear to do under normal circumstances, namely, the "regeneration of albumin."

Still peptonuria does occur, and the proteolysis that leads to the formation of the peptone does not occur within the alimentary canal, but in the tissues or in new growths, and here micro-organisms are the digestive agents. The condition occurs in a great variety of diseases, but the majority are those in which suppuration is a prominent pathological process.

Though one still uses the term peptonuria, it is now recognised that peptone in the correct sense of the term is hardly ever or never found. Urinary peptone is one of the proteoses, namely, deutero-proteose, which is the proteose nearest to the peptones, both in its reactions and its method of formation.

When true peptone is injected into the blood it appears as such in the urine. When deutero-proteose is injected into the blood

of carnivorous animals it appears in the urine as peptone; this is not found to be the case with herbivorous animals like rabbits. The carnivorous urine is rich in pepsin, but such urine produces no further digestive action when mixed with proteoses, first, because free acid is absent, and secondly, because many of the salts of urine exert an inhibitory influence on the ferment. Neumeister suggests that the change occurs in the act of secretion, when there is a momentary occurrence of free acid.

The human being appears in this particular to resemble the rabbit rather than the dog. If any proteolytic action occurs in the kidney itself converting primary proteoses into secondary (*i. e.* deutero-) proteoses, the action does not proceed so far as to lead to the formation of true peptone.

We must distinguish next between this condition which may still be called peptonuria, and the condition generally termed albumosuria. The condition was first described by Bence Jones, and the proteid is still often labelled Bence Jones albumin. This is a very rare condition, and the characters of the proteid are closely akin to, though not absolutely identical with, those of Kühne's hetero-proteose, one of the primary proteoses, distinguished from the other primary proteose, proto-proteose, by its insolubility in pure water. There have only been about a dozen cases of this condition which have been adequately described. They were nearly all in males, and in three cases the disease was osteomalacia. In Ribbink's case there was a sarcomatous growth in bones and other parts; the marrow was red and jelly-like, and the bones exhibited osteoporosis. In other cases, again, the disease was multiple myeloma of the bones. In all cases bone disease of one kind or another was present. A full description of the urine in five of these cases is given in the last edition of Neubauer and Vogel's *Analyse des Harns*, and what the reader will perhaps be most struck with are the differences in the character of the proteid in the various cases. I think this is in part to be explained by the admixture of the albumose (or globulose as Kühne appears to think it was in his case) with nucleo-proteid; some of the analyses certainly show the presence of phosphorus.

Huppert, who is the editor of the last edition of the book just mentioned, includes a sixth case, namely, that of Byrom Bramwell and Noël Paton. This was not a case of bone disease, and the

proteid of the urine was globulin in a crystalline form. It is curious that this mistake should have occurred. Huppert concluded the proteid was hetero-proteose on entirely insufficient grounds, namely on *a priori* reasoning from Noël Paton's paper. When he actually examined the proteid sent to him by Noël Paton he admitted the accuracy of Paton's description, and withdrew his previous opinion. I suppose this was too late for him to include it in the work on urinary analysis which he edited.

I understand, however, that other instances of this curious condition are to be brought before us this evening. Dr. Hutchison brought before the Physiological Society a short time ago a preliminary account of his case.[1] So far as my memory serves me, it was a case of bone disease (sarcoma) and the urinary proteid, though presenting some puzzling characters, agreed on the whole with the hetero-proteose of other observers. Dr. Bradshaw's case was also one of bone disease (myeloma); here, again, the proteose, though nearest in its reactions to hetero-proteose, possessed some characteristic features of its own.[2]

I have now nearly completed what I have to say. I have not attempted to do more than present an outline sketch of the subject. I have endeavoured to mention the principal proteids that may pass under abnormal circumstances into the urine, and have alluded to one or two which seldom make their appearance there. Under this last head I should like to include caseinogen. It is well known that lactose is frequently to be found in the urine during lactation. It is perhaps not remarkable that caseinogen, the other main characteristic constituent of milk, should be conspicuous for its absence, for of all known proteids in which the question has been specially investigated, caseinogen is the most indiffusible; it will not pass, unless its molecular aggregates have been broken up by the use of an alkali, like soda, through the pores of a Chamberland filter under pressures which permit the pressure-filtration of egg albumin, serum albumin, or even of the serum globulin of some animals (D. F. Harris).

The clinical significance of albumin and other proteids in the urine I have hardly endeavoured to touch, merely because I have not felt confidence in my ability to do so with the justice such an important subject demands. I hope, however, that this will not lead others to follow my example in the discussion. As

[1] See p. 146. [2] See p. 140.

I stated at the outset, the usefulness of a debate such as this will be greatly impaired if the pathologists of the bedside do not compare notes with the workers in the laboratory.

I have also purposely avoided any description of methods of analysis. I have felt it necessary to do this merely from considerations of time. It will, however, be useful if we could hear what tests are nowadays considered the most trustworthy. A new test for albumin in urine is one of the most frequent headings of papers on albuminuria, and it has been my fate to examine a large number of these new tests. Although I am not naturally conservative, I feel bound to say that in my experience this is a case where the old is the best. I consider that as general working tests, and those that one can absolutely trust, nothing can beat the time-honoured boiling test after acidulation with acetic (not nitric) acid, and Heller's well-known nitric acid reaction. Special cases of the rarer proteids naturally must be examined by all the modern armament of recent methods and latest knowledge. But it is always the common cases that possess the most important interest to the practitioner, and though the intelligent physician is always on the look out for the rare cases, it is one of the disappointments of life to discover how seldom they arise.

I hope what I have been able to say will form a stimulus to those who follow me to say something more interesting, and in sitting down I have again to express my gratitude to the Society for giving me the opportunity of placing my ideas on proteids in the urine in a consecutive manner before such an important section of the medical profession.

PRINTED BY ADLARD AND SON, BARTHOLOMEW CLOSE, E.C.

[*Reprinted from the Journal of Physiology.*
Vol. XXVI. Nos. 2 & 4, February 28, 1901.]

THE PHYSIOLOGICAL EFFECTS OF EXTRACTS OF NERVOUS TISSUES.

By W. D. HALLIBURTON, M.D., F.R.S., *Professor of Physiology, King's College, London.* (Twenty-one Figures in Text.)

(*From the Physiological Laboratory, King's College, London.*)

Schäfer and Moore[1] appear to have been the first who noticed that an extract of brain, when injected into the circulation, produces a fall of blood-pressure. Mott and I[2] suggested that this was probably due to cholin, especially as both cholin and brain extract produce a remarkable increase of the splenic waves. We were still further strengthened in this view by the work of Gulewitsch[3] which appeared about the same time. This observer found cholin by chemical tests in extracts of fresh ox brain, and naturally found more after the lecithin had been broken down by a process of previous saponification. Gulewitsch further found other basic substances in his extracts which were not identified; neurine however was absent.

Other workers at the same or allied subjects are:—I. Ott[4], who has shown that extracts of spinal cord produce a lowering of blood-pressure; Cleghorn[5], who finds that extracts of sympathetic ganglia have a similar action; Schäfer and S. Vincent[6], who find that extracts of the nervous portion of the pituitary body contain both a pressor and depressor substance; Howell[7], who has also worked at the pituitary; Hunt[8], who agrees with my contention that the substance chiefly responsible for the fall of blood-pressure in extracts of brain and sympathetic ganglia is cholin.

[1] This *Journal*, xx. p. 26. 1896.
[2] *Phil. Trans.* cxci. B. p. 243. 1899.
[3] *Zeit. physiol. Chem.* xxvii. p. 50. 1899.
[4] *Contrib. from the Physiol. Lab. Medico-Chir. College, Philadelphia*, 1898–9.
[5] *American Journ. of Physiol.* ii. p. 471. 1899.
[6] This *Journ.* xxv. p. 87. 1899.
[7] *Journ. of Exper. Medicine*, iii. p. 2. 1898.
[8] *Proc. Amer. Physiol. Soc.* Twelfth meeting, 1899. *Amer. Journ. of Physiol.* iii. p. xviii. 1900.

I have now made a considerable number of experiments on the subject and it was particularly in view of some of Cleghorn's statements that these were originally undertaken. In his first paper he advances no opinion as to the chemical nature of the depressor substance contained in extracts of the sympathetic ganglia, but in a second paper[1] he states it cannot be cholin, as the effect is not abolished by atropin. I propose to examine his evidence for this statement later. He also says that extracts of other portions of the nervous system (nerve, brain, cord, spinal ganglia) have no effect.

I made a preliminary statement of my results at a meeting of the Physiological Society last year[2]. At the same meeting W. A. Osborne and S. Vincent[3] read a paper on the same subject. We arrived at the same result, namely that extracts of all the portions of the nervous system named produced a considerable but temporary fall of blood-pressure. The only point on which we differed was unfortunately a fundamental one; they admitted cholin was present in their extracts, but did not admit that it was the principal cause of the fall of pressure because such fall was not lessened, or only slightly lessened by the previous administration of atropin. I have made no experiments with extracts of pituitary, so that that subject need not come under discussion.

I have made altogether twenty-three experiments. Some of these were performed previous to the meeting just referred to, and some have been done since. Cats anæsthetised with A.C.E. mixture were used throughout.

Action of extracts from ganglia.

It will be convenient to take these first. Cleghorn prepared his extracts from either fresh or dried sympathetic ganglia, and used either glycerine or physiological saline solution as the extracting agent. He used 1 c.c. of the extracting agent for every gramme of ganglia. Before using the glycerine extract for injection he diluted it with saline solution; he does not state the amount of dilution. He usually employed the glycerine method.

I have repeated these experiments, and can fully confirm Cleghorn's statement that a temporary fall of blood-pressure is always observed. In using the glycerine extracts, these were always diluted with physio-

[1] *Journ. Boston Society of Med. Science*, iv. p. 239. 1900.

[2] *Proc. Physiol. Soc.* Feb. 17, 1900. This *Journal*, xxv. p. vii.

[3] *Ibid.* p. ix. They subsequently published their results more fully in this *Journ.* xxv. p. 283.

logical saline solution, so that the proportion of glycerine was 1 in 20. Boiling the solution, and previous section of the vagi, make no difference in the experimental result.

Precisely similar results were obtained from spinal ganglia. The sympathetic and spinal ganglia used were obtained from cats.

My experiments fully confirm those of Cleghorn, that the active substance produces its effect, partly by action on the heart, and partly by causing a dilatation of the peripheral arterioles; and that this dilating action is due to action on the local neuro-muscular mechanism of the vessels themselves. In all these points the similarity to cholin is apparent. Further with saline extracts it can be shown that this action is abolished by atropin. Figs. 1, 2, 3, and 4 illustrate this. Fig. 1 shows the fall of pressure produced by the injection of 3 c.c. of a saline extract of sympathetic ganglia. The extract was made by boiling the crushed fresh ganglia with physiological saline solution, in the proportion of 5 c.c. of saline with every gramme of ganglia. Fig. 2 shows the

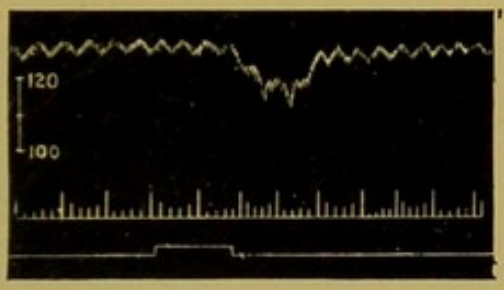


Fig. 1.

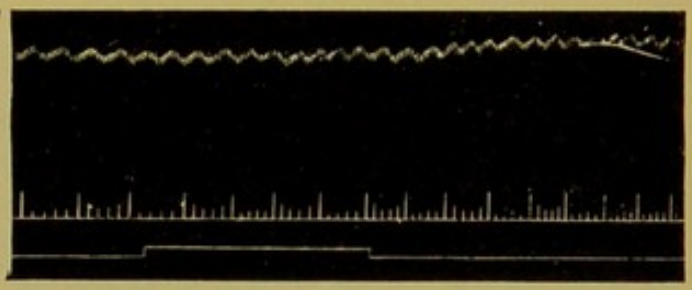


Fig. 2.

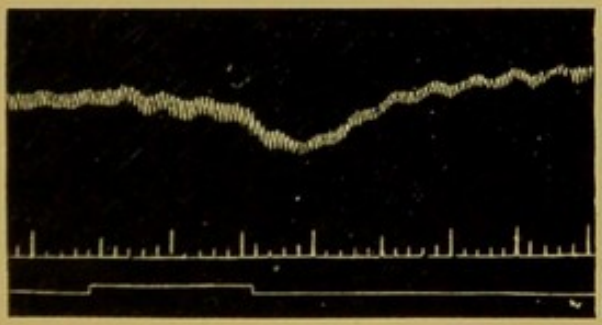


Fig. 3.

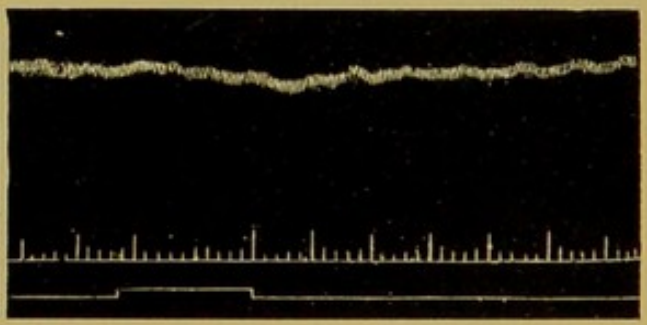


Fig. 4.

Fig. 1. Effect on blood-pressure of injecting 3 c.c. of saline extract of sympathetic ganglia; time in seconds; the raising of the signal line indicates the time of injection. This like all the subsequent tracings was obtained from a cat. All tracings read from left to right. The height of arterial pressure is indicated in the first of each group of tracings.

Fig. 2. The same after atropine.

Fig. 3. Similar effect of 3 c.c. of saline extract of spinal ganglia.

Fig. 4. The same after atropine.

result of the injection of the same amount of extract after the cat had received an injection of 1 c.c. of a 0·5 per cent. solution of atropin subcutaneously. There is now no fall but a slight rise of blood-pressure. Figs. 3 and 4 show a corresponding result with extract of spinal ganglia prepared in the same way before and after atropin respectively.

The next series of tracings (Figs. 5, 6, 7, 8, 9, 10) give typical results showing the effect of injection of glycerine and glycerine extracts of ganglia; 5 c.c. were injected in each case. Fig. 5 shows the result of injecting glycerine (1 to 20 of saline solution). I have other tracings which show that the fall of arterial pressure which this produces is accompanied with dilatation of the splanchnic blood vessels as evidenced by the intestinal plethysmograph. Fig. 8 is the result after atropin. Atropin does not abolish the fall produced by glycerine. Figs. 6 and 7 show the fall of pressure produced by glycerine extracts of sympathetic and spinal ganglia respectively; the slowing of the heart-beat (cholin effect) is here very well seen. After atropin, Figs. 9 and 10 show that a fall of pressure still occurs but the slowing of the heart is absent;

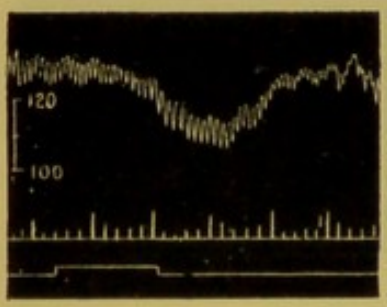


Fig. 5.

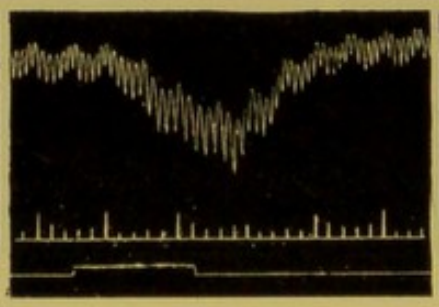


Fig. 6.

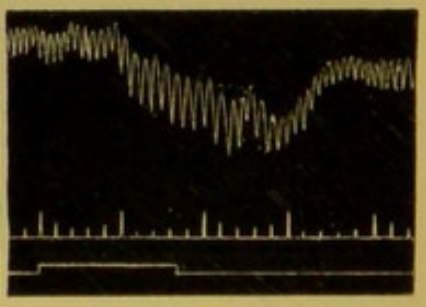


Fig. 7.

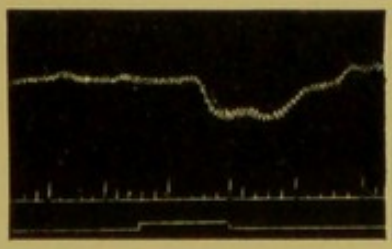


Fig. 8.

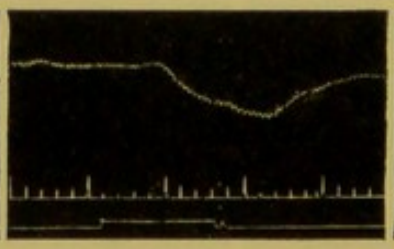


Fig. 9.

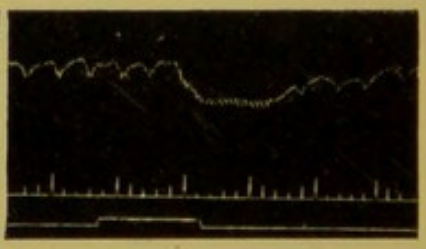


Fig. 10.

Fig. 5. Effect of injecting 5 c.c. of glycerine diluted with physiological saline solution (1 in 20). In all subsequent tracings the amount injected was 5 c.c.

Fig. 6. Effect of injecting glycerine extract of sympathetic ganglia, diluted so that the proportion of glycerine was 1 in 20. Note slowing of heart.

Fig. 7. Similar effect of corresponding glycerine extract of spinal ganglia.

Fig. 8, 9 and 10. Effects of glycerine, glycerine extract of sympathetic ganglia, and glycerine extract of spinal ganglia respectively in the same animal after the subcutaneous injection of atropin. Note the fall of pressure due to glycerine, but absence of heart effect. These Figures are reduced rather more than Figs. 5, 6, 7.

and the fall is here doubtless produced by the glycerine. Glycerine extracts are therefore objectionable, and do not lend themselves well to an investigation of the problem before us.

In his first paper, Cleghorn does not mention whether or not atropin abolishes the fall of pressure. In his second, he states that atropin does not abolish the fall. If he had relied on the action of glycerine extracts, it would be easy to explain his result, but by the time Cleghorn wrote his second paper he appears to have realised the disadvantage of employing glycerine extracts, and adopts a different method. He extracts the ganglia with alcohol, filters, evaporates off the spirit, and takes up the residue with saline solution. He admits that cholin is present in this solution, and finds that it produces the usual fall of arterial pressure. Then he removes the cholin by precipitation with platinum chloride, filters off the precipitate, evaporates the filtrate to dryness, and extracts the residue with saline solution. Injection of this solution produces a fall of blood-pressure even after the administration of atropin.

There appears to have been no attempt made to free the solution from excess of platinum chloride, which must undoubtedly have been present. However, I have thought it well to repeat the experiment just as Cleghorn describes it, and have obtained exactly the same results not only with ganglia, but also with brain extracts. But, I have also adopted the additional precaution of performing a control experiment with a solution of cholin hydrochloride; and this control explains the result obtained. I took a 0·2 solution of cholin hydrochloride in alcohol and added alcoholic solution of platinum chloride until no further precipitate was obtainable; the precipitated double salt of cholin was filtered off; the filtrate evaporated to dryness, taken up with saline solution, and the solution so obtained was injected intravenously. There was still a considerable fall of blood-pressure, and this was not abolished by atropin. This is obviously to be explained in one of two ways; it is either the excess of platinum chloride that is responsible for the effect, or else the double platinum salt of cholin is slightly soluble in alcohol, and it is this that produces the result. In some cats, extremely dilute solutions of platinum chloride in saline solution will produce a fall of arterial pressure. In other animals in which I have tested it little or no fall occurs. If the fall does occur, it is not abolished by atropin. I am however inclined to think that the other explanation, namely that the double platinum salt of cholin is somewhat soluble in alcohol, is the more probable. For, I always find

that in attempting to make quantitative estimations of the amount of cholin in solution by weighing the platinum compound there is always considerable loss, this loss is more marked when one attempts to estimate the cholin in organic mixtures like blood, or organic extracts.

The fact, however, remains, whichever explanation is correct, that Cleghorn's method does not really prove that the fall of pressure he observes is not due to cholin. Indeed after the experiments I have just described which show that pure solutions of cholin after treatment with platinum chloride, will produce a fall of pressure even after the animal has been atropinised, Cleghorn's results tend to prove the exact opposite to the conclusion he draws from them.

Cleghorn investigated the action of his extracts on other physiological functions. He found that they had no action on the pupil, and that after subcutaneous injection in frogs, the simple muscle curve obtained from the gastrocnemius was much prolonged, so that the curve resembled that obtained from a muscle poisoned with veratrin.

Mott and I in our work on cholin did not examine the effect of this alkaloid on the pupil or on the muscle curve. I have now tested these points, and find that so far as the subject can be tested in anæsthetised cats, cholin has no effect on the pupil. Cholin moreover has no effect on the simple muscle curve (tested in brainless frogs which had received subcutaneous injection of from 1 to 4 c.c. of 0·4 per cent. solutions of cholin hydrochloride). I have also found that saline extracts of sympathetic ganglia or of brain have no effect on the simple muscle curve, but glycerine extracts produce the result described by Cleghorn. This, however, is entirely due to the glycerine. When I had found this out I placed the matter in the hands of Dr Willoughby Lyle to work out the details, and he has already made a preliminary communication on the veratrin-like action of glycerine, to the Physiological Society[1].

So far I have spoken only of the physiological tests for cholin, and so far as they go these entirely support my contention that cholin is the active substance in extracts of ganglia. But physiological tests by themselves are insufficient, especially since Dixon[2] has shown that the physiological effects of spermine are very similar. I have therefore throughout supplemented these by performing the chemical tests for cholin. By far the most delicate and characteristic of these

[1] Jan. 26, 1901. The veratrin-like action has also been observed by Langendorff. *Du Bois Reymond's Archiv*, 1891, p. 480.

[2] This *Journal*, Vol. xxv. p. 356. 1900.

reactions is the obtaining of the octahedral crystals of the platinum double salt[1]. These can be obtained abundantly in all the specimens examined. I have always performed this test as follows. A small quantity, usually 5 c.c. of the extract, was evaporated to dryness and the residue taken up with absolute alcohol. The extract after filtration was again evaporated to dryness and again taken up with absolute alcohol, and this procedure repeated twice more. The final alcoholic solution was then treated with alcoholic solution of platinum chloride, and the precipitate allowed to settle, and washed with absolute alcohol by decantation. The precipitate was then dissolved in 15 per cent. alcohol, and the solution after filtration was allowed to evaporate to dryness at 40° C. in a watch-glass; the relative amount of the octahedra may be taken as a rough measure of the cholin originally present. In all the specimens examined (both of glycerine and saline extracts of both varieties of ganglia) the crop of crystals was very abundant.

These experiments have shown me that cholin is not the only substance present. A considerable amount of the precipitate produced by the addition of platinum chloride is not soluble in 15 per cent. alcohol, or in water, and in the final crop of crystals, there are in addition to the octahedra other crystals mostly of needle shape as well. This is only what one would anticipate; in a complex material like nervous tissue one would expect that substances other than cholin would enter into solution in alcohol; no doubt lecithin would be one of these, and there are also other basic substances obtained to which Gulewitsch called attention.

I do not therefore claim that cholin is the only substance of physiological importance in extracts of ganglia, but I do claim that it is at present the only substance which can be readily identified there, and that all the effects described by Cleghorn and myself are explicable on the assumption that it is the active agent in producing such effects.

Such a statement would be more convincing if I could present accurate estimations of the amount of cholin present. I have already alluded to the difficulty in doing this. I have found the relative abundance of the crystals as prepared in the manner described, and which can be easily obtained by a microscopic survey of the watch-glass, a very trustworthy guide to what one would expect in the amount of fall

[1] The trustworthiness and delicacy of this test is also insisted upon in the recent work of F. Gumprecht (*Verhandl. des Congr. f. innere Med. Wiesbaden*, 1900, pp. 326–348). He finds cholin in extracts of brain.

of blood-pressure in any given extract. So far as my weighings go, they show that in saline extracts of ganglia prepared by boiling 1 grm. of ganglia with 5 c.c. of physiological saline solution, that the percentage of cholin varies from 0·05 to 0·08 per cent., and this amount is ample to produce the fall of pressure observed.

Action of extracts from other portions of the nervous system.

I have prepared extracts similar to those just described from various portions of the nervous system; namely, cerebrum, cerebellum, spinal cord and sciatic nerve. In some cases I have used human brain obtained as fresh as possible, but in most cases have employed fresh nervous tissue from recently killed cats. In some cases I used glycerine extracts, but although these produce a great fall of pressure, I soon abandoned the method on discovering that part of the effect is due to the glycerine. In most cases I have used physiological saline (in the same proportion as described in connection with ganglia) as the extracting agent; in some cases I used the absolutely fresh tissue; in other cases this was first dried, and then extracted. In some cases I have used the saline at the ordinary temperature of the air; in other cases I have boiled it with the tissue. In other cases still the saline extract was evaporated to dryness; the residue taken up with alcohol, filtered, and evaporated to dryness; the residue was then extracted with saline, filtered and used for injection. The result in all cases was practically the same but different in degree. The results may be briefly summarised as follows:—

1. The fall of blood-pressure is partly due to effect on the heart, and partly to dilatation of peripheral vessels. This was tested by the intestinal plethysmograph.

2. The effect is more marked, the greater the proportion of grey matter in the tissue used.

3. The effect is more marked when extracts are made with boiling than with cold saline solution.

4. It is not abolished by section of both vagi.

5. It is, however, abolished if the animal has been effectively atropinised. Sometimes the abolition of the fall of pressure is not absolute; it generally is, and is often replaced by a rise of pressure.

6. There is little or no effect on the respiration.

7. These physiological tests which are all consonant with the hypothesis that the active material is cholin have been supplemented in

every case by chemical tests. The iodine test for cholin is not very delicate nor in the presence of other organic substances very trustworthy. I therefore rely principally on the platinum chloride reaction already fully described in connection with my experiments on ganglia. So far as the method can be used quantitatively, and I have already pointed out its deficiencies in this respect, the extracts obtained from brain are richer in cholin than those of ganglia, the amount in extracts of grey matter usually approaching 0·1 per cent. This would be quite in agreement with the greater effect observed with extracts of grey matter. What I have said in respect to the admixture of cholin with other substances in extracts of ganglia holds equally well in regard to extracts of other nervous tissues. Among the substances identified was frequently a small amount of lactic acid. The depressor effect of lactic acid is not abolished by atropin.

Again, all I claim from my experiments is that although extracts of nervous tissue probably contain numerous substances, the results described which follow injection of the extracts can all be explained on the hypothesis that they are mainly produced by the only substance which has been completely identified in such extracts, and that this substance is cholin.

I may add that in all the experiments I have performed I only observed that the fall of blood-pressure was not altered by the action of atropin in one case. This was the first experiment I did with extract of brain. The brain in this case had not been dried very rapidly and had a strong putrefactive odour before the extract was made. I therefore feel justified in doubting the value of this single exception.

It now only remains for me to present and briefly describe some

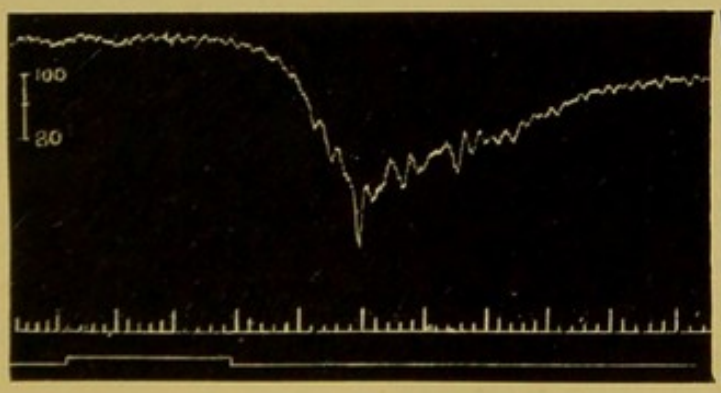


Fig. 11.

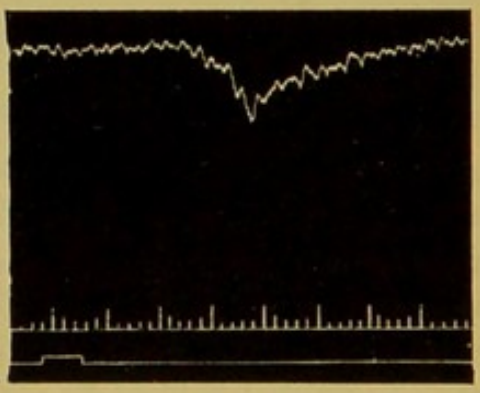


Fig. 12.

Fig. 11. Effect of injecting extract of cat's brain, made with boiling salt solution.
Fig. 12. Smaller effect produced in same animal of an extract made with cold salt solution.

typical tracings to illustrate the results just described. In all cases 5 c.c. of the extract were injected into the jugular vein, or if an alcoholic extract of the tissue was employed, the residue was taken up with saline solution, in such a proportion that the 5 c.c. injected corresponded to 5 c.c. of the original extract.

Figs. 11 and 12 were obtained from the same animal; and show that an extract made with boiling salt solution (Fig. 11) is more powerful than one made with cold salt solution. In both cases cat's brain was the tissue extracted.

The next pair of tracings illustrate the abolition of the effect after atropin. Here again fresh cat's brain was employed. Fig. 13 shows the effect before, Fig. 14 after atropin. The fall seen in Fig. 13 is replaced by a slight rise in Fig. 13.

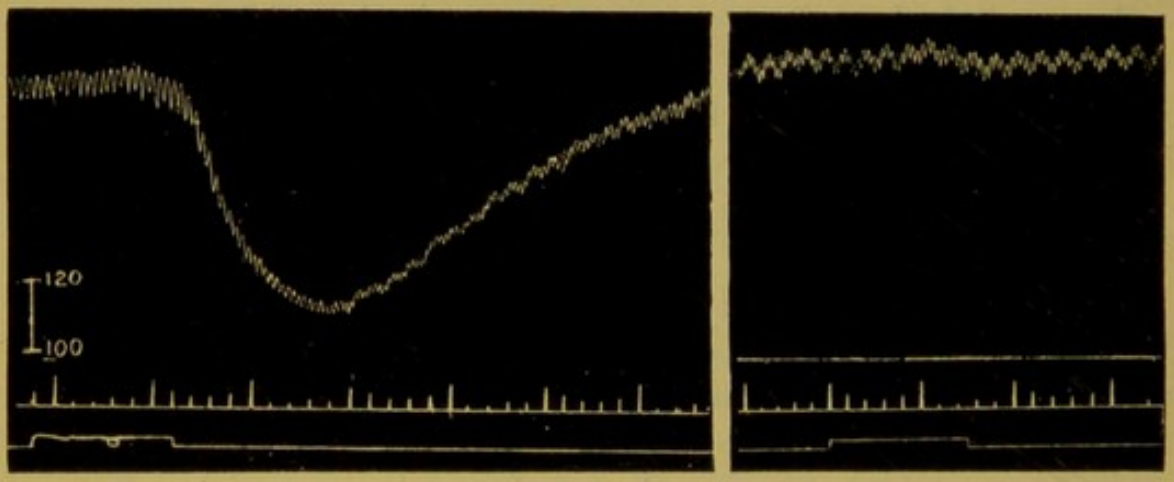


Fig. 13. Fig. 14.

Fig. 13. Effect of injecting extract of cat's brain before atropin.
Fig. 14. Effect after atropin of the same extract in the same animal.

The next three tracings (Figs. 15, 16, and 17) were obtained with extracts of human brain. Fig. 15 is the result of injecting extract of grey matter; Fig. 16 that of injecting extract of white matter; here the fall is much less. Fig. 17 shows the result of the same dose of grey matter extract after atropin. Here the fall is practically abolished though not replaced by a rise of pressure. The uppermost tracing in these three cases of the respiration taken by the tambour method shows that breathing is not affected by the injections.

Figs. 18 and 19 show much the same results except that the tracing of the intestinal plethysmograph is given also. Before atropin brain extract causes the usual fall of blood-pressure accompanied by the

expansion of the intestinal vessels (Fig. 18). After atropin the fall is absent, though there is still a peripheral dilatation (Fig. 19).

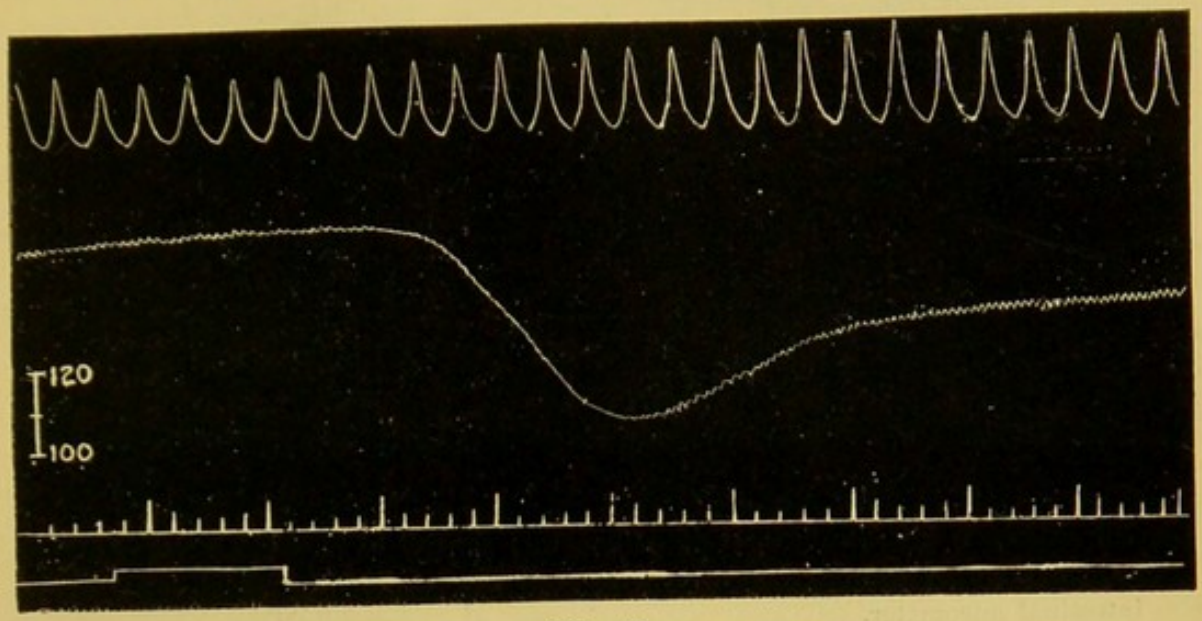


Fig. 15.

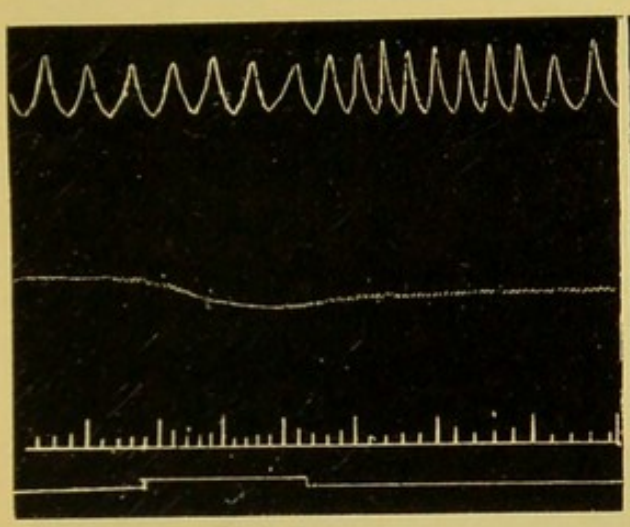


Fig. 16.

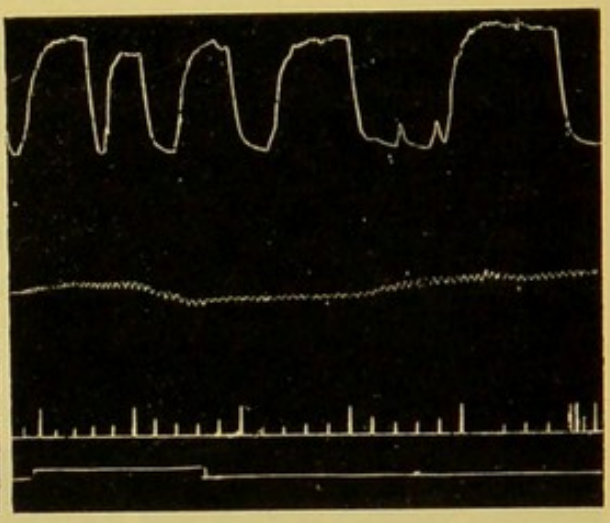


Fig. 17.

Fig. 15. Effect on injecting extract of human grey matter. The top line is respiration.
Fig. 16. Effect of the same dose of extract of human white matter, in the same cat.
Fig. 17. Effect of the same dose of human grey matter extract in the same cat after atropin.

I have not thought it necessary to present any illustrations of the effects of spinal cord or cerebellum extracts: it is sufficient to say that the effects are exactly similar to though not so pronounced as those produced by extracts of cerebral grey matter, and are like them extinguished by atropinisation.

I give in conclusion a couple of illustrations to show the effect of injecting an extract of cat's sciatic nerves. The effect before atropin

(Fig. 20) is a very marked fall. This cat was peculiarly susceptible both to cholin hydrochloride and extracts of nervous tissue; 2·5 c.c. of

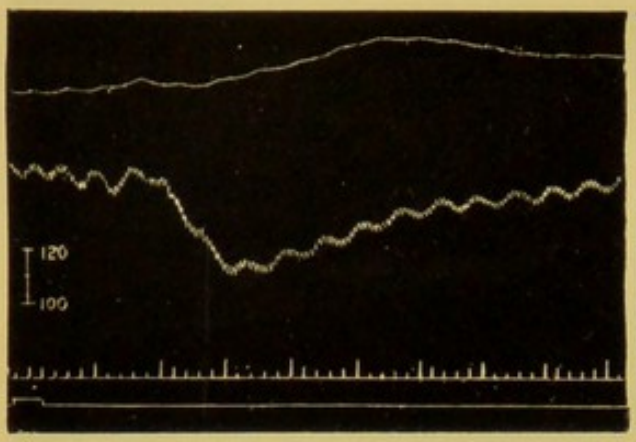


Fig. 18.

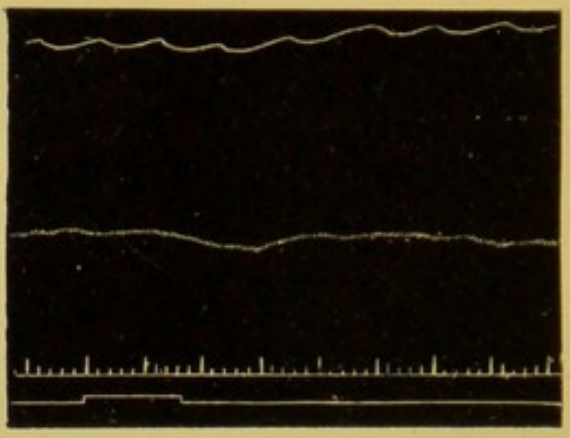


Fig. 19.

Fig. 18. Effect of injecting extract of cat's brain; the fall of pressure is accompanied by dilatation of peripheral vessels as seen in the tracing (uppermost line) of the intestinal oncometer.

Fig. 19. The same after atropin. The fall in pressure is abolished.

a 0·2 per cent. solution of cholin hydrochloride, or 5 c.c. of an extract of cerebral grey matter, produced falls almost twice as great as that shown in the figure. As a rule extracts of nerve produce about as much effect as those of cerebral white matter. Fig. 21 shows the effect of the same extract after atropin. There is still a slight fall, and this is the most marked fall I have ever obtained after atropin; but in this particular animal cholin hydrochloride itself caused a similar slight fall after atropin.

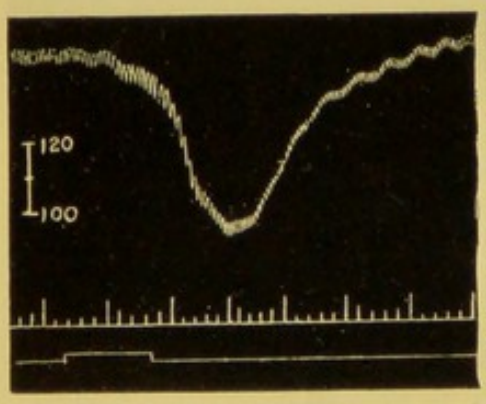


Fig. 20.

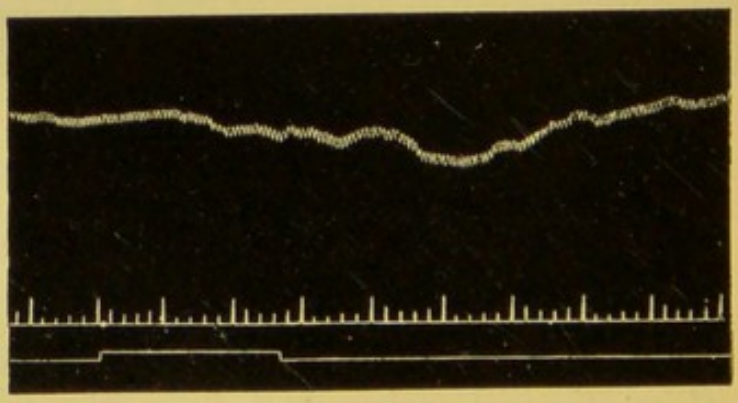


Fig. 21.

Fig. 20. Effect of injecting extract of sciatic nerve.

Fig. 21. The same after atropin.

I now come to a much more difficult problem, and that is to account for the difference in my results from those obtained by Osborne and

Vincent. I would first call attention to one of their experiments; they treated a saline extract of alcoholic residue from brain with sulphuric and phosphotungstic acids to precipitate alkaloids like cholin. The precipitate was filtered off, and the filtrate treated with barium hydrate, and then with dilute sulphuric acid, to free it from phosphotungstic acid and excess of barium respectively. The final filtrate was acid, and produced a distinct depressor effect when injected. I have had some experience of this method of separating alkaloids, and I know only too well the difficulty of getting rid of the last traces of barium; and barium salts have a very marked depressor effect. This will probably explain this result, but as they do not appear to have made any extensive use of this method, I will freely confess that it will not explain their other experiments. They appear to have used principally saline extracts prepared from the absolutely fresh brain, and injected these before and after the use of atropin. Though they used hot saline as the extracting agent, it is possible that their extracts contained more nucleo-proteid than they bargained for, and more than my extracts happened to contain. If this was the case, the cholin effect would be greatly masked. If this was not so, their results are inexplicable to me at present. For the reasons previously stated, I do not attach much value to their estimations of the amount of cholin in their extracts.

My experiments with brain extracts have been repeated by Dr W. E. Dixon, who was interested in them in view of his own work on spermine. He informed me privately that his results coincide with mine, namely that the depressor effects are abolished by atropin.

One other author has been referred to who has done similar work, namely I. Ott. He has used aqueous infusions of spinal cord, and states that the fall of blood-pressure occurs after the injection of atropin. His figures do not support this view; the fall after atropin varied from 2 to 4 mm. Hg. and quite as big a fall, sometimes a greater one, occurred after stimulation of the peripheral end of the cut vagus. His experiments merely show that the animal was not effectively atropinised.

Summary of conclusions.

1. Extracts of sympathetic ganglia produce a fall of blood-pressure as Cleghorn states.

2. Extracts of other portions of the nervous system (spinal ganglia, brain, spinal cord, nerve) have a similar action.

3. Glycerine should not be used in the making of extracts, for this reagent is not physiologically inactive; it produces a fall of arterial pressure which is not abolished by atropin, and has a veratrin-like effect on the contraction of striped muscle.

4. The effect of saline extracts can be explained on the hypothesis that cholin is the principal active agent in the solutions.

5. There are doubtless other substances present as well, but these have not been accurately separated or identified, or indeed shown to possess any important physiological action, at any rate, on blood-pressure.

6. All observers are agreed that cholin is present; the only doubt is whether sufficient is present to account for the fall of blood-pressure. In the absence of any accurate method of estimating cholin in organic mixtures this cannot be definitely decided; I am therefore in the meanwhile entitled to my opinion that sufficient is present.

7. Several observers state that the depressor effect of nervous extracts is not abolished by atropin. My own experiments show that it is. In the case of Cleghorn and I. Ott I think my explanations of their negative results are sufficient. I confess I am unable to explain all the results obtained by Osborne and Vincent, but have been entirely unable to confirm their experiments in this direction, though I think we are agreed in all other particulars.

It may perhaps be asked in conclusion, Do such experiments teach us anything useful, or have they any bearing at all in the solving of any important physiological problem, or are they merely of academic interest?

To myself the most interesting result is the fact that cold physiological saline solution is able to extract cholin from nervous tissues. By more violent means, as for instance by the use of boiling saline, it is possible to extract more. But the use of vigorous methods of this kind will break up the nervous tissues, and so we should expect to find

the products of their disintegration afterwards. The use of physiological salt solution at the ordinary temperature is more instructive, for this reagent of all others produces least disturbance, and the presence of cholin in solution appears to show that lecithin especially in the most active part of the nervous system, namely grey matter, is in that continual unstable condition of chemical equilibrium which we call metabolism. Thus Gumprecht finds it in the normal cerebro-spinal fluid in minute quantities, and confirms the observation made by Mott and myself that it is immensely increased in diseased conditions in which the katabolic side of nervous action is preponderant. Mott and I have been recently continuing our work, and hope to publish our results more fully, but I may here say that one result of our work has been to show that normal blood in the cat contains minimal amounts of cholin. This entirely confirms the view I have just advanced concerning lecithin metabolism.

THE VERATRINE-LIKE ACTION OF GLYCERINE.

By H. WILLOUGHBY LYLE, M.D.

(Reprinted from the "Journal of Physiology," vol. xxvi., Proc. Physiol. Soc., 1901, p. xxvi.)

THESE experiments were undertaken at the suggestion of Prof. Halliburton in view of A. Cleghorn's statement[1] that glycerine extracts of sympathetic ganglia produce, after injection into the dorsal lymph-sac of the frog, a veratrine-like action on the simple muscle curve obtained from the gastrocnemius.

Prof. Halliburton has found that saline extracts of the ganglia and of brain do not have this effect. Choline also has no similar action. He therefore suggested to me that I should ascertain whether the result may not simply be due to the glycerine. I have found that it is so. The latent period is somewhat increased, the time and amount of contraction is also increased, but the most marked effect is great prolongation of the relaxation period. I have, however, never seen the initial twitch which veratrinised muscles usually exhibit; moreover, the effect does not pass off on repeated stimulation. Further experiments on the influence of heat and cold are now in progress. At the time these experiments were started, I was not aware that Langendorff[2] had previously performed experiments with glycerine and noted the main fact of its veratrine-like action.

[1] *American Journal of Physiology*, II., p. 480.
[2] Dr. Bois Reymond's "Archiv," 1891, p. 480.

[*Reprinted from the Journal of Physiology,*
Vol. XXVI. Nos. 1 & 2, *December* 31, 1900.]

THE ACTION OF ETHER AND CHLOROFORM ON THE NEURONS OF RABBITS AND DOGS.

By HAMILTON WRIGHT, M.D., *Director of the Pathological Institute of the Federated Malay States.* (Plate I. and Four Figures in Text.)

The following investigation was undertaken to determine whether chloroform and ether produce any transient or permanent changes in the cortical and spinal neurons.

In the first series of experiments, rabbits were used. The animals were handled as gently as possible in order to avoid abdominal congestion and consequent cerebral anæmia. The drug was administered by a tube in the trachea. In the case of rabbit No. 1, the animal was killed by excess of ether; in the remaining cases the organs (brain and cord) were removed during the anæsthesia, and this operation was the obvious cause of death.

Fifteen minutes before each animal was killed, administration of the anæsthetic was suspended, and the time of return of the conjunctival reflex noted. The administration of the anæsthetic was then resumed until the animal was killed. The following table gives (1) the time during which anæsthesia was kept up, (2) the time taken for the return of the conjunctival reflex; this may be regarded as a rough indication of the functional depression in the neurons concerned in the reflex, and so also as an index of the depression in all the nerve-cells implicated by the anæsthesia.

	Anæsthetic		Time of return of reflex
Rabbit 1.	Ether	35 minutes	2 minutes
,, 2.	,,	1 hour	$2\frac{3}{4}$,,
,, 3.	,,	2 hours	4 ,,
,, 4.	,,	4 ,,	$5\frac{1}{2}$,,
,, 5.	,,	6 ,,	$6\frac{3}{4}$,,
,, 6.	Chloroform	30 minutes	2 ,,
,, 7.	,,	1 hour	$2\frac{1}{2}$,,
,, 8.	,,	2 hours	4 ,,
,, 9.	,,	4 ,,	6 ,,
,, 10.	,,	6 ,,	$7\frac{1}{4}$,,

In a second series of experiments which were performed on dogs, the additional precaution was taken to keep up the body temperature by placing the animal on a hot water bed, and covering it with cotton wool. I did this because it had been suggested by Dr Head at the International Physiological Congress, at Cambridge, when I made a preliminary communication on my experiments with rabbits, that some of the changes I there described might be due to lowering of temperature. The following table gives the details of length of anæsthesia, time of return of conjunctival reflex, and body temperature.

	Anæsthetic		Time of return of reflex	Maximum and minimum temp.
Dog 1.	Ether	30 minutes	½ minute	101—101°
„ 2.	„	1 hour	1½ minutes	101—99·2°
„ 3.	„	2 hours	2½ „	101·5—98·75°
„ 4.	„	4 „	3½ „	101—98°
„ 5.	„	6 „	5 „	101—98·25°
„ 6.	Chloroform	30 minutes	½ „	101·5—101·5°
„ 7.	„	1 hour	1½ „	100·5—100°
„ 8.	„	2 hours	2¾ „	101—99·8°
„ 9.	„	4 „	4 „	101—98·5°
„ 10.	„	6 „	5½ „	101—97·8°

I did not measure the amount of anæsthetic actually given in the various experiments; some preliminary attempts to do this did not yield satisfactory results.

The organs removed from the animals (brain, and cervical region of the spinal cord) were divided into two parts longitudinally; one half was placed in absolute alcohol, the other in Müller's fluid. The tissues fixed in alcohol were worked out by Nissl's methylene blue method, by Weigert's hæmatoxylin and by the hæmatoxylin-eosin stains. Those fixed in Müller's fluid were investigated by Berkeley's modification of Golgi's method in the case of the rabbits, and by Cox's method in the case of the dogs.

The following paragraphs give the result of the microscopic examination in each animal. The sections from the tissues fixed in alcohol were cut to a uniform thickness of 15μ, and any description given is from observation of those cells that were cut fairly through their median planes.

Rabbit 1. Ether for half-an-hour.

(*a*) *Cerebrum.* All the cortical cells are seen by the aniline dyes to have undergone a change. This is most marked in the layer analogous to the

pyramidal layer in man. Nissl's bodies have almost entirely disappeared from the apical and basal dendrons, and many are absent from the cell bodies, leaving clear untinted spaces. Those yet *in situ* are pale, granular looking and reduced in size; the hyaloplasm between the Nissl's bodies is unstained as in normal cells. There is no breaking down of the chromophilic granules into dust-like particles and diffuse staining of the hyaloplasm such as occurs in degenerating cells. The chromatic substance seems to have merely undergone some change so that it no longer reacts in the usual way to methylene blue. The term *rarefaction* may be employed for the appearances observed.

By the silver-chrome method many processes stain as they do in normal tissue, but in the majority of cases the most distant visible part of the apical dendron and its primary and secondary branchlets, show small moniliform swellings (see fig. 1 in text). The cells from which they originate appear normal by this method.

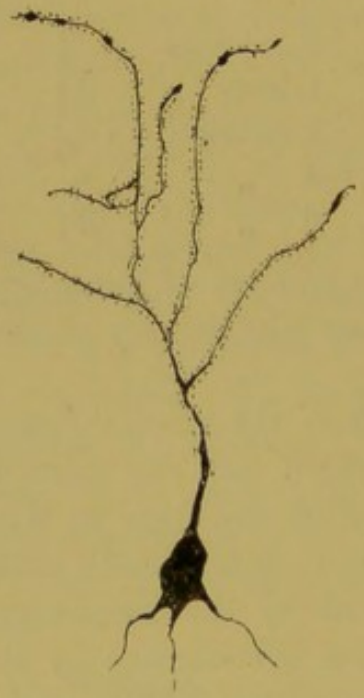


Fig. 1. Cortical cell of rabbit; moniliform enlargement of the distal portions of the apical dendron is seen. Ether anæsthesia for half-an-hour.

The basal dendrons are but rarely affected.

(*b*) *Cerebellum.* No change.

(*c*) *Spinal cord.* No change was seen in any cells by the methylene blue process. In this, as in the cords of the remaining rabbits, I was not successful in obtaining satisfactory preparations by the silver method.

(*d*) *Vascular conditions.* In brain and cord and their membranes, there is capillary anæmia and venous engorgement. The peri-capillary spaces

are widened and contain a scant number of leucocytes. In the cerebellum the capillary anæmia is not so marked.

Rabbit 2. Ether for one hour.

(*a*) *Cerebrum.* The 'rarefaction' just described is now more general throughout the cell layers, but the pyramidal cells are especially involved (see Plate 1, fig. 1). A few of these have the appearance of mere skeletons, but their outlines are firm, and their nuclei and nucleoli sound. Occasionally in these 'skeleton cells' a faintly stained network with irregular meshes is seen. Glia cells appear swollen, but are not increased in number.

By the silver method, only a few of the apical processes of the pyramidal cells are normal. The majority show the moniliform enlargements noted in the first rabbit. The change is, however, more marked; the enlargements are more numerous and extend farther down the main stem. A large number of basal dendrons are also implicated. Almost all the dendrons arising from the median layer of cells are in some degree moniliform.

(*b*) *Spinal cord.* There is 'rarefaction' in a small number of anterior cornual cells, but no change in the cells of the posterior horn.

(*c*) *Vascular conditions.* The same as in the first case.

Rabbit 3. Ether for two hours.

(*a*) *Cerebrum.* A process of restitution seems to have set in during the second hour. The dendrons are still totally denuded of Nissl's bodies, but the cells themselves appear to have become rehabilitated to a noticeable degree. A few, however, show as great a 'rarefaction' as in previous cases. All the cortical glia cells are swollen and pale; they appear to be augmented in number and tend to aggregate in the vicinity of the rarefied cells.

By the silver method, nearly every dendron is seen to be moniliform; and the swellings have increased in size, notwithstanding the apparent restitution in the cell bodies. The cells from which the affected dendrons spring look healthy, though probably those which are most implicated spring from the cells which by the methylene blue process would have been shown to be most rarefied.

(*b*) *Cerebellum.* No change.

(*c*) *Spinal cord.* The cells are not more deeply implicated than at the end of one hour of etherisation.

(*d*) *Vascular conditions.* The same as before except that the number of leucocytes in the peri-vascular spaces has slightly increased. There are no observable lesions in the vessel walls.

Regarding the restitution which occurred in this case, I was at first inclined to the opinion that the process was *ante-mortem* and represented a successful effort on the part of the cells to throw off the effects of the drug

My subsequent work did not confirm this view. I now believe the effect occurred after the removal of the organs from the body; there was in this case an unavoidable delay of five or six minutes in transferring the organs to the fixing solutions. During this pause, there was doubtless an escape of ether from the blood and lymph, and consequently from the 'surviving' cell bodies.

Rabbit 4. Ether for four hours.

(*a*) *Cerebrum.* The greater number of cells are again rarefied, and many are reduced to mere skeletons. The layers of cells above and below the pyramidal layer are slightly rarefied. The pyramidal cells are markedly rarefied, and a certain number of these show a more marked change than had been noticed in the previous animals; the margins of these cells are disintegrated, their nuclei are eccentric, swollen and granular, the nucleoli are enlarged and irregularly stained. One would hesitate to describe such cells as degenerated, for there is nothing to indicate that the change is a permanent one, or that any cells have completely broken down. The glia cells are indubitably augmented in number and noticeably turgid; they with many leucocytes cluster about and in not rare cases actually penetrate within the most profoundly affected cells.

By the silver stain, all the apical dendrons are seen to be moniliform. In numerous instances the swellings are larger than any observed in previous cases. The lower portions of the dendrons are also more frequently affected. No rupture is apparent in any dendrons. The method reveals no change in the cell bodies.

(*b*) *Cerebellum.* A few of the cells of Purkinje are decidedly rarefied, but the vast majority are normal.

(*c*) *Spinal cord.* The posterior cornual cells are for the first time noticeably rarefied, though the majority are still normal. The anterior horn cells are rarefied (Plate 1, fig. 2), and a small number of these show the pseudo-degeneration just described in the cortical cells. The glia cells show the same change and behaviour as in the cortex. Leucocytes are numerous in the tissue, and mixed with swollen glia cells in the peri-cellular and vascular spaces.

(*d*) *Vascular conditions.* These are practically the same as in previous cases, except that more leucocytes are seen in the peri-capillary spaces, and also a few swollen pale glia cells. There is no appearance of stasis. The nuclei of the capillaries slightly bulge, and stain more deeply than normal.

Rabbit 5. Ether for six hours.

(*a*) *Cerebrum.* Here the same general condition obtains as in the last case, but the number of skeleton cells (Plate 1, fig. 3) is greater, and those

in which the disintegrative change has gone further are also more numerous. Small masses of débris with occasionally a pale swollen nucleus can be seen in any section; these are encompassed by swollen glia cells, and leucocytes; in other instances a mass of enlarged, pale glia cells and leucocytes filled with products of degeneration, and scattered débris amongst them are seen. These possibly mark destroyed cells (Plate 1, fig. 4). Compared with the one hour case, the number of glia cells is enormous.

By the silver method, practically the same results are noted as in the four hour animal, though occasionally the bulbous enlargements are larger (fig. 2 in text).

Fig. 2. Cortical cell of rabbit. The moniliform enlargements have increased in size and extent after six hours' etherisation.

In the cells lying outside the pyramidal layer fine moniliform swellings are not infrequently seen in the most distal parts of their dendrons.

(*b*) *Cerebellum.* A larger number of cells of Purkinje are rarefied than in the last case, and some are reduced to mere skeletons.

(*c*) *Spinal cord.* Only a few anterior horn cells remain normal. A small number show extreme change (Plate 1, fig. 5), and quite two-thirds of the remainder are markedly rarefied. Skeleton cells are more numerous than in the last case, and more of the posterior horn cells, especially the larger ones, are affected. The glia cells appear as in the last case.

(*d*) *Vascular conditions.* These are as before. There are many more leucocytes in the peri-capillary spaces and mingled with them swollen glia cells containing granular material. The latter feature is not so remarkable in the cord as in the cortex.

Rabbits 6, 7, 8, 9, 10. Chloroform for $\frac{1}{2}$, 1, 2, 4, 6 hours respectively.

The changes in the chloroformed animals differ so little in kind or even in degree from those anæsthetised by ether, that it is only necessary for me to note the few differences that were observable.

In the two first of the series (chloroform for $\frac{1}{2}$ and 1 hour) the cellular rarefaction is slightly greater than in the corresponding ether cases.

At the end of two hours (Rabbit 8) the rarefaction is rather more obvious, and there was no diminution of the degree of rarefaction as in the corresponding ether case (Rabbit 3).

After four and six hours the changes noted in the corresponding ether cases are present to a more marked degree, in cerebrum and cerebellum, whereas the changes in the spinal cord are about the same whichever anæsthetic is used.

By the silver method, moniliform swelling of the dendrons is present from first to last, and increases in degree *pari passu* with the anæsthesia.

The vascular conditions are the same as in the ether cases, except that the veins were like the capillaries found to be almost empty and contracted.

Dog 1. Ether for half-an-hour.

No change is observable in any cells by either method. Venous engorgement and capillary anæmia are present as in the rabbits.

Dog 2. Ether for 1 hour.

No change is observable in any cells by Cox's method. By the methylene blue method no change is seen in the cells of cerebellum and cord, but in the brain the Nissl's bodies in many of the pyramidal cells are pale and granular in appearance, and the dendrons of these cells are denuded of Nissl's bodies. The same vascular conditions are present as in the first dog.

Dog 3. Ether for two hours.

Practically the same appearances are present as in dog 2. More pyramidal cells are, however, affected, but moniliform enlargement of the dendrons is not seen. The glia cells are slightly swollen, but stain well. Spinal and cerebellar cells are still normal.

Dog 4. Ether for four hours.

(*a*) *Cerebrum.* The large pyramidal cells are decidedly rarefied (Plate 1, fig. 6). The main apical processes are usually denuded of Nissl's bodies; the basal dendrons occasionally show the same change. The edges of such cells are ragged. A few skeleton cells are seen. The glia cells are more swollen, but show no augmentation. By Cox's method the extreme tips of the apical processes are moniliform, but the swellings are small (fig. 3 in text). The basal dendrons are still normal.

(*b*) *Cerebellum.* A small number of the cells of Purkinje are rarefied.

(*c*) *Spinal cord.* Very occasional rarefied cells are seen in the anterior horn, but the majority of these cells, and all the cells in the posterior horn, are normal.

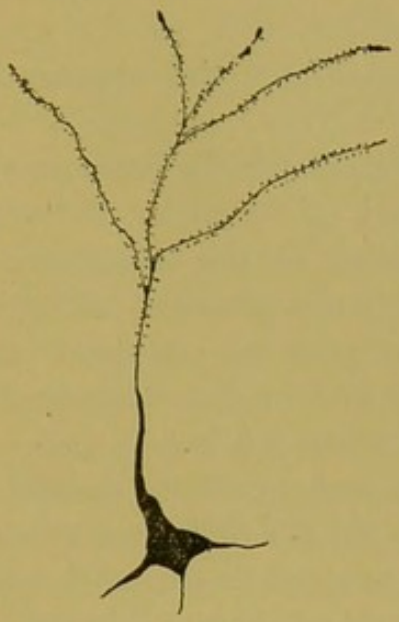


Fig. 3.

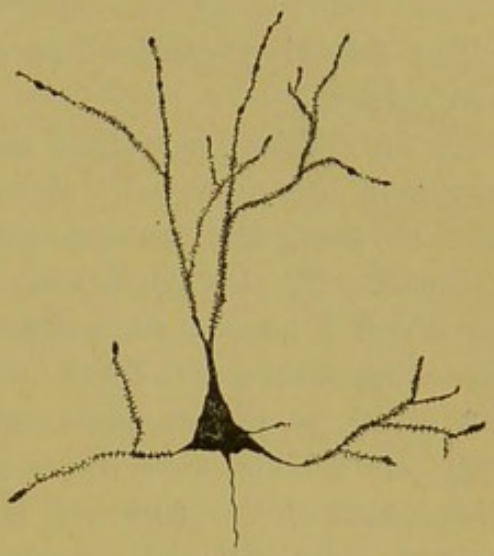


Fig. 4.

Fig. 3. First detected appearance of moniliform change in apical dendron of dog's pyramidal cell after four hours' etherisation.

Fig. 4. Moniliform swellings in dog's pyramidal cell after six hours' etherisation.

(*d*) *Vascular conditions.* Capillary anæmia and constriction are present as in the previous cases. The nuclei of the capillaries bulge and stain rather more deeply than normal.

Dog 5. Ether for six hours.

The condition seen is simply an exaggeration of that in the previous case. The change in the cortical cells has not advanced beyond 'rarefaction' but the glia cells are more numerous and cluster about the markedly affected cells. The next figure in the text (fig. 4) shows the increase in moniliform change. The cells of cerebellum and spinal cord have about the same condition as in the four hour case.

Dogs 6, 7, 8, 9, 10. Chloroform for $\frac{1}{2}$, 1, 2, 4, 6 hours respectively.

The changes observed in these animals are of a precisely similar character to those just described in the case of ether. At the end of four hours the rarefaction is slightly more general and more advanced. Plate 1, fig. 7, shows one of the pyramidal cells in the skeleton stage at the end of six hours' anæsthesia. With regard to the vascular condition, there is less marked capillary anæmia, and the veins are less congested. This agrees with what was observed in rabbits.

It should be mentioned that the anæsthetics were administered to dogs by the mouth, except in the six hour cases (dogs 5 and 10), where a tracheal tube was used.

Conclusions.

The following conclusions can be drawn from the appearances described:

1. In rabbits both ether and chloroform anæsthesia cause certain changes in the nerve-cells of both brain and spinal cord. These are slight at first, but become more pronounced as the anæsthesia is continued. By the methylene blue method, the principal change is that which I have described as 'rarefaction'; in the advanced cases, I have introduced the term 'skeleton cell,' and in the most marked cases of all, a 'pseudo-degenerative' change has set in.

2. In rabbits also there is an early and constant moniliform enlargement of the tips and stems of the chief dendritic extensions of many pyramidal cells; these enlargements grow in size and spread along the dendrons towards the cell body as the anæsthesia is continued. Though it was of course impossible to examine the same cells by both the methylene blue and the silver processes, I brought under observation the cells of nearly identical parts of the two hemispheres, and I consider it justifiable to assume that the moniliform dendrons spring from the cells that show rarefaction.

3. In dogs, there are practically no changes up to two hours, but between that time and four hours changes occur in the nerve-cells similar in kind to though less in degree from those observed in rabbits; these changes become more marked as the anæsthesia is continued.

4. Corresponding to this, the nerve-cells of dogs show no moniliform enlargement of their dendrons up to two hours of anæsthesia, but after this point and presumably at the time that changes occur in the cell-bodies, a few dendrons show moniliform change and this becomes more pronounced as the anæsthesia is continued.

I feel inclined to the view that the lesser degree of the affection in dogs is due not so much to my care in keeping up the body temperature of these animals, but rather to the fact that the neurons of dogs have more inherent power of resistance to the drugs than those of rabbits. In dogs the time of the return of the conjunctival reflex is shorter than in the corresponding rabbits, so the degree of narcosis is less, and it is a common experience that rabbits succumb more readily to anæsthetics than dogs.

I also regard the changes observed in the cells and their processes to be due directly to the influence of the anæsthetics, and not indirectly due to the capillary anæmia which is produced. Mott[1] has produced changes somewhat resembling those I have described by suddenly cutting off the arterial supply to the brain, but there is an obvious difference between such an experiment, and the capillary anæmia I have described. I should regard the anæmia in my experiments as a 'conservative process,' secondary to a diminution in the activity of the nerve centres.

The venous congestion again appears to be a secondary phenomenon, and cannot be regarded as the cause of the changes in the nerve-cells. I think this is quite clear when one compares the effects of chloroform with those of ether; for under chloroform the effect on the nerve-cells is greater, whereas the amount of venous congestion is less.

Ether and chloroform are generally stated to circulate in the blood as such and no bio-chemical change in the blood has hitherto been described as a result of their administration. I am therefore forced to the conclusion that the neuronal changes are bio-chemical in nature, and are produced by the anæsthetic that reaches them *via* the blood stream.

Nissl's theory is that healthy nerve-cells fixed and stained in a constant manner will appear the same under constant optical conditions, and the appearance seen is the equivalent of such healthy nerve-cells during life. It follows that if nerve-cells under the same constant conditions present a difference from the equivalent or symbol of healthy cells, the difference is the measure of some change that occurred during life. To decide how such changes are produced by anæsthetics is a difficulty; there is nothing to suggest that chloroform or ether could cause these changes mechanically, and the supposition that they act chemically is extremely probable. It is possible for instance that the anæsthetic forms a compound with the Nissl bodies and thus prevents them from reacting to stains in a normal way; the degree of this change is proportional to the length of time to which they are exposed to the action of the drugs.

It is generally admitted that Nissl's bodies, forming as they do so large a component of the cell body, are in some way nutritional and energy-producing. Changes in them must necessarily be followed by some modification visible or invisible of the other structures in the cell

[1] *Lancet*, June 30th. 1900.

body and its branches; this is probably the explanation of the moniliform swellings of the dendrons. A large experience in the histological methods employed in this research has convinced me that the varicosities are not due to *post-mortem* changes, and I have seen nothing in my work which confirms a widely spread belief that the enlargements are primarily the bio-physical basis of loss or modification of consciousness. The change is essentially nutritional or bio-chemical, affecting primarily the cell body and secondarily the processes that spring from it. The puffing out of the dendrons occurs first at the points furthest removed from the nutritional centre in the cell, and it is not until the dose of the drug is increased that the moniliform enlargements grow in size, become more numerous and encroach upon the gradually weakening dendritic stem. The phenomenon, whether it is due to simple hydration at the swollen parts, or to a more profound chemical change, appears to be quite analogous to, though less in degree than the degenerative changes that occur when the neuronal body atrophies; the first changes are observed at the most distal portions of the nerve fibre that originates from the cell body, and one may even compare the moniliform enlargements to the increase and swelling of the protoplasm around the nuclei of the sheath of Schwann that occurs in Wallerian degeneration. The bio-physical theory rests principally on preparations made by the use of one or other modification of Golgi's method. Important light is thrown on the question when the aniline stains are employed as well, as by these alone can changes in the cell body be detected.

Lugaro denies the existence of dendritic varicosities as the result of chloroform anæsthesia, and has substituted for the bio-physical theory of Demoor and other writers, another which appears to be still more difficult of proof. According to Lugaro varicosity of dendrons and retraction of neurons is the condition on which consciousness depends, and loss of consciousness depends on the absence of varicosities and retraction, and therefore on the contact of neurons.

If Lugaro's dogs had been anæsthetised for a sufficiently long time, he would doubtless have found moniliform swellings; he makes no mention of the time during which anæsthesia was kept up, and I must conclude that the length of time must have been insufficient. His method of fixing the tissues I regard as a point of secondary importance. He injected Cox's fluid by the carotid, and thus the tissues were fixed *in situ*. If the varicosities I observed were the result of *post-mortem* changes due to my placing the pieces of brain in Cox's fluid in the ordinary way, the varicosities would have appeared in all cases, but

Edwin Wilson, Cambridge

they did not appear until anæsthesia has been prolonged for four hours.

Whether the facts I have described as occurring in dogs and rabbits, occur also in the human subject under the influence of anæsthetics, it is obviously impossible to say. But I do not consider that there is any analogy between the changes I have described, and those bio-chemical anabolic and katabolic changes that occur in daily life and mark our sleeping and waking hours. I regard the action of narcotics such as those I have employed as pathological, not very intensely pathological it is true, but still something which is remote from physiological processes. In sleep there is probably an opportunity for the constituents of the nerve-cells to undergo anabolic changes, whereas in the unconsciousness produced by anæsthetics, the process appears to be associated with an exhaustion of them.

The expenses of this work were defrayed by a grant made to me as John Lucas Walker Exhibitioner of the University of Cambridge. The experiments themselves have been performed partly in the University of Heidelberg, partly in the Pathological Laboratory at Cambridge, and partly in the Physiological Laboratory at King's College, London. A good deal of the microscopic examination of the tissues was carried out in the London County Asylums Laboratory, Claybury, and a few experiments have been completed since my arrival in the Malay States.

EXPLANATION OF PLATE I.

Fig. 1. Moderate 'rarefaction' of large pyramidal cell of rabbit after one hour's administration of ether.

Fig. 2. Rarefied anterior cornual cell of rabbit after four hours' etherisation.

Fig. 3. 'Skeleton' pyramidal cell of rabbit after six hours' etherisation.

Fig. 4. Pyramidal cell of rabbit after six hours' etherisation in which the change has advanced beyond the skeleton stage.

Fig. 5. Anterior cornual cell of rabbit after six hours' etherisation showing the same advanced change.

Fig. 6. Rarefied pyramidal cell of dog's cortex after four hours' etherisation.

Fig. 7. 'Skeleton' pyramidal cell of dog's cortex at the end of six hours' chloroform anæsthesia.

[*Reprinted from the Journal of Physiology.*
Vol. XXVI. No. 5, April 24, 1901.]

THE ACTION OF ETHER AND CHLOROFORM ON THE CEREBRAL AND SPINAL NEURONS OF DOGS.

Supplementary paper. By HAMILTON WRIGHT, M.D.,
Director of the Pathological Institute, Federated Malay States.

In my first paper on this subject[1], I showed that ether and chloroform produce in dogs and rabbits certain changes in the nerve-cells. The Nissl granules lose their affinity for methylene blue, and the cells present a rarefied or, in extreme cases, a skeleton-like appearance. By Cox's method the dendrons are found to present moniliform enlargements. These changes which I attribute to the bio-chemical action of the anæsthetic on the substance of the nerve-unit become more pronounced as the anæsthesia is prolonged, and are more readily induced in rabbits than in dogs. The nerve-cells of dogs show little or no change until the anæsthesia has been prolonged for more than four hours.

I now present five more experiments on dogs which supplement and extend these observations. The object of these experiments has been twofold:—

1. To determine whether a still more prolonged period of anæsthesia renders the changes more intense.

2. To ascertain whether the pseudo-degenerative change is permanent, by examining the tissues of the animals, after the anæsthesia has passed off.

I may briefly state at the outset, that the answer to the first question is in the affirmative, and to the second in the negative.

The plan of the experiments was the same as in the former cases. Care was taken to prevent the body-temperature of the animals from an undue fall. The 'return-time' of the conjunctival reflex I take as an index of the depression of the neurons affected by the anæsthesia.

The following table gives the duration of anæsthesia, the return-time of the conjunctival reflex, and the body-temperature in each of the five animals.

[1] This *Journal*, xxvi. p. 30. 1900.

	Anæsthetic	Time of return of conjunctival reflex	Minimum and maximum temp.
Dog 1.	Ether 8 hours	14½ minutes	97°—101·5° F.
Dog 2.	„ 9 „	15 „	96·9°—101·7° F.
Dog 3.	„ 8½ „	15½ „	97°—102° F.
Dog 4.	Chloroform 8 hours	16½ „	97·3°—101° F.
Dog 5.	„ 8 „	16 „	98°—101·3° F.

In these five animals, the anæsthetic was administered for from 2 to 3 hours longer than in any of my previous experiments; the return-time of the conjunctival reflex had increased from 5 to 5½ minutes in the previously recorded 6 hours cases to 14½—16½ minutes.

Dogs 1 and 4 were killed while the animals were still under the influence of the anæsthetic, in order to test the first question I had set myself to answer. The other three dogs were allowed to recover from the anæsthetic, and killed at later periods, in order to test the second question.

Brains and cords were as before placed in hardening fluids in order to examine them microscopically later. The microscopic methods were in the main the same as those previously used.

The following are the principal facts in each of the experiments :—

Dog 4. Chloroform for eight hours. The animal was then killed by the operation of removing the brain and cord while it was still under the action of the anæsthetic.

Cerebrum. Hardly a cell was normal in appearance when treated by the methylene-blue process. The change was the same as those previously noted, but more intense. The nuclei even participate in the difficulty of staining, and the nucleoli are almost achromatic, exhibiting distinctly their nucleolar particles. By Cox's method, there is also intensification of the moniliform condition of the dendrons and dendrites; this is especially well seen in the apical dendrons, which were traced into the molecular layer where they bend off to right and left. A larger number of basal dendrons were also affected.

Cerebellum. A few of Purkinje's cells are in the skeleton condition; a large number are slightly rarefied. Cox's method showed but little change.

Spinal cord. Here also nearly every cell was markedly affected. The same change in the nuclei and nucleoli noted in the cerebral cells was seen here also. The anterior cornual cells were those in which the change is most marked, but the large cells of the dorsal horns and intermediate grey matter were also affected. The preparations by Cox's method were a failure.

Glia cells. These were much increased in number in both cerebrum and spinal cord.

Dog 1. Ether for eight hours. The animal was then killed in the same way. In this animal the venous congestion and capillary anæmia noted in all etherised dogs was again seen. In the chloroformed dog, the venous congestion was slight. Otherwise, the changes in the microscopic appearances were practically the same as in the corresponding animal of the chloroform series just described.

Dog 2. Ether for nine hours. The animal was kept alive for 48 hours after the cessation of anæsthesia, and then guillotined. He showed the usual signs which an animal exhibits when recovering from an anæsthetic, and somewhat more than an hour after the administration had ceased he walked with grotesque unsteady movements to his kennel. During the night he barked and growled incessantly, and did not eat, but the next morning he was in a perfectly normal condition. Microscopic examination of the nerve-cells showed that they were practically normal; a few cerebral cells continued to be slightly rarefied, and a small number of the processes were still swollen. All the spinal cells were normal. Forty-eight hours thus suffices for a practically complete recovery from the effects of nine hours' ether narcosis.

Dog 3. Eight and a half hours' ether narcosis.

Dog 5. Eight hours' chloroform narcosis.

These two animals were kept alive for eight days after the anæsthesia, and were then killed by the guillotine. The nervous tissues were found to be absolutely normal in every respect. There was no trace of venous congestion, no rarefaction in any nerve-cell, and no degeneration of any nerve-fibre in brain or cord, as tested by Marchi's reaction.

Conclusions.

The observations confirm my previous work and show that ether and chloroform act directly upon the chromatic substance of the peri-karyon, chemically changing it so that it loses its affinity for aniline dyes. This bio-chemical change is more intense in the experiments now recorded than in those previously described, the anæsthesia having been kept up longer. In these later cases even the nuclei and nucleoli are affected. The nucleolus is the last part of the cell to show the effect of the drugs. The slow return of the conjunctival reflex in these cases appears to indicate that after a certain period of anæsthesia (six hours in the dog) the depression of neuronal function becomes more rapidly profound, and that there is a limit to the time of safe anæsthesia. The histological changes observed support this view, for a greater alteration occurred in the cells during the three hours between the sixth and the ninth of

anæsthesia, than during the five hours between the first and sixth hours.

The experiments also show that the changes described in the cells are only transitory; they disappear with the disappearance of the drugs from the circulation and tissues, or soon after. Certainly after nine hours' ether narcosis, the cells are practically normal 48 hours later. There is no permanent deformity in the cells, and no degeneration of their processes.

This observation affords further evidence that the moniliform swelling of dendrons is not due to simple retraction of neurons but is the result of a pathological change in the trophic centre of the neuron; it is analogous to the swelling observed in the first stage of atrophy in axons when cut off from their trophic centres.

The rarefaction of the cell-substance and the formation of moniliform swellings, may of course modify nervous function, and to such changes may perhaps be attributed those losses of memory, slight manias and melancholias that are now and then reported to follow prolonged anæsthesia in the human subject.

[*Reprinted from the Journal of Physiology.*
Vol. XXVII. No. 6, *January* 31, 1902.]

SPECIFIC GRAVITY OF THE BRAIN.

By R. H. C. GOMPERTZ, B.Sc., *Assistant Demonstrator of Physiology, King's College, London.*

(*From the Physiological Laboratory, King's College, London.*)

THERE are considerable variations in the statements which have been made concerning the specific gravity of the brain (Bischoff[1], Danilewksy[2]). The most complete research on the subject appears to have been that of Danilewsky, who examined various regions of the central nervous system. He attempted among other things to determine the area of the whole cortex by a comparison of the weight of the brain, its specific gravity (1038), the specific gravity of the grey matter (1033), and white matter (1041), and the average thickness of the grey matter (2·5 mm.) De Regibus made similar calculations from the amount of water in the whole brain and in its two constituent substances[3].

Among more recent writers, Halliburton[4] has determined the percentage of water in various parts of the nervous system; this is especially high in the grey matter, and in those regions where grey matter is abundant.

The present research was undertaken at Professor Halliburton's suggestion, as the matter had attracted his notice from an address on "Sex in Education" delivered by Sir James Crichton Browne[5] some years ago. Among the differences between the brains of men and women, Sir James stated he had found that the specific gravity of the female brain is less than that of the male brain; this difference was due to a difference of specific gravity in the grey matter, that of the white matter being the same in both sexes.

It was, however, pointed out in some correspondence that followed the publication of the address that this generalisation rested on very

[1] *Sitzungsberichte d. K. Bayer. Akad. d. Wiss.* II. p. 347. München, 1864.
[2] *Centralbl. f. d. med. Wiss.* XVIII. p. 241. 1880.
[3] See *Quain's Anatomy*, III. Part 1, p. 177. 1893.
[4] *This Journal*, XV. p. 90. 1894.
[5] *British Medical Journal*, I. p. 949. 1891.

few observations, the brains of two healthy men and one healthy woman having been investigated. Observations on the brains of lunatics, which were mainly used, can hardly be considered as likely to yield trustworthy results of what obtains in the normal state.

It was accordingly thought advisable to examine the brains in a larger number of cases. The experiments have been limited to the cerebral hemispheres of adult men and women who suffered from no brain disease. The cause of death and the age of each patient is stated in the following tables.

The brains were obtained as fresh as possible from the post-mortem room, and protected from evaporation until the observations were made.

As will be seen immediately there are considerable variations, which must in part be due to varying amounts of blood and lymph in the cerebral tissue. The only way to eliminate a changing factor is to obtain the average of a large number of observations.

The first observations I will describe are those in which I attempted to ascertain the specific gravity of white matter and grey matter separately; only instead of taking the brains of two men and one woman, I took those of two women and one man. The weighings were made in air, and in water, and the following are my results:

		Specific gravity of		
	Cause of death	White matter	Grey matter	
Male, aged 35	Typhoid	1·0381	1·0263	(Optic thalamus)
Female, aged 51	Scirrhus	1·0392	1·0335	(Cortex)
Female, aged 49	Fractured spine	1·0391	1·0329	(Optic thalamus)

If these numbers were relied on, they would prove the exact contrary to what Sir James C. Browne stated to be the case. As a matter of fact they also showed me that there are considerable variations, and pointed to the necessity of making a larger number of observations in order to obtain an average.

These further observations were made in a rather different way. There is considerable difficulty in obtaining sufficient of the grey matter, especially from the cortex, so as to be quite certain that there is no white matter mixed with it. If one shaves off the grey matter a little too deep in parts, there will be a variable admixture with white matter which will vitiate the results. Accordingly it was thought better to take a large piece of the brain from several regions, and estimate the specific gravity of the whole pieces. These were cut as

nearly as possible from the same regions in each brain. Care was taken to completely and rapidly remove the membranes, to select pieces where there was no portion of the ventricles or other cavities where air might be imprisoned, to leave them in the water long enough to displace all entangled air, but not long enough to cause any solution of the brain substance. No correction was made in the weighings in air, for the small amount of air displaced. This, however, is a very small error, and may be regarded as practically constant; the resnlts are, therefore, correct from a comparative point of view.

I will give my results in detail for the one case only.

Male, aged 40. Died of fracture of skull.

		Grammes
Frontal pole.	Weight in air	59·1115
	„ „ water	1·8270
	Specific gravity ... 1·0318.	
Rolandic area.	Weight in air	41·2275
	„ „ water	1·4155
	Specific gravity ... 1·0355.	
Occipital pole.	Weight in air	41·9805
	„ „ water	1·4900
	Specific gravity ... 1·0368.	

It will be sufficient to give the other results with this one in tabular form.

Specific gravities of male brains.

Age	Cause of death	Frontal	Rolandic	Occipital	Whole brain
50?	KCN poisoning	1·0348	1·0359	1·0390	1·0366
40	Fractured skull	1·0318	1·0355	1·0368	1·0347
35	Phthisis	1·0336	1·0349	1·0344	1·0343
24	Typhoid	1·0366	1·0379	1·0385	1·0373
58	Pneumonia	1·0351	1·0358	1·0326	1·0345
46	Pneumonia	1·0366	1·0365	1·0364	1·0365
43	Pulmonary embolism	1·0381	1·0393	1·0387	1·0387
	Average sp. gr.	1·0352	1·0365	1·0366	1·0361

Specific gravities of female brains.

Age	Cause of death	Frontal	Rolandic	Occipital	Whole brain
72	Cancer	1·0368	1·0405	1·0382	1·0385
50	Run over	1·0339	1·0351	1·0318	1·0336
51	Scirrhus	1·0363	1·0333	1·0348	1·0343
24	Stone	1·0368	1·0383	1·0405	1·0385
49	Fractured spine	1·0361	1·0371	1·0373	1·0368
	Average sp. gr.	1·0360	1·0368	1·0365	1·0364

In addition to the twelve brains included in the foregoing tables I have only examined one other, namely from a man aged 55 who died of uræmia, and in whom the brain was markedly œdematous. The results obtained from this brain are:

Frontal pole	sp. gr.	1·0280
Rolandic area	„	1·0350
Occipital pole	„	1·0307

This low specific gravity is obviously due to the œdema.

It will be noticed from the main results[1] shown in tabular form, that:

1. My numbers are somewhat lower than those given by Danilewsky[2].

2. There are considerable variations between the specific gravities of different parts of the same brain, and of different brains in both sexes.

3. That the average specific gravity is practically identical in the two sexes, a small difference in the fourth place of decimals being negligeable.

In conclusion, I may point out that a low specific gravity of the brain does not necessarily imply a poor quality, for the part of the brain which is most important and most active—the grey matter—has a lower specific gravity than the white matter[3].

Dec. 4, 1901.

[1] Since the above was written I have found in Poirier's *Traité d'Anatomie humaine* (Paris, 1899) a large number of observations on the specific gravity of the brain made by Peacock, Sankey, Bastian, Buckill, and others. The only one who touches the question of sex-difference is Peacock; he gives the average specific gravity of the male cerebrum as 1·034, and of the female cerebrum as 1·035. This difference which is in the same direction as the smaller difference I found is in Poirier's opinion too slight to be considered as definite, especially in view of the great individual variations.

[2] They accord very well with the numbers given in *Morris's Anatomy*, p. 726 (Ed. 1898); the source of the figures there quoted is, however, not given.

[3] A fallacy of this kind underlies many of the statements made on this subject in the discussion on the sex question. It is also seen in a communication made by J. P. H. Borleau (*Lancet*, II. p. 485, 1882), in which he draws attention, in the examination he made of the brain of a highly gifted man, not only to the great weight of the brain, but also to its *high* specific gravity.

[*Reprinted from the Journal of Physiology.*
Vol. XXVII. No. 6, *January* 31, 1902.]

RETICULIN AND COLLAGEN. By M. CHRISTINE TEBB.

(*From the Physiological Laboratory, King's College, London.*)

The fibres of reticular or retiform tissue are anatomically continuous with those of areolar tissue, and are not distinguishable from them on microscopic examination[1]. Mall[2], however, stated that no gelatin was obtainable from the fibres of reticular tissue, and that they were, therefore, chemically different from ordinary white fibres. The fact that gelatin is obtainable from reticular tissue was first demonstrated by R. A. Young[3], and subsequently by Siegfried[4]. Siegfried, however, confirmed Mall's view that the fibres contained something special, and separated from them a material which he called reticulin. If such a chemical substance does exist, the point is by no means proved that reticular fibres are different from white connective tissue fibres; it is at least equally possible that reticulin is not specially characteristic of reticular fibres, but is also present in all white connective tissue fibres.

Accordingly, at Prof. Halliburton's suggestion, I sought by following Siegfried's directions as closely as possible to separate reticulin from a typical form of connective tissue, namely, tendon. My failure to obtain this substance from tendon led me next to repeat Siegfreid's experiments on the tissue with which he himself worked, namely, the mucous membrane of the intestine. Here, again, I failed to obtain reticulin; the only organic substance present in appreciable quantity in the connective tissue basis is collagen. My experiments have led me to believe that Siegfried's reticulin is an artifact, and is mainly produced by the effect of ether and alcohol on collagen. These reagents render collagen very insoluble and hinder greatly its subsequent conversion by hot water into gelatin.

[1] Schäfer. *Quain's Anatomy.* 10th Ed. I. p. 239. 1891.

[2] *Anat. Anz.* III. No. 14, 1888; *Abhandl. d. math. phys. Cl. d. k. Sächs. Gesellsch. d. Wissensch.* XIV. No. 3, 1887; XVII. No. 4, 1891.

[3] This *Journal*, XIII. p. 332. 1892.

[4] "Ueber die chemischen Eigenschaften des reticulirten Gewebes," *Habitationsschrift.* Leipzig. 1892.

Such being the main results of the investigation, I propose to support my statements by recording a number of typical experiments. It will, however, be first necessary to describe those of Siegfried rather more in detail. He used the *mucosa* of pig's intestine, which he always obtained from a butcher whose assistant had been instructed in separating it from the *submucosa*. The *mucosa* of 8—17 pigs was placed in about 40 litres of water at 37° C. with 25—30 grms. of Parke Davis and Co.'s pancreatin, 50 grms. of sodium bicarbonate, and some sodium carbonate and thymol or chloroform. After 48 hours the tissue was well washed with water, collected by a centrifugal machine and freed from water by alcohol. It was then extracted with ether in a Soxhlet's apparatus for several days. The tissue was again digested with pancreatin with less water for a further period of 48 hours. After washing with water and drying with alcohol and ether, Siegfried describes the reticular tissue as being in grey strands, which swelled in water to porous membranes having the structure of the original tissue, and he says that the microscope showed pure reticular tissue free from connective tissue fibres and lymph cells. Siegfried's principal statements concerning reticular tissue prepared in the manner described are as follows:

(1) If it is boiled for half-an-hour, it loses its structure and is transformed partly into a loose powdery substance, which is the author's reticulin, and partly into gelatin.

(2) Almost all the gelatin-yielding material can be extracted from the tissue by boiling for 20 minutes; and from this it is inferred that the material converted into gelatin is something other than collagen, which cannot be converted into gelatin in so short a time.

(3) The percentage composition of the so-called reticulin is given as: C, 52·88; H, 6·97; N, 15·63; S, 1·88; P, 0·34; ash, 2·27. It differs from collagen in containing phosphorus, in yielding no gelatin on boiling, and in yielding little or no glutaminic acid on being boiled for 72 hours with hydrochloric acid.

I now pass to the description of my own experiments, and will first take those in which I sought for reticulin in fibrous tissue, with negative results.

Exp. I. Three-quarters of a pound of tendon (ox) was superficially freed from fat, cut into small pieces and left in water over night; after which it was finely divided and soaked in about 7 litres of saturated lime-water, occasionally renewed, with some thymol, for six weeks. It was then washed in a large quantity of water and for three minutes in 0·2 % acetic acid (which

did not more than neutralize the adherent lime), and finally in more water. It was then digested in a 2 % solution of Benger's *liquor pancreaticus*[1] (to which 0·5 % sodium carbonate and some chloroform were added) for 48 hours at a temperature of 37° C. The digestive fluid was then renewed, and the tendon was digested for a further period of 96 hours. The volume of fluid used in each case was about 15 litres. During digestion the quantity of tendon apparently diminished, pointing to the inference that some of the collagen itself had suffered digestion in spite of its never having undergone any preliminary treatment with acid[2] or boiling water. The tendon was ground in a mortar and digested finally for 2 days. It was then well washed and boiled in water for periods of half-an-hour at a time, the fluid being removed by decantation, and the tissue well washed after each period of boiling. The extracts always yielded satisfactory jellies. After 3 hours the collagen had all been converted into gelatin, leaving only a powder which I found to be carbonate of lime with hardly a trace of organic matter; it gave the faintest possible xanthoproteic reaction.

Exp. II. In this instance the tissue was not subjected to the action of lime-water, nor to pancreatic extract. 25 grms. of finely divided tendon were boiled in 250 c.c. of water; the water was renewed once an hour or oftener for 7 hours, when the tissue was nearly all dissolved. The small residue examined microscopically proved to be of the structure of tendon fibres and showed the rows of shrunken tendon cells. The extract after the first 20 minutes of boiling, yielded, on concentration, a firm jelly, showing that some conversion of collagen to gelatin had already taken place during that short time, in spite of Siegfried's statement to the contrary.

It would seem from these experiments that one of the results of the action of pancreatic digestion is to render collagen more easily convertible into gelatin. Siegfried notices, in the case of the *mucosa* of the dog, that previous treatment with pancreatic extract causes reticular tissue to fall to pieces on boiling much more rapidly than undigested tissue, and he says he has performed other experiments to show that pancreatic digestion has an influence on the rapidity of formation of gelatin from reticular tissue.

Failing to detect reticulin in tendon, I endeavoured to obtain it from reticular tissue itself. I procured the intestines of two pigs, but in each case I found that the *mucosa* was difficult to distinguish and to separate from the *submucosa*, and that it was very thin, and I have, therefore, preferred to work with the tissues of dog and cat. In these animals

[1] I am indebted to Mr Benger for specially active preparations of *liquor pancreaticus*.

[2] The acetic acid mentioned above was never in sufficient quantity to render the tendon acid to litmus.

the *mucosa* is thick and very easily distinguished and separated from the whiter and firmer *submucosa*. As digestive fluids I have used either Benger's *liquor pancreaticus* (usually in 25 % solution in 0·75 % sodium carbonate), or else Parke Davis and Co.'s pancreatin which I obtained direct from the manufacturers at Detroit; of this I used generally 2·5 grs. to a litre of 0·5 % sodium bicarbonate with some carbonate, this being a much larger proportion of pancreatin than Siegfried used. The mucous membrane contains a considerable quantity of mucin, which cannot be removed by simply washing for a few hours. This forms a slimy mass when alkali is added and impedes digestion. I therefore removed the greater part of the mucin before digestion by soaking the tissue for 24 hours in a 1 % solution of sodium carbonate. and then washing, repeating the treatment for a second period of 24 hours in some cases. It is remarkable that Siegfried makes no mention of the presence of mucin or mucin-like substances.

I have usually found that digestion of the cell substance takes much longer than the time Siegfried allowed, often as much as a fortnight, the digestive fluid being renewed every few days; and in many cases I have regarded it as impossible to remove the remains of broken-down cells; and this especially so, when I have attempted further digestion after the tissue has been extracted with ether.

When digested tissue is boiled 24 hours with water I have always obtained a minute residue which gives the xanthoproteic reaction, and is probably coagulated undigested proteid or some substance corresponding to anti-albumid.

The following are typical of many experiments performed:

Exp. III. The mucous membrane of the small intestine of a cat was washed in water and put into 250 c.c. of 1 % sodium carbonate over night; it was next day washed until no longer slimy, and put into 400 c.c. water in which 2 grs. sodium bicarbonate, 1 gr. carbonate, and 1 gr. Parke Davis and Co.'s pancreatin were dissolved, chloroform being used as antiseptic. The whole was kept at 37° C. for 5 days. The tissue was washed, and boiled in 50 c.c. water for 20 minutes; the extract was removed by decantation and was concentrated. The tissue was washed and then boiled with 50 c.c. fresh water; and so on repeatedly. Each extract before concentration gave a precipitate with tannin; the first 6 extracts mixed together yielded a jelly; the 7th, 8th, and 9th extracts together yielded a jelly; the 10th gave a small quantity of tender jelly, and so did the 12th extract. After boiling the tissue for 2 hours, 40 minutes the fluid became more difficult to decant, as the

pieces of tissue were softer, but they were still curled as when the boiling commenced. The tissue now appeared to gradually wear away, little pieces breaking off; but there was never at any time a falling to powder as described by Siegfried; and the fact of obtaining a jelly from the 12th extract shows that all the gelatin-yielding substance had not been converted into gelatin in 3 hours, 40 minutes. After boiling all night (with a long vertical tube fitted into the cork of the flask to avoid loss by evaporation), a small residue was left, which gave the xanthoproteic reaction. This was not reticulin, for according to Siegfried, reticulin when boiled for many hours continuously forms an opalescent solution.

The next experiment is one in which the mucous membrane was extracted with ether.

Exp. IV. The mucous membrane of the small intestine of a dog was soaked for 24 hours in 500 c.c. of 1 % sodium carbonate solution, and was then digested, after washing, with 400 c.c. of 0·25 % pancreatin in 0·5 % sodium bicarbonate solution for 4 days and again for further periods of 7 and 4 days. The tissue having been digested for 15 days, was well washed with water and then with alcohol, and was left during 23 days in ether. After this treatment the tissue was boiled during about 8 hours in successive volumes of water. The amount of water used during each operation was 50 c.c., and the water was changed at intervals of from 20 to 80 minutes. After such boiling, the tissue was still unchanged in appearance, and the extracts did not yield jellies, even when several were concentrated together. The tissue was allowed to boil all night in 100 c.c. water and there was next day a slight residue; the fluid in which it had been boiled gave a precipitate with acetic and with nitric acid, and with tannin, and gave a distinct xanthoproteic reaction.

From this experiment it would seem that prolonged treatment with ether had hindered the formation of gelatin when boiling water acted on the tissue; and I proceeded to test this quantitatively.

Exp. V. The mucous membrane of the small intestine of a cat was soaked for a few hours in 1 % solution of sodium carbonate and was afterwards well washed in water and each piece was wiped with linen. It was divided into two equal parts (12·9 grs. in each). One part was put into 400 c.c. of water already boiling and kept at 98° C. for 4 hours. Small pieces of tissue (probably villi) became detached as soon as the tissue was put into boiling water. At the end of the 4 hours these were collected on a dried weighed filter, and they were later washed with water till there was no longer any cloudiness when tannin was added to the washings; afterwards with alcohol and with ether; the filter was then dried at 110° C. and

weighed. The tissue weighed 0·02 gr. The larger pieces of tissue were severally washed well in water and were left in strong alcohol for 18 hours, in absolute alcohol for one hour and in ether for 7 days; they were then dried at 110° C. and weighed. The weight was 0·35 gr. Adding to this the weight of the small pieces, the whole weight of residue was 0·37 gr.

The other portion of mucous membrane was put into strong alcohol for 1 hour, absolute alcohol for 1·5 hours and ether for 7 days. It was then heated in 400 c.c. water at 98° C. for 4 hours—no small pieces broke off as in the case of tissue not previously hardened with ether—the residue was washed with water and left in strong alcohol 18 hours, absolute alcohol 1 hour, and ether half-an-hour, after which it was dried at 100° C. and weighed. The weight was 0·45 gr.

It will be observed that the tissue was in each case under ether for the same length of time, so the loss of weight due to the removal of fat was in each case the same. The difference in weight may at first sight appear small; but it must be remembered that only a portion of the mucous membrane is composed of a gelatin-yielding material, there being much proteid present.

It now seemed desirable to ascertain whether ether has a similar "coagulating" effect on tendon fibres. Prof. Halliburton tells me that some years ago Dr T. G. Brodie performed in this laboratory some experiments with alcohol. The main results of Dr Brodie's work, hitherto unpublished, are as follows:

(1) Alcohol has practically no "coagulating" action on gelatin. Gelatin may be kept for weeks or months under alcohol, but after that lapse of time it is still readily soluble in hot water and the solution gelatinizes on cooling.

(2) The effect of alcohol on collagen is, however, different. Even after a few days under spirit it is difficult to extract much gelatin with boiling water. After a few months none at all can be obtained.

I have now found that ether has a similar effect on collagen. The following experiment shows the result in a quantitative way:

Exp. VI. Some tendon was finely divided and in the fresh condition three portions of 20·0 grs. each were weighed. One portion was put into alcohol direct. Another portion was stirred for a quarter of an hour in alcohol and was then put into ether. The third portion was treated as follows:

The tendon was put into a flask and 600 c.c. of water already boiling were added, and the whole kept at 98·5° C. for $3\frac{1}{2}$ hours, the flask being fitted with a cork and a long upright tube to prevent loss by evaporation.

The contents of the flask were poured on to a filter, previously dried to constant weight at 110° C., and the residue, being collected on a filter, was washed with cold water until the washings no longer gave any cloudiness with tannin. It was then washed with spirit (the tube of the funnel being fitted with a cork) which was allowed to remain all night. Next day the spirit was allowed to drain through, and the residue on the filter was treated with absolute alcohol for an hour, and with ether for a quarter of an hour. It was very hard and appeared dry; it was now heated in an oven at 100° C. until the weight was constant.

As a result of heating 20·0 gr. of moist tendon with 600 c.c. water at 98·5° C. for 3½ hours, I obtained 4·00 grs. of dry residue.

(In the above experiment I always used cold water for washing the tendon; because hot water, even when it does not boil, converts collagen to gelatin, as I showed thus: 5 grms. of tendon were kept in 25 c.c. water in presence of chloroform at 60° C. for 23 hours. The clear liquid was separated and the albumin in it precipitated by boiling with a few drops of acetic acid. This precipitate was filtered off and tannin was added to a portion of the filtrate; a heavy precipitate was produced; the remainder of the filtrate on concentration yielded a firm jelly.)

The portion of tendon which was left under spirit was after 45 days dried at ordinary temperature and treated with water exactly as above. The residue weighed 6·11 grs.

The portion which was left under ether was after 43 days treated in exactly the same way; 6·54 grs. was left.

This experiment proves quantitatively that ether and alcohol, acting for a prolonged period, render collagen much less easily convertible into gelatin.

In no experiment on the boiling of reticular tissue with water, have I observed the falling to a powder described by Siegfried. On boiling the *mucosa* of dog after pancreatic digestion, he reports the falling to pieces in a quarter of an hour, whereas, if it were not previously digested this did not take place for one hour. I have also boiled undigested tissue, but even after 4 hours it was apparently unchanged; though when the tissue has been digested and not extracted with ether, I have noticed that it gets softer and begins to wear away during the second or third hour of boiling.

In no case have I extracted nearly all the gelatin that can be obtained from a sample in 20 minutes as described by Siegfried. Where I have obtained jelly from the first extract I have obtained it in apparently equal quantities from later extracts; and where the tissue has been for a prolonged period under ether I have not obtained more than traces of gelatin in any extract.

Siegfried says that he boiled reticulin for 72 hours with hydrochloric acid and tin chloride, and after removal of the tin saturated the concentrated fluid with hydrochloric acid at 0° C. After standing for many days in one instance no glutaminic hydrochloride had crystallized out, and in another instance there was only a trace.

Not being able to obtain reticulin, I have treated reticular tissue, after it has been boiled in water 1½—2 hours (which presumably Siegfried would consider long enough to remove all the gelatin-yielding substance), in a similar way, and have always been successful in obtaining a considerable deposit of small crystalline needles, which I believe to be glutaminic hydrochloride.

As one of many experiments I will cite the following:

Exp. VII. Some mucous membrane of the small intestine (cat) after digestion with 25 % Benger's *liquor pancreaticus* in 0·75 % sodium carbonate for nearly 3 weeks, the digestive fluid being renewed every few days, was washed in water and left to soak in distilled water, several times changed, in presence of chloroform, for 7 days. The prolonged washing with water was to remove the digestive fluid and products of digestion as far as possible. When examined microscopically the tissue appeared to be particularly free from cell substance. After treating for 2 hours with alcohol, the tissue was kept under ether for 7 days. It was dried at the temperature of the laboratory and its weight was 0·27 grms. This quantity was obtained from the mucous membrane of 9 cats.

The dried tissue was boiled in 50 c.c. of water for four periods of half-an-hour, being well washed after each period of boiling. From every extract I obtained a firm jelly. Since Siegfried states that almost all the gelatin is removed in 20 minutes, I conclude that if glutaminic hydrochloride could now be produced, he would not consider it to have come from any remaining gelatin-yielding substance.

After boiling for 72 hours with 50 c.c. of 15 % hydrochloric acid and a little tin dichloride, subsequently removing the tin by sulphuretted hydrogen, and concentrating the fluid to about 20 c.c., I saturated with hydrochloric acid gas. In a few minutes there was a deposit of cubical crystals of an inorganic nature—presumably of sodium chloride—and later, after surrounding the vessel containing the fluid with a mixture of ice and salt, I obtained a considerable deposit of small needles.

It is not possible to state positively without an elementary analysis that these crystals consist of glutaminic hydrochloride. The small amount of material at my disposal rendered this impossible; the difficulty of obtaining a larger yield will be evident from the fact that

the mucous membrane of 9 cats only gave me 0·27 gr. of raw material to start with. I, however, consider it extremely probable that they did consist of glutaminic hydrochloride; and my evidence is that their method of formation at a low temperature and their ready solubility on exposure to air when the hydrochloric acid evaporates, seems to preclude the possibility that they can be anything else. In crystalline form also they are similar to those which can be obtained from gelatin. I prepared crystals from gelatin itself under the same conditions, and in all the foregoing points they were identical with those obtained from the reticular fibres. The crystals obtained from gelatin I purified by recrystallizing twice, and determined the percentage of nitrogen in them by Kjeldahl's method. The results of two experiments were 7·51 and 7·57 per cent. of nitrogen. The calculated percentage of nitrogen in glutaminic hydrochloride is 7·64, and I think I am justified in concluding that glutaminic hydrochloride is the body with which I have been dealing.

Horbaczewski[1] obtained glutaminic hydrochloride by the action of hydrochloric acid on gelatin, and this he identified by making carbon, hydrogen, and chlorine determinations.

Conclusions.

It will be seen that I have been able to confirm very few of Siegfried's statements; and my main result is that reticulin does not exist either in ordinary white fibrous tissue (tendon) or in the reticular tissue of the intestinal mucous membrane. Both consist of fibres which are chemically and histologically identical; the main material of which they are composed is the gelatin-yielding substance called collagen.

I regard Siegfried's reticulin merely as collagen which has been "coagulated" by the reagents he employed (especially alcohol and ether) *plus* proteid and nuclein residues of cells. After treatment with these reagents the conversion into gelatin is much more difficult, not only in the case of a finely stranded tissue like reticular tissue, but even in such a dense material as tendon.

The fact that Siegfried's reticulin contains a small amount of phosphorus I attribute to the nuclei and other residue of cells. These (especially the cells of Lieberkühn's crypts) are much more difficult to get rid of by pancreatic digestion than Siegfried considers; on

[1] *Akad. d. Wiss. Sitzber. Wien.* LXXX. 2, p. 117. 1879.

microscopical examination of the digested tissue many parts will be found free or all but free from cells, whereas on making further search other pieces of the same mucous membrane will show a great many; on the whole, this is more marked in the dog than in the cat. Siegfried himself states that the phosphorus cannot be contained as nuclein because shaking reticulin with dilute nitric acid at 25° C. for half-an-hour will not produce phosphoric acid. Prof. Halliburton's experience with nuclein from animal cells is that considerably more vigorous treatment than this is necessary to produce phosphoric acid from it.

Siegfried states that reticulin yields to dilute alkali an organic compound containing phosphorus, soluble in chloroform and alcohol, and this he is inclined to attribute to something that resembles lecithin; the body in question might on my hypothesis be also derived from cell-residues.

With regard to the high percentage of sulphur, I would suggest that as he had previously removed from the tissue part of the collagen as gelatin (a substance poor in sulphur) the residue which he termed reticulin would contain a relatively high proportion of sulphur on account of the proteid admixture which would not have been removed by the boiling.

Coming next to the decomposition products of Siegfried's reticulin, he states that glutaminic acid is absent. I have certainly always found a body which I believe to be glutaminic hydrochloride in the decomposition products of reticular tissue which has been treated in a manner which Siegfried states to be sufficient to remove all collagen.

I have already alluded to the difficulty of being quite sure on this point, for glutaminic acid gives no tests by which it may be readily recognized, and the amount I obtained was too small for elementary analysis.

There are some small points on which my work differs from that of Siegfried; for instance, as to the relative length of time necessary to obtain gelatin from white fibrous tissue and reticular tissue respectively. I have also never observed the falling to pieces of reticular tissue which Siegfried states occurs on boiling it.

[*Reprinted from the Journal of Physiology.*
Vol. XXVIII. No. 3, May 28, 1902.]

FATIGUE IN NON-MEDULLATED NERVES.

By T. GREGOR BRODIE, M.D., *Director of the Laboratories of the Royal College of Surgeons (Eng.) and Royal College of Physicians (Lond.)*, and W. D. HALLIBURTON, M.D., F.R.S., *Professor of Physiology, King's College, London.* (Six Figures in Text.)

The experiments, on which the assertion rests that nerve-fibres are not fatiguable, have nearly all been performed with medullated motor nerves. The method adopted in such experiments has been to excite the nerves for a number of hours, and to exclude fatigue in the terminal structures by preventing the impulses reaching the peripheral organ. On removing the block by means of which this is accomplished the activity of the peripheral organ is still manifested with undiminished force. The blocks employed have been curare[1], a galvanic current[2], the application of ether[3], and in the case of secretory fibres, atropine[4].

A few investigators have employed non-medullated nerve-fibres in their experiments. Eve[5] used the cervical sympathetic nerve in the rabbit, and after exciting this for twelve hours found that the vaso-constrictor apparatus in the ear vessels was still in action at the end of this time. The main object of his experiments was to ascertain whether any histological change is demonstrable in the cells of the superior cervical ganglion. To this end he excited the preganglionic fibres. He therefore only incidentally touched the question of fatiguability of non-medullated fibres. He was also exciting other structures (nerve-cells and medullated preganglionic fibres). But so far as can be judged from his experiments he showed that non-medullated fibres were not fatigued, and even that fatigue of the peripheral vaso-motor mechanism is difficult to demonstrate.

[1] Bowditch. This *Journal*, VI. p. 133. 1885.

[2] Bernstein. *Pflüger's Archiv*, XV. p. 289, 1877; Wedenski, *Centr. f. d. med. Wiss.* XXII. p. 65. 1884.

[3] Maschek. *Sitzungsber. d. k. Acad. d. Wiss. Wien.* XCV. Abth. 3, p. 109. 1887.

[4] Lambert. *Compt. rend. Soc. Biol.* 10th Ser. I. p. 511. 1894.

[5] This *Journal*, XX. p. 340. 1896.

Howell, Budgett, and Leonard[1], in a paper on the influence of temperature on the irritability and conductivity of nerve-fibres, also incidentally touched upon the question; they state that vaso-constrictor and cardio-inhibitory fibres show no functional fatigue; but as the longest time during which they applied continuous excitation was one hour, their contention can hardly be considered to be satisfactorily proved.

Another set of experiments has been performed by Miss Sowton[2]. In 1897, Dr Waller[3] alluding to his well-known theory that carbonic acid is produced during the activity of nerve, writes, "You have seen that such a (medullated) nerve is inexhaustible, and yet that it exhibits very clear symptoms of chemical change after action (increase of the action current similar to that produced by minute doses of carbonic acid). All these things (*i.e.* including the facts of Wallerian degeneration) reconcile themselves with the notion that the active grey axis both lays down and uses up its own fatty sheath and that it is inexhaustible, not because there is little or no expenditure, but because there is an ample re-supply."

If this tentative suggestion is correct, and the absence of fatigue is due to the presence of the fatty sheath, fatigue ought to be demonstrable in nerve-fibres which have no fatty sheath. Miss Sowton selected the olfactory nerve of the pike as the non-medullated nerve with which to try the experiment, and the tracing she has published shows that the galvanometric replies of this nerve become slightly feebler after repeated stimulation.

As some doubt has recently been cast on the absolute trustworthiness of the electrical response as a sign of nervous activity[4], recourse to some other method appeared to us imperative.

The splenic nerve seemed to be the most convenient for this purpose, since it consists mainly of large bundles of postganglionic non-medullated fibres. Our first experiments were, therefore, conducted upon this nerve.

The following is the method of procedure we adopted:

A dog is anæsthetised with morphine and A.C.E. mixture or ether, the abdomen opened, the spleen exposed, and the splenic nerves which lie by the side of the main splenic artery are laid bare. It is quite easy to dissect out

[1] This *Journal*, XVI. p. 298. 1894.

[2] *Proc. Royal Society*, LXVI. p. 379.

[3] *Lectures on Physiology. First Series. Animal Electricity*, p. 70. 1897.

[4] See Prof. Gotch's article 'Nerve' in Schäfer's *Text-book of Physiology*.

a length of nerve sufficient for the experiment (1 to 1½ inches). The nerve is cut as far from the spleen as possible, and the spleen is enclosed in an air oncometer, as in Schäfer and Moore's[1] experiments; our instrument differed from theirs in that the splenic mesentery with its vessels, &c. enters through a long slit at the bottom of the box, instead of through a groove at the side. On stimulating the nerve with a faradic current the organ contracts and the recording lever of the writing apparatus falls. In our earlier experiments we sometimes used a Marey's tambour, or a piston-recorder. As, however, the range of these instruments proved to be too limited for our experiments we abandoned them for a light flexible bellows[2] made of cardboard and peritoneal membrane. This is brought into communication with the spleen box by a piece of stout india-rubber tubing, and can be very accurately calibrated; the amount of the fall or rise of the lever attached to the upper surface of the bellows thus gives us a measure in c.c. of the decrease or increase of the splenic volume.

It is not absolutely necessary to use any recording apparatus at all; the shrinkage of the spleen when the nerve is excited is perfectly evident to the eye, and is limited to that portion of the spleen which

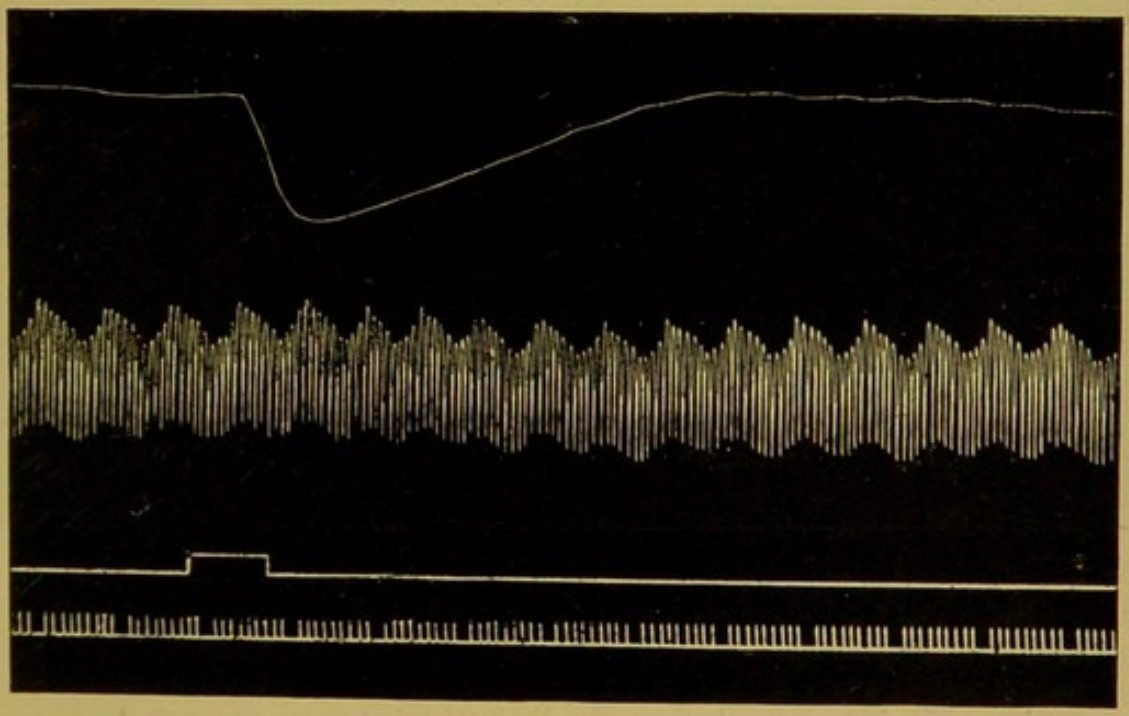


Fig. 1. Dog. Morphine and ether. The upper line gives the splenic volume, the second the carotid blood-pressure. The signal line is 6 cms. above the zero of blood-pressure. Time tracing in seconds. During the time indicated by the signal a large splenic nerve was stimulated. Coil at 2 cms. The decrease in volume of the spleen amounted to 6 c.c. This tracing is the same size as the original.

[1] This *Journal*, xx. p. 1. 1896.

[2] This piece of apparatus was exhibited by T. G. Brodie at the International Congress of Physiologists. Turin, 1901. See also this *Journal*, xxvii. p. 473. 1902.

is innervated by the nerve-fibres on the electrodes. Still it appeared to us advisable to employ the exact method of registration just described, as it gave us quantitative differences in the contractions recorded at different times.

Simultaneously with the splenic tracing we always took a tracing of the arterial blood-pressure from the carotid artery. This we did because most of our experiments lasted many hours, and the condition of the arterial pressure furnished us with a convenient means of gauging the general condition of the animal. We give (Fig. 1) a typical tracing of the result obtained on stimulating the nerve.

A slight rise of arterial pressure usually occurs simultaneously with the contraction of the spleen (see Fig. 3, *D*), an effect the cause of which is discussed later (p. 197).

We next found that a longer stimulation does not keep the spleen contracted, but that the organ gradually returns to its original volume in spite of the stimulation. The production of this condition is also clearly shown by recording the successive contractions produced by short excitations repeated after brief intervals of rest. This is shown in the next tracing (Fig. 2).

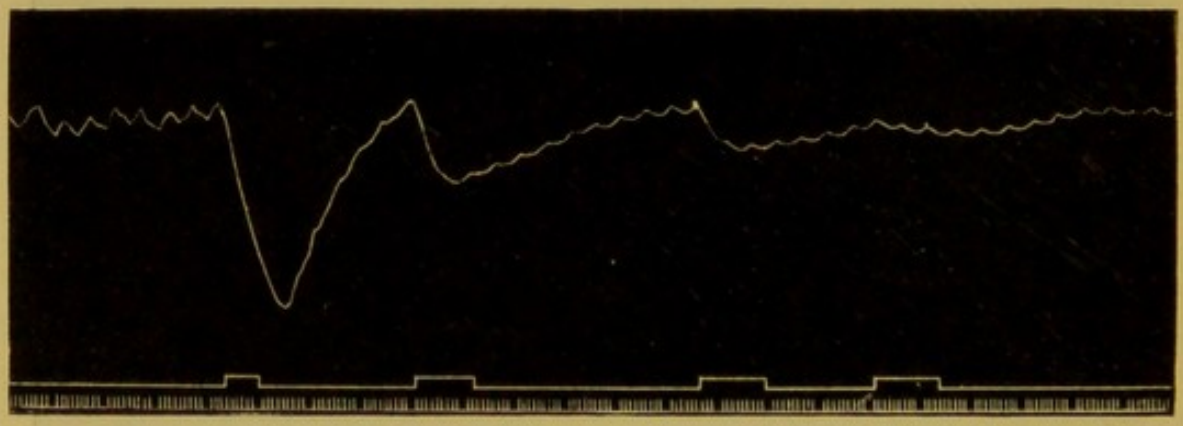


Fig. 2. Dog. Morphine and ether. Splenic volume. At the times indicated by a rise of the signal line a splenic nerve was stimulated. The strength of the stimulus was the same in all four instances. Du Bois coil, 2 Leclanché batteries, coil at 6 cms. Time tracing records seconds. This figure is rather more than half the size of the original tracing. The first contraction caused a diminution in volume amounting to 14 c.c.

Here it will be seen that the effect of each stimulation is smaller than the preceding, and in the end hardly any response is noticeable. This is due to some change analogous to fatigue in the terminal structures, for the fourth excitation of the series in the record (Fig. 2) was applied to a piece of nerve nearer to the spleen than that which was excited during the first three excitations. That it was not due

to fatigue of the nerve-trunk was excluded by our subsequent experiments.

It was, therefore, evident that we must have recourse to some form of block so as to prevent the nerve-impulses reaching the spleen. Curare and atropine are both ineffective, and so we tried the constant current. This was sent in by means of platinum electrodes, placed between the exciting electrodes and the spleen, and it proved a very effective block. But it has this disadvantage; the polarisation of the nerve lasts so long that a considerable time elapses after the removal of the current before the fibres can once more conduct nerve-impulses. After quite a weak current ($\frac{1}{3}$ of a Daniell cell) has been sent in for two minutes the nerve does not transmit impulses for an hour or more; it then slowly recovers. If, therefore, faradic excitation of the nerve is kept up all the time the constant current is in, and fails to cause a contraction of the spleen when the constant current is removed, it is impossible to say whether this is due to fatigue of the nerve-fibres on the proximal side of the block, or whether it may not be due to the fact that the block created by the constant current is still effective.

Non-medullated fibres are much more sensitive to the polarisation effects of a constant current than medullated nerves are. We made control experiments on the dog's sciatic nerve; the block created in the sciatic passes off much more rapidly, and even when six Daniell cells are employed has disappeared in twenty minutes after five minutes' exposure. The prolonged injurious effect produced by a constant current was observed by Wedenski, who avoided it by reversing the direction of the current at fairly frequent intervals. We tried this with our non-medullated nerves, but were never able to abolish the effect, so as to be certain the block was removed immediately the current was broken.

We had, therefore, to employ a block which would be at once effective and immediately removable. We found that cold fulfils these requirements admirably. The nerve rests on a pair of platinum electrodes, and on a metal tube through which cold brine can be kept flowing. This tube is interposed between the electrodes and the spleen. The electrodes and tube are mounted on a small block of wood which can be easily kept in position, and the nerve is kept moist by a cover of thin rubber. If the temperature of the fluid is just about freezing point[1],

[1] As the nerve and tube lie within the body the fluid is warmed several degrees when circulating through the tube; it is therefore necessary to employ a colder solution (a few degrees below zero) than would suffice to block the impulses if the nerve was freely exposed to the air.

nerve-impulses are completely blocked. Immediately the cold fluid is replaced by warm (36° C.) the nerve conducts the nerve-impulses again.

We ascertained first the effect of these variations of temperature without using the exciting current. The effect is almost inappreciable; sometimes we have noticed a slight slow expansion of the spleen when the cold saline is turned on, and sometimes an increase in the amplitude of the splenic waves. Turning on the warm solution subsequently produces the opposite effect, but this is always extremely small.

If the brine in the tube is kept at the low temperature mentioned, and the nerve is being excited with strong induction shocks all the time, the spleen remains irresponsive; the nerve-impulses are able to reach the block but not to pass it. If now warm water is passed through the tube, thus removing the block, and the spleen continues to be irresponsive, we have a proof that the piece of nerve between the electrodes and the tube has been fatigued.

Our experiments have, however, shown us that the splenic nerves are just as difficult to fatigue as medullated motor fibres. We have done a number of experiments in which we varied the length of time during which the nerve was stimulated, but even after the longest period (six hours) the nerve is practically as excitable as it was at the start, for a good splenic contraction is obtained when the cold block is removed. One of our numerous graphic records is shown in the next tracing (Fig. 3).

At the commencement of this experiment the spleen gave the contraction reproduced in Fig. 1. The duration of the stimulus was 9 seconds and the secondary coil was 2 cms. from the primary. The nerve was next blocked by cold, and after a short time the secondary coil was pushed home on the primary, and the stimulation continued with short intervals at the end of each hour to test whether the nerve remained excitable. After the total duration of the excitation had amounted to 3 hours the tracing of Fig. 3 was taken. At the beginning of this tracing the nerve was still being stimulated. At *A* the current of cold saline was stopped and replaced by warm water at *B*. It is seen that 7 seconds later the spleen commenced to contract. The stimulation was stopped at *C*, and 44 seconds later, *i.e.* at *D*, the nerve was again excited for 10 seconds when a very marked contraction resulted. This contraction was larger than that of Fig. 1, for as already pointed out the strength of the stimulus had been increased a little. In this experiment the nerve was still further excited, until the sum of the

times of stimulation amounted to 4½ hours but still with the same result.

From experiments such as this, and from others in which the time of excitation amounted to more than 6 hours, we conclude that the splenic nerve-fibres cannot be fatigued.

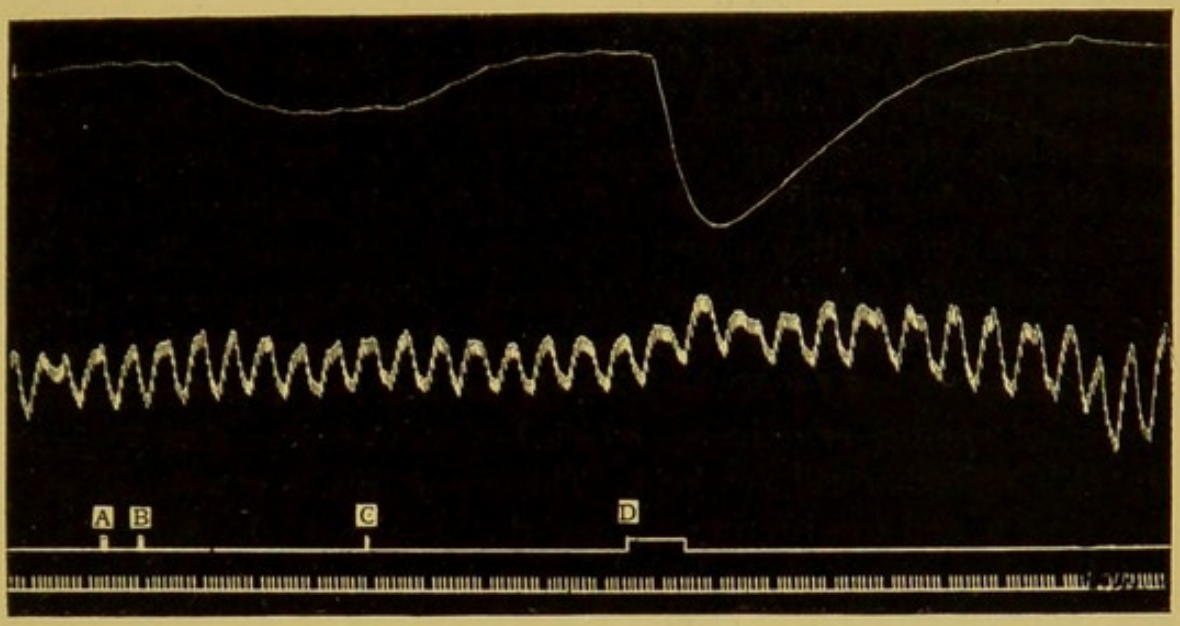


Fig. 3. Dog. Morphine and ether. A large splenic nerve has been excited continuously for three hours. This excitation is still continued at the beginning of the tracing. The upper tracing is that of the splenic volume. The second, the blood-pressure taken from the carotid. The next line is a signal line. The zero-abscissa of blood-pressure is 5 cms. below this line. The time record marks seconds. This figure has been reduced to about two-thirds of the original size.

We examined histologically some of the nerves which had been excited, and found the vast majority of the fibres were non-medullated, but that there were always a few fine medullated fibres. In one specimen, for instance, we found as many as 50 medullated fibres, whereas the non-medullated were present in thousands. In reply to a letter from us regarding these fibres Dr Langley kindly wrote to us as follows:

"The medullated fibres peripheral to the solar plexus are partly afferent, partly preganglionic, which have not yet reached their cell-stations (very few of these go to the spleen), and partly medullated post-ganglionic. They all, but especially the last, vary in number in different animals."

"The external carotid branch of the superior cervical ganglion in the rabbit, which amongst other structures supplies the ear, contains

very few medullated fibres, half-a-dozen perhaps, and possibly these are all afferent."

Even if all those fibres of the splenic nerve which are medullated were motor it is hardly possible to imagine that the effect we obtained could be due to them, having regard to their insignificant number, and the very large area of the spleen which contracts.

A possible source of fallacy in our experiments was pointed out by Prof. Gotch at a meeting of the Physiological Society when we gave a demonstration on this subject. He suggested that the distance between the exciting electrodes and the cooling tube, *i.e.* five millimetres, was possibly too short, and that the effect of the cold might spread to the position of the exciting electrodes, and annul the excitability of the nerve to faradic stimulation at this spot. If this were so we should not have been starting nerve-impulses at all, and our method would, therefore, be useless for determining the problem. Since then we have performed another experiment, which has convinced us that Professor Gotch's objection will not hold. The experiment to test the point is very simple, and consists in reversing the position of the cooling tube and the electrodes. The cold brine was kept flowing for 46 minutes, and its temperature 0° C. at first was depressed until it reached – 5° C. (the outflow water having a temperature of – 3° C.)[1]. The effect did not spread to the exciting electrodes; and at each test excitation during this period, between one and two hours, the spleen executed as large contractions as it had shown before the cold brine was allowed to run.

We have extended our observations to other nerves, and our first experiments in this direction were performed upon the cervical sympathetic.

The external carotid branch was dissected out, and left in connection with the superior cervical ganglion; all the other branches of the ganglion were divided. The nerve in question breaks up into twigs around the artery very soon, so that it is not possible to isolate more than about half-an-inch: the ganglion forms a convenient handle by means of which the nerve can be lifted on to the electrodes. We at first placed the nerve beyond the electrodes on a tube through which cold brine was kept flowing, and found that cold here as in the splenic nerves formed a convenient and perfect block to the impulses. If no block is employed some amount of constriction of the ear vessels persists throughout the period of excitation, and flushing of the ear

[1] These are temperatures considerably lower than those we have found it necessary to employ in our experiments.

takes place when the stimulation ceases. In one experiment we continued stimulating for six hours, with occasional brief stoppages to observe the relaxation.

We found that some constriction persisted during the whole period of excitation, but that the constriction gradually decreased, and the longer the stimulation was kept up the less pronounced became the final flushing.

We therefore thought it better to test the question in a region where it would be possible to obtain a graphic record of the result. Accordingly we selected the hind leg of the dog as the subject of our experiment. The animal was anæsthetised in the usual way, and sufficient curare was given to prevent the contraction of voluntary muscles on stimulation of their nerves. The limb was enclosed in a plethysmograph, and the sciatic nerve was exposed and stimulated. The resulting fall of the plethysmograph lever persists for about a minute, but in spite of the continuance of the excitation the lever gradually ascends and in a period varying from five to ten minutes has nearly returned to its original level. When the faradisation is stopped the plethysmograph lever rises above its previous level, showing that vascular dilatation has occurred. If a few minutes are now allowed to elapse, a second period of excitation leads practically to the same result. If only half-a-minute's interval is allowed, the constriction is less marked, and the return is more rapid. But there is usually a small amount of residual constriction, and dilatation occurs on cessation of the stimulation. The excitation was kept up in one animal for $4\frac{1}{2}$ hours, with occasional brief intervals to see if dilatation occurred. In another animal the excitation was maintained for 5 hours with a similar result.

The results we had obtained upon the blood vessels of the rabbit's ear and the dog's leg led us to still further extend our experiments to other vessels and we therefore performed some upon the intestine. The splanchnic was exposed at the lower part of the thorax by resection of the posterior portions of two ribs. About $1\frac{1}{2}$ to 2 inches of the nerve were then dissected out and having been cut centrally the nerve was placed upon shielded electrodes. A loop of intestine was enclosed in a plethysmograph and its volume changes recorded by a small bellows-recorder. The results of these experiments are in the main similar to those we had found with the sciatic. The constriction is at first great and remains so for a variable time. In our experiments this time has varied from 3 to 8 minutes. A gradual dilatation then sets in and

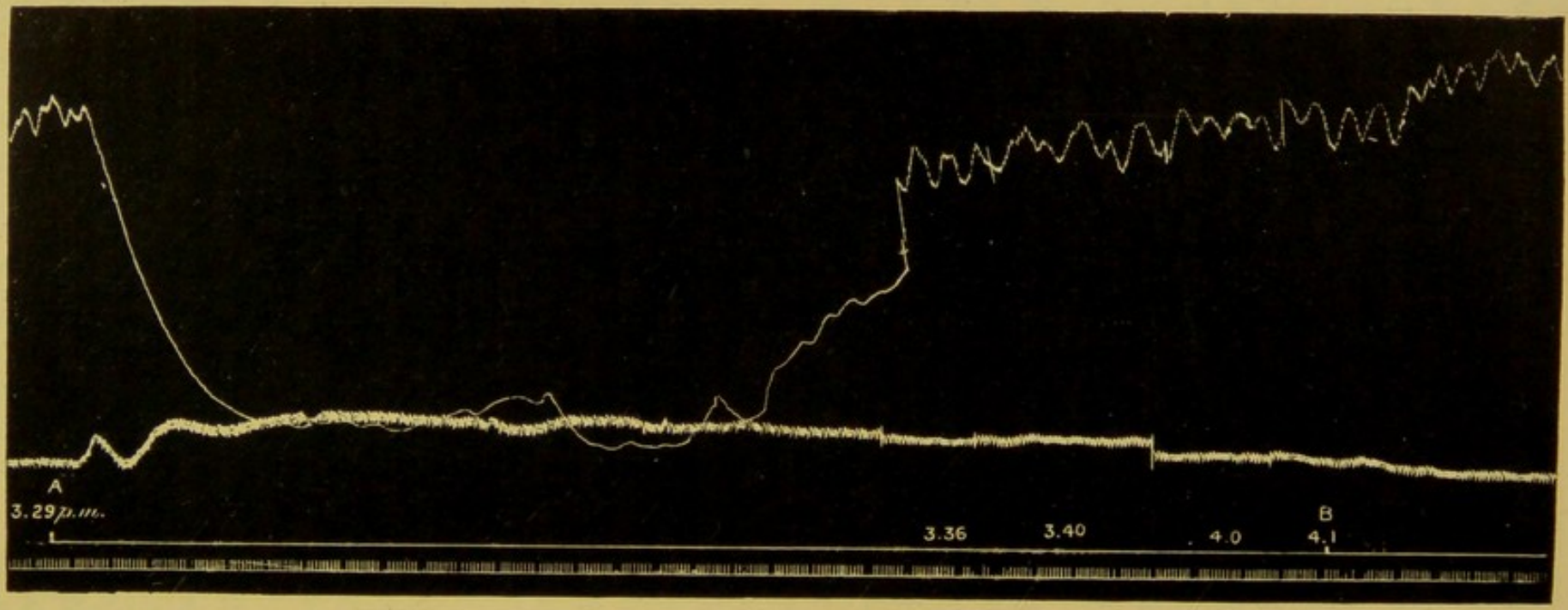


Fig. 4. Dog. Morphine and A.C.E. mixture. The uppermost line is a tracing of the intestinal plethysmograph; the next is blood-pressure. Time tracing shows seconds. At *A* on the signal line faradic excitation of the splanchnic nerve was commenced (3.29 p.m.). The intestinal vessels almost immediately contracted and the lever of the bellows-recorder fell, and remained in this position for about 3 minutes; the two large movements noticed during the last minute of this time were produced by slight struggling movements of the animal. The lever then began to gradually ascend, and short pieces of the record were taken at intervals; the time is shown under the tracing; at 4.1, *i.e.* 32 minutes after the observation began, the stimulation was stopped (*B* on signal line), and a slight further relaxation of the vessels took place. This figure is rather more than half the size of the original.

after a time varying from 6 to 25 minutes the organ has nearly returned to its original volume. If the stimulation is stopped soon after this stage is reached a further dilatation is recorded which may lead to a rise of the recording lever to a higher level than that at the start of the experiment. If the stimulation be continued for a considerably longer time this dilatation is much less marked and may even be absent. The result of a typical experiment is shown in Fig. 4. If the nerve is again stimulated within a minute of the cessation of the first stimulation, as a rule no contraction is recorded, and if any occurs it is very small in amount. If, however, we wait about 15 mins. and then stimulate again, we record just the same series of changes as before but with the following differences. The amount of contraction is only about one-half that of the first, is slower in onset, and recovery begins much earlier. Even in these cases, however, it is common to observe a small amount of dilatation when the stimulation is stopped. In a number of experiments of this kind we have repeated the stimulations several times until the total duration of excitation has added up to between 5 and 6 hours, and we have still been able to observe, in a few cases, a dilatation on cessation of the stimulation, but the longer the time of stimulation has been the less marked is the ultimate dilatation. This is well seen in Fig. 5. We have in all cases controlled our results by inspection of the intestine itself. The blanching with the first excitation is of course very evident and so is the dilatation which comes on during the course of the stimulation, but the dilatation occurring when the excitation is stopped is at best very difficult to observe, and in many instances was quite undetectable although a reference to the record proved that one had taken place.

We see, therefore, that in all our experiments upon blood vessels we invariably found that the constriction passed off in spite of a continuance of the excitation, and that this was especially the case when the splanchnic or the sciatic was stimulated. Also, on cessation of stimulation there may be no further dilatation (Fig. 5), though usually some was recorded. When obtained it was never great in amount, rarely exceeding $\frac{1}{10}$th of the amount of the original constriction. Since the completion of our experiments we have found that the same effect has been observed by Howell, Budgett, and Leonard[1] for several different sets of sympathetic fibres. They, too, record it for the blood vessels of the hind limb when the sciatic is stimulated, and have

[1] *Loc. cit.*

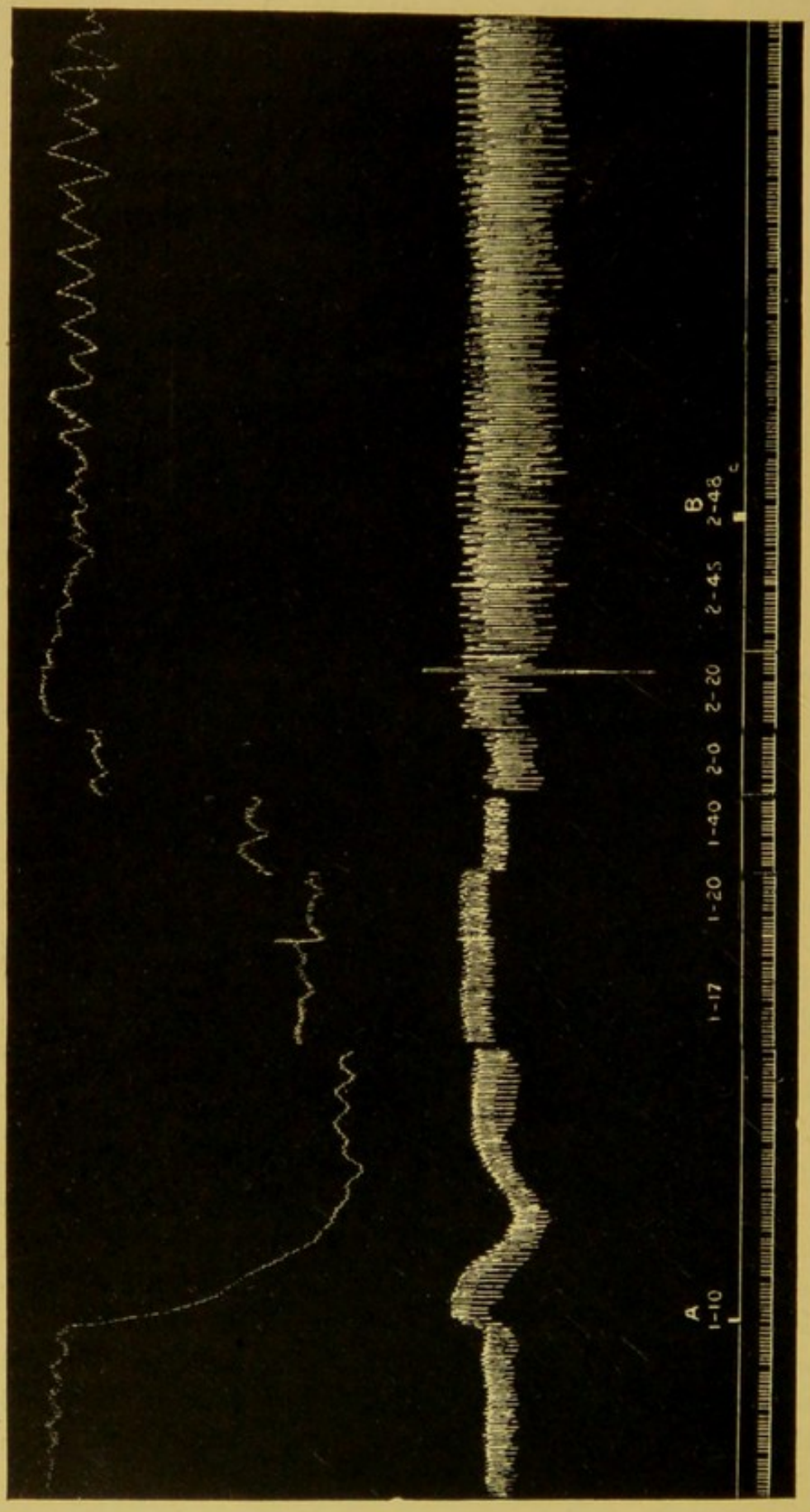


Fig. 5. Dog. Morphine and A.C.E. mixture. The uppermost line is a tracing of the intestinal plethysmograph. The next is blood-pressure. Time in seconds. At *A*, 1.10 p.m. the right splanchnic was excited and this excitation maintained until *B* at 2.48. The tracing gives short lengths of tracings at successive intervals of 20 mins. It is seen that dilatation had commenced at 1.17, and was complete at 2.20. The figure also shows that there is no dilatation occurring after cessation of the stimulus at *B*. This figure is two-thirds the size of the original.

observed a similar effect when watching the dilatation of the pupil following stimulation of the cervical sympathetic in the rabbit.

In attempting to determine to what cause this effect is due we have several possibilities to consider. It may be a peripheral effect, *i.e.* it may be due to fatigue of the muscle or nerve endings. Or again, it may be a local effect upon the nerve at the spot excited, or an effect upon the whole length of nerve down to the vessels. If a local effect upon the nerve it may be due to mechanical injury, to polarisation, or to local fatigue. Of these possible causes we consider that a local polarisation due to the current is the most probable explanation, though there are indications that other factors take some part in its production. Thus we found that, if the electrodes are shifted nearer the periphery after we had excited the nerve for a time long enough to allow dilatation to occur, a good constriction could be obtained. This constriction was only in rare cases as extensive as the original, but was usually less in amount and slower in development, indicating as we think some degree of fatigue either of the muscles or nerve endings. If, after a prolonged stimulation, we tested the different parts of the nerve we found, as above stated, that stimulation below caused constriction, stimulation at the original spot caused no effect even if the strength of stimulation was increased, and finally that stimulation above the original level also caused constriction in the majority of cases. In this latter case the constriction was never quite so marked as that produced by exciting below.

The condition of the nerve therefore was, that it was inexcitable at the original spot stimulated, but excitable above and below that spot, and further that though inexcitable it could still conduct, but to a somewhat less degree, than in the parts which had not been stimulated. Howell noted that the effect was mainly a local one and pointed out that by moving the electrodes peripherally a further good contraction could be obtained. They speak of the effect as "stimulation fatigue," but the use of the term fatigue in this connection is we think scarcely a wise one. We have so frequently observed the extreme sensitiveness of these non-medullated fibres to electrical currents that we are much rather of the opinion that we are here dealing with a polarisation effect. This is also supported by the fact that the nerve just below the part stimulated is still excitable and gives no evidence of fatigue. To avoid polarisation we performed experiments in which we used the Helmholtz modification and non-polarisable electrodes, but here again obtained the same results as before. We also excluded the possibility

that they were due to the use of too strong a current of stimulation or to mechanical pressure on the nerve.

In order to avoid any possibility of polarisation we have attempted mechanical and chemical stimulation but have not been successful. All the forms of mechanical stimulation have proved most ineffectual. Thus stimulation by dropping mercury on the nerve or by v. Uexkull's tetanometer very rarely excited at all, and if any effect was produced it was minimal. Pinching the nerve is effective, but is obviously useless for our experiments for it destroys the nerve and the stimulus lasts but a short time.

With regard to chemical stimulants, we obviously could not employ any reagent which would affect the nerve injuriously. Of the chemical agents employed by previous workers, sodium fluoride appeared to us to be the one we were in search of, since its effects can be abolished by washing it away with physiological salt solution. We used solutions of this salt varying in strength from 1 to 4 per cent. but with absolutely negative results. Dilute sulphuric acid also caused no excitation. It may be mentioned here that one of us (T. G. B.) has tried dilute acids and alkalis, sodium sulphate, sodium chloride, and magnesium sulphate of various strengths upon the vagus in the cat but in all cases with negative results.

It is a very curious thing that different nerves vary in the readiness with which 'stimulation fatigue' may be produced. Of the nerves tested by Howell, Budgett, and Leonard it was shown most clearly by the vaso-constrictor and sweat fibres of the sciatic nerve. Vaso-constrictor fibres at their origin from the cord, and in the cervical sympathetic where they are medullated do not exhibit this phenomenon. Such a distinction between medullated and non-medullated nerve-fibres was, however, not found to be of universal application; for instance 'stimulation fatigue' was as readily produced in the sweat fibres in the part of their course where they are medullated, as in the part where they are non-medullated.

To this we have to add, that various non-medullated fibres show a similar difference; the non-medullated splenic nerves do not show any 'stimulation fatigue' even after six hours' excitation, or if any is at all noticeable it is exceedingly slight. In the investigation of this phenomenon there is of course no necessity to have recourse to a block. After the completion of our experiments on the sciatic and splanchnic nerves we returned once more to the splenic nerves, and some experiments without a block fully confirmed our earlier results; little or no

stimulation fatigue was discoverable. In the case of the splenic nerves 'peripheral fatigue' is much more easily produced.

When we consider how insensitive mammalian nerve-fibres are to chemical, mechanical, or thermal stimuli, whereas they respond very readily to electrical stimuli, it becomes probable that electrical stimulation must produce some very considerable disturbance at the spot excited. If this is so, we can readily understand that prolonged stimulation by electrical currents of high potential may produce marked injury at the excited spot, especially if the nerve is a very sensitive one. In this connection, too, it is important to emphasize the point already noted, that though a nerve in which "stimulation fatigue" has been produced is inexcitable it can still conduct. That is, that though it cannot respond to the very abnormal mode of excitation by an electrical stimulus it can still carry on its normal and proper function, viz. that of conduction, in which the spot is excited by the proper physiological impulse.

To sum up, although we are inclined to regard the phenomenon of 'stimulation fatigue' as probably due to the injurious polarisation effects produced by electric currents at the spot of nerve stimulated, we are unable to be quite positive that this is the correct explanation, first because of our inability to control our experiments by the methods of mechanical or chemical stimulation, and secondly because we are unable to explain the difference just noted between various nerves.

The main object of our research was to test Dr Waller's hypothesis that the presence of a medullary sheath explains why fatigue in nerve-fibres cannot be demonstrated. By the use of our methods of investigation we have shown that functional fatigue is just as difficult to produce in non-medullated as in medullated nerves.

Although some recent observers[1] have once more affirmed that the non-medullated fibres possess a thin medullary sheath, we consider that Tuckett[2] has satisfactorily proved that this is not the case.

We are therefore convinced that Waller's hypothesis cannot hold as a correct generalisation for all nerve-fibres.

On *a priori* grounds we should not have expected to find that non-medullated fibres are peculiarly susceptible of fatigue, seeing that some of them, especially the vaso-constrictors, are continually in action, and that dilatation of the vessels always occurs when they are cut.

[1] Gad and Heymans. *Arch. f.* (*Anat. u.*) *Physiol.* p. 531, 1890; Ambronn and Held. *Arch. f. Anat.* (*u. Physiol.*) p. 201. 1896.

[2] This *Journal*, XIX. p. 267. 1895–6.

Certain side issues of our work have attracted our attention; one of these is the remarkable sensitiveness of non-medullated fibres to the polarising effect of a constant current; another is the rise of arterial blood-pressure which accompanies splenic contraction; and a third is the question whether on prolonged activity non-medullated fibres become acid.

We will take these three points one by one.

1. The duration and intensity of the blockage created by quite weak constant currents has already been alluded to. Two minutes' exposure to a current equal to one-third of a Daniell cell creates a block which lasts for more than an hour, and yet in the case of the same nerve (the splenic nerve with which most of our experiments have been performed, and in which so-called 'stimulation fatigue' is not a noticeable phenomenon), several hours' excitation with alternating induction shocks produced in the usual way by the Wagner's hammer of the inductorium has very slight polarising effects. We thought it worth while to ascertain whether a succession of induction shocks all in the same direction would produce an effect comparable to that caused by the constant current. In order to do this we used the key previously described by one of us[1]. In some experiments we used only make shocks, the break shocks being all short-circuited. In others we used break shocks only. We found, as we anticipated, that the effect produced is comparable to that caused by the constant current, though it naturally takes longer to develop. We used the same induction coil as before with two cells, the secondary coil being pushed home. The rate of stimulation was about twenty times per second.

In one dog, the piece of splenic nerve excited was polarised, or to put it another way, manifested loss of excitability due to 'stimulation fatigue' after 35 minutes' excitation; in another animal about an hour and a half was necessary to produce the same effect. The block created in this way extends some little distance (three or four sixteenths of an inch) beyond the excited spot and lasts for about an hour. We consider that such experiments support the hypothesis that 'stimulation fatigue' is a polarisation phenomenon.

The sensitiveness of non-medullated nerve to electrical currents suggests to us two possible explanations of the effect observed by Miss Sowton on the non-medullated fibres of the pike's olfactory nerve. In the first place the diminution she found in the galvanometric

[1] T. G. Brodie. *Proc. Physiol. Soc.* p. x. 1896. This *Journal*, XIX.

response in this nerve after repeated stimulation is probably not an indication of true functional fatigue. For by repeated closure of the galvanometer circuit the nerve is being repeatedly submitted to the influence of a weak galvanic current, namely, the action-current of the nerve itself, and the diminution of the amount of galvanometric response may be simply the result of the polarising effect of a constant current. In the second place, she may have been dealing with an instance of 'stimulation fatigue.' She at any rate does not mention in her paper that she shifted the position of her exciting electrodes.

2. The second side issue of our work to which we have devoted attention is the rise of blood-pressure which occurs on stimulating the peripheral end of the cut splenic nerves.

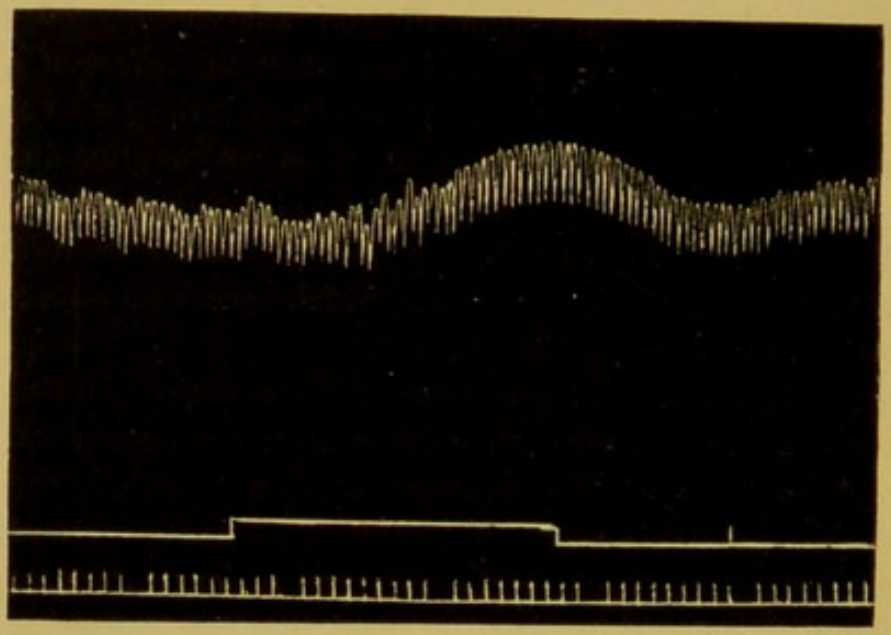
Using the calibrated bellows recorder, the amount of blood squeezed out by the contracting spleen into the general circulation was found to average 10 to 15 c.c. We doubted at first whether this slightly increased flow, acting through the liver and pulmonary circuit, could be sufficient to account for the rise of carotid blood-pressure observed, but we found on actually testing the point that this simple explanation is the correct one, and that it is unnecessary to suppose that there is any nervous reflex concerned. We injected 10 c.c. of defibrinated blood into the splenic vein at a rate comparable to that produced by the contracting spleen, and invariably found a slight rise of arterial pressure similar to that seen in our previous tracings.

Fig. 6 shows the graphic record of such an experiment; in *A*, the rise of arterial pressure which accompanied a splenic contraction (equal to 10 c.c.) is shown; *B* shows the similar effect produced in the same animal by injecting 10 c.c. of defibrinated blood into the splenic vein.

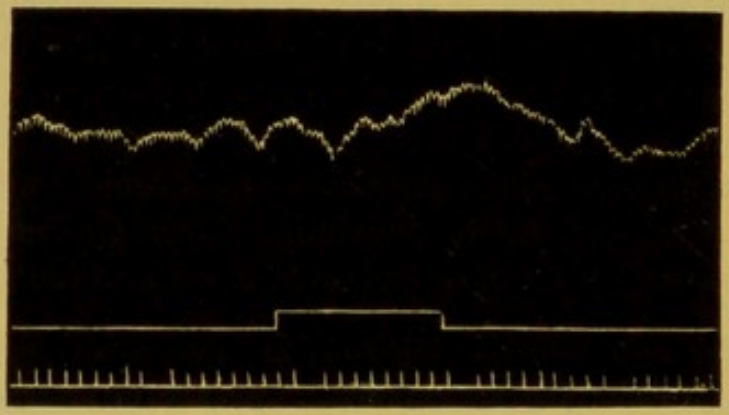
Physiological salt solution injected in the same way produces a similar result. In one dog we employed, this rise of arterial pressure was always in the case of salt solution, even if warmed to body temperature, followed by a marked fall; the same occurred if the injection was made into the crural vein. We have very seldom noticed this effect in other animals, and we are unable to account for it; no doubt this particular animal was in some way peculiarly sensitive to salt solution. Defibrinated dog's blood produced no such fall.

3. We tested the reaction of the stimulated nerves, thinking that here was a favourable opportunity of ascertaining whether on long-continued excitation there is a development of acid similar to that described by Eve and others in the central grey matter. The absence

of acidity in medullated nerve might be possibly due to the masking effect of a mass of myelin. We, however, never found that the splenic nerves even after the longest stimulation became acid to litmus. We



A



B

Fig. 6. Dog. Morphine and ether. Tracings of carotid blood-pressure; time in seconds. *A*. During the time indicated by the rise of the signal line, excitation of a splenic nerve caused contraction of the spleen; in *B*, during the time indicated by the rise of the signal line, 10 c.c. of defibrinated dog's blood was injected into the splenic vein. In both *A* and *B* there is a slight rise of pressure. Original size.

did not investigate the question of carbonic acid production. There was also no histological difference between the portion of the nerve which had been excited and that which had not.

General Conclusions.

The main results of our work are the following.

1. Using cold as a block in the same way that Bowditch used curare and Bernstein a galvanic current in their well-known experiments which showed the non-fatiguability of motor medullated nerve-fibres, the non-medullated nerves of the spleen even after many hours of stimulation give no demonstrable evidence of fatigue.

2. Non-medullated nerve-fibres do not become acid to litmus as the result of the prolonged stimulation described in our experiments.

3. The small rise of arterial blood-pressure which occurs soon after the splenic contraction begins is due to the increased flow through the liver produced by the increased output of blood from the contracting spleen.

4. In experiments on fatigue in non-medullated nerves, the constant current is not suitable as a blocking agent. Non-medullated fibres like those in the splenic nerve are remarkably sensitive to the constant current and do not transmit nerve-impulses for a considerable time after quite weak currents have been passed through them for short periods. Induction shocks in one direction produce a corresponding polarising effect only more slowly, but if one uses induction shocks in alternating directions any appreciable amount of 'stimulation fatigue' does not occur in the splenic nerve even after several hours' excitation.

5. The term 'stimulation fatigue' introduced by Howell, Budgett, and Leonard indicates that certain nerves (mainly but not entirely of the non-medullated variety) are injuriously affected by prolonged faradic stimulation, so that the spot which has been excited is no longer excitable until a considerable period of rest has elapsed.

6. We have noticed in the spleen that 'peripheral fatigue' is induced somewhat readily. We have seen but little evidence of this in vaso-motor nerves; the vaso-motor nerve-fibres we have mainly tested are those in the sciatic and splanchnic nerves. These nerves do not manifest signs of 'functional fatigue' in the course of the nerve-fibres. They, however, differ from the splenic nerves in that the phenomenon of 'stimulation fatigue' is produced with comparative readiness.

7. A portion of nerve in which 'stimulation fatigue' has led to a complete loss of excitability can still transmit nerve-impulses, though its power in this direction is somewhat impaired.

8. We think that the use of the term fatigue in this connection is not correct. We are inclined to believe that it is due to the injurious polarisation produced by electric currents. We were not however able to establish this hypothesis by control experiments in which mechanical or chemical excitation was employed, for non-medullated fibres show little or no response to this form of excitation, unless the mechanical means adopted are such as to injure the nerves. We are also unable to explain why different nerves like the splenic and the intestinal should manifest so great a difference in the readiness with which this so-called 'stimulation fatigue' is manifested.

9. The diminution of the action-current observed by Miss Sowton after repeated stimulation of the non-medullated nerve-fibres of the pike's olfactory nerve is probably not an indication of true functional fatigue, but is possibly produced either by the polarising effect of a weak constant current due to the closure of the action-current through the galvanometer circuit, or else is a case of 'stimulation fatigue.' Possibly a combination of both factors comes into play.

Some of the expenses of this work have been paid from a grant from the Government Grant Committee of the Royal Society.

24 *March*, 1902.

[*Reprinted from* Vol. II. of "The Archives of Neurology."]

THE COAGULATION-TEMPERATURE OF CELL-GLOBULIN, AND ITS BEARING ON HYPER-PYREXIA.

BY W. D. HALLIBURTON, M.D., F.R.S.,

AND

F. W. MOTT, M.D., F.R.S.

It is well known that there are various factors that influence the temperature of heat-coagulation of proteid substances. Among these the rate of the rise of temperature is one of some importance. This was clearly demonstrated in the work of Corin and Ansiaux,[1] and of Hewlett.[2] These observers showed in connection with serum and egg-white respectively that if the temperature is maintained long enough below the point at which heat-coagulation is usually stated to occur, not merely opalescence but the formation of flocculi will take place.

In performing the process of fractional heat-coagulation with extracts of various organs and tissues, one of us[3] has shown that in nearly all of them a proteid is present that coagulates at an extremely low temperature, which varies in different cases from 45° to 50° C. This proteid is a globulin, and has been variously named. Thus, in muscle Hammarsten terms it musculin, and one of us has named it para-myosinogen; in liver cells it has been called hepato-globulin; in extracts of nervous tissues, neuro-globulin; in extracts of lymph-cells, cell-globulin, and so on. There can be very little doubt that such a globulin is characteristic of protoplasmic structures, and even if it is not absolutely the same proteid in all cases, the term cell-globulin may be provision-

[1] *Bulletin de l'acad. roy. de Belgique*, xxi., 3, 1891.

[2] *Journ. of Physiol.*, xiii. 494, 1893.

[3] See "Schäfer's Text-Book of Physiology," vol. i., Art., "The Chemistry of the Tissues and Organs," by W. D. Halliburton.

ally employed in a general sense to indicate that cells, as a rule, yield to saline solvents a proteid with characteristically low coagulation-temperature.

One might, however, object that the behaviour of saline extracts of cells does not necessarily teach us the condition of the proteids as they are actually present in the complex we call protoplasm. In view of such a criticism we attach special importance to the researches subsequently carried out by Brodie and Richardson,[1] and later by Vernon.[2] These investigations show in the case of muscle that the shortening which occurs in the process of heat-rigor is not a single one, but takes place in a series of steps; the temperatures at which these steps occur are the same as those at which the individual proteids separate out during the fractional heat-coagulation of an extract of muscular tissue. Thus in mammalian muscle the two principal shortenings occur at 47° and 56° C., the coagulation-temperatures of the two principal muscular proteids. In frog's muscle there are three steps at 40°, 47°, and 56° C. respectively, which correspond to the three proteids that can be separated out in a saline extract of this variety of muscular tissue.

Brodie and Richardson also showed another important point, namely, that after the first step has occurred in the shortening, the muscles lose their irritability; in other words, in order to destroy the vitality of muscular tissue, it is not necessary to raise the temperature sufficiently high to coagulate all its proteids, but that when one of the muscular proteids has been coagulated, the living substance as such is destroyed. It therefore appears to be the case that the proteids of muscle are not independent units. The unit is protoplasm, and if one of its essential constituents is destroyed, protoplasm as such ceases to exist.

These experiments in connection with muscle would lead one to suppose that the same is true in regard to other protoplasmic structures; that is to say, the results which have been obtained by the examination of saline extracts of such structures can be applied to the elucidation of the composition of the protoplasm of which they are composed.

[1] *Phil. Trans.*, vol. 191 B, 127, 1899. [2] *Journ. of Physiol.*, xxiv., 239, 1899.

Our attention has been directed to a consideration of this subject in connection with the question of hyperpyrexia. One of us (F. W. M.) has made observations on the condition of the nerve-cells after death has occurred in this condition. The cells show a disappearance of the Nissl granules; both cell-bodies and processes show a diffuse blue staining with methylene blue.[1]

It is a familiar fact that very high body temperature is incompatible with life. Marinesco[2] has pointed out, in experiments on hyperthermia in animals, that a temperature of 47° C. is immediately fatal; a temperature of 45° C. kills in an hour or two; a temperature of 43° C. kills after a longer lapse of time. Moreover, the occurrence of death is coincident with the break-down of the nerve-cells in the manner just indicated. It is possible that analogous changes occur in other cells of the body also, but these do not seem to have been specially investigated. The nerve-cells are undoubtedly essential to healthy life, and lend themselves very readily to microscopic investigation, especially by the methylene blue process. A temperature of 47° C. leads to a practically instantaneous disappearance of the chromatophile granules; the same change occurs at 45° C. in a few hours; at 43° C. a longer lapse of time is necessary.

We have been struck with the coincidence of the fatal temperature (47° C.) with that of the coagulation-temperature of neuro-globulin;[3] and we argue that as in muscle, the coagulation of even the lowest coagulating proteid of nerve-cells would produce a destruction of the life of their protoplasm; a distinct chemico-physical cause can therefore be found for death due to hyperpyrexia.

Still a temperature as high as 47° C. (117° F.) in man is unknown; and we thought it possible that the proteid in question would coagulate at a lower temperature if it was

[1] In saying this we do not commit ourselves to the opinion that the Nissl granules are existent as such in the normal cells. The diffuse staining is probably due to a diffusion of the nucleo-proteid material throughout the cell owing to the break-down of the protoplasm.

[2] "Recherches sur les Lésions des Centres Nerveux consécutives à l'Hyperthermie Expérimentale et à la Fièvre." *Revue Neurologique*, 1899. See also Goldscheider and Flatau, "Normale und Pathologische Anatomie der Nervenzellen," Berlin, 1898.

[3] See "Proteids of Nervous Tissue," by W. D. Halliburton, *Journ. of Physiol.*, xv., 90, 1893.

kept at that temperature a sufficient length of time. We proceeded to put the suggestion to the test of experiment, fully anticipating, in the light of the work of Hewlett and others, alluded to in the opening paragraph of this paper, that the supposition would turn out to be correct. Experiment has shown that this is the case.

The first experiments were made with the brains of cats.[1] After the animal had been killed by bleeding (sufficient chloroform having been given to render it unconscious), the brain was rapidly removed; the grey matter was finely minced and ground up in a mortar with 0·9 per cent. solution of sodium chloride. We selected this solvent as the one likely to produce least change in the constituents of the protoplasm. After repeated filtration the extract remained somewhat opalescent; it was fairly rich in proteid as tested by rapidly boiling a sample. It did not prove at all difficult to see any increase in the opalescence when the extract was carefully heated in a water-bath. The extract was faintly alkaline, but we judged it best not to add any acid to neutralise this, in order that we might deal with as natural conditions as possible.

When the rate of observation is fairly rapid, the first crop of flocculi was observed to separate out at 47° C. These are removable by filtration, and the filtrate is practically clear.

In our next experiment the temperature was not allowed to rise higher than 45°, and was kept between 44° and 45° C., being more frequently nearer the lower than the higher of these limits. In somewhat less than two hours the separation of flocculi took place, and as good a coagulum was ultimately obtained at this temperature as was obtained in the first experiment at 47° C. Previous to the formation of actual flocculi, there was an increase of opalescence, which became denser as time went on.

In the next experiment an attempt was made to obtain the coagulum at a still lower temperature, namely, 42° C. (108° F.); here again we were rewarded with success; there was at first the gradual deepening of the opalescence,

[1] All experiments involving the use of animals have been carried out by one of us (W. D. H.) at King's College, London.

and in time a distinct separation of minute flocculi, which increased in number and size. The first separation of visible flocculi occurred about three hours after the commencement of the observation, and an hour later the crop was fairly abundant, though the size of the coagulum was not so great as in the previous two experiments. After filtering, the flocculi were, of course, removed, but the filtrate was still distinctly opalescent. Doubtless if we had continued to watch the tube for a longer time the coagulation would have been more complete.

The next experiment consisted in trying a still lower temperature, namely, 40°-41° C. In this case, however, though the tube was watched for eight hours, there was no coagulation.

We have repeated this series of experiments several times, and in some cases instead of grinding up the brain substance with salt solution only, we have employed clean sand or powdered glass as well. By this means one obtains an extract richer in proteid, filtration is easier, and the filtrate clearer. The phenomena of heat coagulation are exactly the same as in the experiments just described, but the proteid being more abundant they are more readily seen.

In a further series of experiments we have employed human grey matter, removed from the cadaver as soon as possible after death. We have selected the optic thalamus as a convenient mass of grey matter for this purpose. The results absolutely agree with those already given.

We had hoped to have had an opportunity of similarly investigating the grey matter after death had supervened in hyperpyrexia; but since we began this work no such case has come under our notice. We have accordingly had to be content with experiments on animals. A cat was, after anæsthetisation, rapidly killed by bleeding; the brain was removed as quickly as possible and divided into two equal halves; this was first done roughly, and then the two halves were accurately made equal by removing fragments of the white matter from the heavier moiety. Each weighed about 9·5 grammes. One half was immediately ground up with powdered glass and normal saline solution, and the extract examined. The other half was first heated to 47° C.

for an hour, and then similarly treated, the same volume of saline solution being used. In the extract of the first (the normal) half, fractional heat coagulation revealed the presence of coagula, which came down at 47°, 56°-60°, and 72° C. respectively.[1] In the extract of the second (the heated) half, the 47° coagulum was absent, but the other two were obtained. The total amount of proteid in the two extracts was also estimated in the usual way, by weighing the precipitate produced by excess of alcohol. 100 cc. of the first (normal) extract contained 0·674 grammes and 100 cc. of the second (heated) extract contained only 0·144 grammes of proteid material. The amount of cell-globulin which passes into solution in normal saline is thus relatively large.

In a second experiment, the half-brain was heated to 42° C. instead of 47° C. It was kept at 42° C. for five hours. Examination of the extracts showed that the extract of the normal half gave the usual crop of coagula, and 100 cc. contained 0·483 grammes of proteid; the extract of the half-brain which had been heated to 42° C. gave as before no coagulum at 47° C.; 100 cc. of this extract contained 0·226 grammes of proteid. The chemical examination of brain tissue as fresh as possible thus gave results which exactly correspond to those obtained in the experiments with saline extracts of brain.

The same is true for the histological examination we have made with "surviving" brain tissue.

We have not repeated Marinesco's experiments on hyperthermia in animals, but we have performed the experiment of exposing the brain *in situ* immediately after death to an elevated temperature. Two cats were anæsthetised and decapitated; the heads were placed in a warm chamber, a thermometer being inserted into the brain through the foramen magnum. In one cat the brain was kept at 44° to 45° C. for one and a half hours; in the second cat, at 42° to 43° for three and a half hours. In each case, and particularly in the first one, the cells exhibit chromatolysis. These experiments completely corroborate the views expressed in the foregoing portions of this paper.

[1] See "Proteids of Nervous Tissues," by W. D. Halliburton, previously quoted.

The following are the details of the microscopical examination of the brains of these two cats :—

Cat 1.—Brain *in situ* was exposed to a temperature of 44-45°C. for 1½ hours immediately after death. Preparations were made of the cerebrum by the methylene blue process.

Neuroglia.—The cells in the first layer seem somewhat unusually large and prominent.

Small pyramids.—A few cells appear fairly normal, but the majority are swollen both as regards nucleus and cell body; the body of the cell is generally stained deeply; protoplasm is breaking down. Sometimes only a small tag is left by the side of a large pale nucleus.

Medium pyramids.—The body of the cell is generally swollen; the nucleus in some is swollen, in others shrunken and distorted, and the processes of the cells are few and indistinct. Various stages of chromatolysis (perinuclear and peripheral) are seen. No distinct granules are seen in any of the cells, and the reticulum is clearly visible in many of them. All that is left of some cells appears to be a nucleus and some shreds of what looks like stained reticulum. Other cells have a dull, diffuse, granular appearance.

Largest pyramids.—Betz cells. Some cells are much swollen; the processes generally are few and short, and when seen are usually pale. The outline of the nucleus is often not clear; in some cases the nucleolus seems large. No Nissl-bodies are seen in any of the cells, but the protoplasm has a finely granular, somewhat honeycombed appearance, often stained a general dull faint blue. The reticulum is clearly visible in places in some cells. In others there appears to be simply a ring of stained protoplasm, with a nucleus at one side and a clear space in the body of the cell.

Practically none of the cells are normal; all show more or less advanced acute changes. The perivascular spaces are somewhat dilated as a rule.

Leucocytes and small round cells (nuclei of neuroglia?) are fairly numerous, and often in close relation to the nerve-cells.

Cat 2.—Brain *in situ* was exposed to a temperature of 42°-43° C. for 3½ hours immediately after death. Preparations of the cerebrum were made by the methylene blue process.

Neuroglia.—The cells seem rather large and prominent in the first layer.

Small pyramids.—These are not generally so deeply stained as in Cat 1, and not so swollen ; many seem fairly normal, others show breaking up of protoplasm.

Medium pyramids.—These also are not, as a rule, so swollen as in Cat 1. The processes are often fairly numerous and distinct, and Nissl-bodies are seen in many of the cells. Some cells are swollen and show chromatolysis, some stain faintly, others deeply and diffusely.

Largest pyramids.—Some cells are practically normal in shape, and show numerous Nissl-bodies, perhaps rather faintly in the processes. Other cells are swollen, and stain diffusely. Some are pale, others dark ; the body of the cell has a granular appearance. The chromatolysis does not seem so advanced in nearly so many of the cells as in Cat 1 ; the lymphatic spaces are dilated.

Leucocytes and small round cells are fairly numerous.

Conclusions.

Our experiments confirm our hypothesis, that the physico-chemical cause of death from hyperpyrexia is due to the coagulation of cell-globulin. When this constituent of cell-protoplasm is coagulated the protoplasm as such is destroyed. The temperature at which such coagulation is most easily produced is 47° C. But temperatures as low as 42° C. will have the same effect, provided the heating is continued long enough. These chemical changes in the brain substance are demonstrable by experiments with saline extracts of that tissue, or with the "surviving" brain of animals just killed. They are coincident with the histological (chromatolytic) changes in nerve-cells, which can be rendered evident by the use of the methylene blue method. The expression coagulation-necrosis employed by Marinesco for this appearance is therefore justifiable, though Marinesco and others who have obtained similar results missed the connection of the temperature necessary to produce it, with that of the coagulation-temperature of cell-globulin. Lastly, though the nerve-cells are those which lend themselves most readily to the histological part of the research, it is by no means improbable (looking at the wide distribution of cell-globulin) that many other cells of the body are affected by a high temperature in a corresponding manner.