ON THE EFFECT OF NARCOTIC AGENTS IN THE DETACHMENT OF ELECTROLYTES FROM CELL PROTEINS¹

By HERBERT E. ROAF, M.D., *Lecturer on Physiology, University of Liverpool*, and E. ALDERSON, M.D.

From the Bio-Chemical and Physiological Departments of the University of Liverpool

(Received July 12th, 1907)

Moore and Roaf, as the result of experiments on the comparative vapour pressure of chloroform when dissolved in water, saline, serum and haemoglobin solution, showed that serum and haemoglobin solution, for the same strength of chloroform, always possessed a lower vapour pressure of chloroform than did saline or distilled water.²

In a later paper the same authors showed that in lowering the vapour pressure of chloroform, emulsions of tissue cells had a greater effect than emulsions of the ethereal extractives from the same source and of the same strength as in the original emulsion of tissue cells.

From these results it was suggested that chloroform formed a loose chemical compound or physical aggregate with proteins and that anaesthesia results from paralysis of the activities of the protoplasm by the formation of such aggregates. Further, from the greater solubility of other anaesthetics in serum than in water, it is probable that they act in a similar manner.³

Observations on the electrical conductivity of saline and serum before and after the addition of chloroform showed that the same

1. The expenses of this research have been defrayed from a grant given by the Government Grant Committee of the Royal Society.

2. *Proc. Roy. Soc.*, Vol. LXXIII, p. 382, 1904, and *Thompson Yates and Johnston Laboratories Reports*, Vol. VI, Part I, p. 151, 1905.

3. *Proc. Roy. Soc., B.* Vol. LXXVII, p. 86, 1905.

percentage of chloroform caused less diminution of conductivity when added to serum than when added to saline, and that under certain conditions the conductivity, instead of being diminished, was actually increased by the addition of chloroform to serum.¹

It was suggested that this increase of conductivity was due to the detachment of electrolytes from the proteins under the influence of the chloroform.

Alcock, using frog's nerve, found that chloroform and ether affected the injury current. If the anaesthetic was applied to the whole nerve the injury current was abolished. When it was applied to the cut end the current was increased, but when applied to the longitudinal surface the anaesthetic diminished the electrical potential. In uncut nerves a current was caused by local application of the anaesthetic, and the direction of the current was such that the anaesthetised portion became negative to the rest of the nerve.²

These electromotive effects could be explained by the same process that Macdonald showed to take place in an injured portion of nerve. Dead or dying portions of nerve have the electrolytes liberated from their 'masked' condition and thus the injury current is caused by the diffusion of the ions from a point of higher to that of lower concentration.³

Alcock states that the electrolytes are not liberated by the anaesthetic as the electrical conductivity was not increased after the nerve was anaesthetised,⁴ but Waller found a diminution of resistance when a nerve was chloroformed.⁵

In a second paper Alcock showed that the resting current of frog's skin was abolished by the application of chloroform to the outer surface, but it was unaffected when the anaesthetic was applied to the inner surface. He also found that the electrical conductivity was increased

1. *Ibid.*, p. 99.

2. *Proc. Roy. Soc., B.* Vol. LXXVII, p. 267, 1906.

3. *Proc. Roy. Soc., B.* Vol. LXXVI, p. 322, 1906.

4. *Loc. cit.*, p. 278.

5. *Journ. Physiol.*, Vol. XXIII, proc. p. 11, 1899.

after the skin was subjected to the action of chloroform vapour.¹ He explains these results on a membrane hypothesis whereby the anaesthetic is supposed to act by altering a layer of lipoid surrounding each cell.² The presence of a resting current shows an unequal distribution of electrolytes in which the ions of the electrolytes are moving in such a direction that the difference of potential tends to be abolished. If the salts of the lymph are less firmly united to the proteins than are those of the epidermal cells, so that the active mass of electrolytes is greater at the inner than at the outer surface, then setting free of salts on the application of chloroform to the outer surface would diminish the electrical potential and at the same time increase the electrical conductivity. There is, therefore, nothing in Alcock's experiments which disproves that chloroform can detach electrolytes from combination or adsorption with proteins.

Stewart in working with muscle extracts found that heat coagulation caused an increase of electrical conductivity due to a detachment of electrolytes, but the effect on the molecular concentration was peculiar. Freezing point determinations showed sometimes a greater and sometimes a less depression after removal of the proteins. These changes were not due to experimental error, as the changes are beyond the error of the method used.³

Ringer⁴ and Locke⁵ showed the effect on the heart beat of various inorganic salts when perfused through the arteries, and suggested that a mixture of salts is more favourable than any individual constituent of the solution. Later observers have demonstrated that this rule holds true for every kind of cell activity. The results of experiments on the influence of various salts on cell activity have led to the ion-protein

1. *Proc. Roy. Soc., B*, Vol. LXXVIII, p. 159, 1906, in a foot note, p. 169, he states, 'Using the apparatus mentioned in the text, it was found that any alteration in the resistance of serum was not detectable after CHCl_3 , but that there was a diminution of approximately 15 per cent. in the case of blood (laked or simply defibrinated); it would seem that the effect of CHCl_3 on haemoglobin would be worth investigating.' Cf. Edie, *Thompson Yates and Johnston Laboratories Reports*, Vol. VI, Part I, p. 195, 1905.

2. E. Overton, *Studien über die Narkose*, Jena, G. Fischu, 1901, and Hans Meyer, *Arch. f. exper. Path. u. Pharm.*, Vol. XLII, p. 109, 1899, and Vol. XLVI, p. 338, 1901.

3. *Journ. Physiol.*, Vol. XXIV, p. 460, 1899.

4. Schäfers, *Text Book of Physiology*, Vol. II, p. 225, 1900.

5. *Journ. Physiol.*, Vol. XVIII, p. 332, 1895.

hypothesis put forward independently by J. Loeb¹ and Pauli² and elaborated by many other workers.

The present research was undertaken to see if anaesthetics and other toxic substances might so alter the chemical activities of proteins that electrolytes would be detached from the cells.

METHOD

The method employed was that of dialysis carried out in a similar manner to that used by Spence and Edie in determining the percentage of sugar in blood serum.³

It was not considered necessary to keep the cells intact, so instead of saline the dialysing fluid used was distilled water. This latter had several advantages. First, it broke up the cells by dialysing out the salts, thus minimising the effect of any impermeable membrane surrounding the cells. Secondly, it did not mask small differences in the salts dialysing out.

As putrefaction, by altering the proteins, would probably change the relationship of the proteins to the electrolytes, precautions against bacterial change were taken. The material was collected as freshly as possible from the animal, and all vessels or instruments with which it came in contact were sterilized. During the period of dialysis the experiments were conducted in an ice chest to reduce bacterial growth to a minimum.

The experiments were carried out as follows :—

A series of sterilized sausage dialysing tubes were placed in sterile wide-mouthed bottles. Equal portions (50 grammes or 50 c.c.) of the material under examination were put into these tubes and the tubes surrounded with equal bulks (100 c.c.) distilled water.

The first of these tubes was not treated further but was kept as a control. The other tubes were acted on in various ways. A known

1. J. Loeb, *Dynamics of Living Matter*, pp. 78-105, New York, 1906.

2. W. Pauli, *Ueber physikalisch Chemische Methode und Probleme in der Medizin*, Wien, 1900. *Cit. nach* Loeb.

3. *Bio-Chem. Jour.*, Vol. II, p. 103, 1907.

volume of chloroform or ether was added to certain of the bottles. In most cases an excess of these reagents was added so that the liquid would be completely saturated and the maximum effect obtained. Certain of the chloroform experiments were performed with smaller quantities to test the effect around the anaesthetising value. As the S.G. of $\text{CHCl}_3 = 1.5$ and the total bulk of liquid = $50 + 100 = 150$, the number of cubic centimetres of chloroform added corresponds to the percentage of chloroform by weight in the liquid. The effect of coagulation of the proteins was studied by placing the bottles (containing the water, sausage tubes and material) into a water bath, heating to boiling and allowing the whole to remain at boiling temperature for ten minutes. Carbon dioxide was used by passing it from a Kipp through a wash bottle and allowing it to bubble through the dialysing fluid for about an hour. Finally in some cases the dialysing fluid instead of distilled water consisted of $\frac{n}{50}$ acetic acid in distilled water. After the tubes had been treated the stoppers were inserted and the bottles placed in the ice chest.

After dialysis had proceeded for two days the bottles were taken from the ice chest, the sausage tubes were removed, and the volume of the dialysate was measured. The dialysate was evaporated to dryness and incinerated at a dull red heat. The ash from the dialysate was weighed, after cooling in a desiccator, and then it was dissolved in distilled water and made up to 100 c.c. Aliquot portions of this solution were taken for analysis. Ten cubic centimetres were titrated for chlorides with $\frac{n}{10} \text{AgNO}_3$, using potassium chromate as indicator. Twenty-five cubic centimetres were titrated with standard uranium acetate, using potassium ferrocyanide as an outside indicator. The 'reactivity' was determined in 10 c.c. by titrating with $\frac{n}{10} \text{H}_2\text{SO}_4$ in the presence of phenol-phthalein. After the pink colour had disappeared di-methyl-amido-azo-benzol was added and the titration continued until the yellow colour changed to orange. The 'reactivity' to di-methyl. is the total amount of acid added from the beginning of

the titration. Finally the electrical conductivity was measured by the method of Kohlrausch, and in certain cases the presence of calcium, magnesium and sulphates was sought, but in none of the experiments were these present in sufficient amount to obtain quantitative measurements. The results obtained were corrected so that the figures given correspond to the amount of substance present in 100 c.c. of the original dialysate. Thus suppose that the original volume of the dialysate was 98 c.c. then, because the ash was made up to 100 c.c., the figures for 100 c.c. of the solution after incineration were multiplied by $\frac{100}{98}$ to express the concentration in 100 c.c. of the original dialysate.¹

The experiments are given under the following heads :—

- A. Experiments with Red Blood Corpuscles.
- B. Experiments with Serum.
- C. Experiments with Muscle.
- D. Experiments with Liver.
- E. Experiments with Kidney.
- F. Experiments with Brain.

A. EXPERIMENTS WITH RED BLOOD CORPUSCLES

For these experiments the blood was collected, at the slaughter-house, direct from an artery into a sterilized pail, and defibrinated by whipping with a sterile whisk. The blood was centrifugalised in sterile glasses and the corpuscles separated as completely as possible from the serum. All the corpuscles were thoroughly mixed, and 50 c.c. was placed into each of the sausage tubes and treated as mentioned in the tables. In some of the experiments an excess of chloroform and ether was used, but in addition in Experiments II and III smaller quantities of chloroform were added to see if the same results could be obtained around the anaesthetising value.

1. The correction in the case of ether is probably too high, as the solubility of ether in water is about 8 per cent. the amount of water in the dialysate would be $\frac{12}{100}$ of the volume of the dialysate.

TABLE I. PIG'S RED BLOOD CORPUSCLES

Results of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P_2O_5 in grams	Chlorine in grams	$\frac{N}{10}H_2SO_4$ to Phenolphthalein	$\frac{N}{10}H_2SO_4$ to Dimethyl	Specific conductivity
1. Control	88 c.c.	0.1320	0.0174	0.0403	1.7 c.c.	6.8 c.c.	0.002061
2. Chloroform—3 c.c. put into dialysing tube	85 c.c.	0.2532	0.0267	0.0710	3.5 c.c.	14.7 c.c.	0.004082
3. Ether—15 c.c. put into the dialysing tube	82 c.c.	0.2234	0.0320	0.0563	2.4 c.c.	12.2 c.c.	0.003378
4. Boiled	87 c.c.	0.1906	0.0302	0.0488	2.3 c.c.	10.3 c.c.	0.003023

TABLE II. PIG'S RED BLOOD CORPUSCLES

Results of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P_2O_5 in grams	Chlorine in grams	$\frac{N}{10}H_2SO_4$ to Phenolphthalein	$\frac{N}{10}H_2SO_4$ to Dimethyl	Specific conductivity
1. Control	94 c.c.	0.0600	0.0093	0.0226	6.4 c.c.	1.91 c.c.	0.001216
2. Chloroform — 0.1 per cent. 0.1 c.c. of $CHCl_3$ put into the dialysing tube	88 c.c.	0.1329	0.0134	0.0383	1.38 c.c.	3.4 c.c.	0.002058
0.5 per cent. 0.5 c.c. $CHCl_3$ put into the tissue in the dialysing tube ...	88 c.c.	0.1100	0.0135	0.0323	3.4 c.c.	9.09 c.c.	0.001870
1 per cent. 1.0 c.c. $CHCl_3$ put into dialysing tube ...	89 c.c.	0.1178	0.0118	0.0379	3.15 c.c.	4.5 c.c.	0.002102
10 c.c. $CHCl_3$ put into dialysing tube	92 c.c.	0.1314	0.0138	0.0516	1.2 c.c.	3.26 c.c.	0.002372
3. Ether—10 c.c. inside and 20 c.c. outside the dialysing tube ...	102 c.c.	0.0404?	0.0086	0.0313	0.98 c.c.	1.96 c.c.	0.002139
4. Boiled	95 c.c.	0.1648	0.0166	0.0568	1.9 c.c.	4.42 c.c.	0.003200
5. CO_2 passed through the dialysing fluid	96 c.c.	0.0918	0.0074	0.0296	2.19 c.c.	5.83 c.c.	0.001656
6. $\frac{M}{50}$ Acetic acid ...	96 c.c.	0.1292	0.0105	0.0370	2.19 c.c.	5.20 c.c.	0.002160

TABLE III. PIG'S RED BLOOD CORPUSCLES

Results of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P_2O_5 in grams	Chlorine in grams	$\frac{N}{10}H_2SO_4$ to Phenolphthalein	$\frac{N}{10}H_2SO_4$ to Dimethyl	Specific conductivity
1. Control ...	81 c.c.	0.1393	0.0161	0.0307	1.6 c.c.	6.17 c.c.	0.00222
2. Chloroform — 0.05 per cent. 0.05 c.c. of $CHCl_3$ put into dialysing tube ...	86 c.c.	0.1326	0.0167	0.0330	2.9 c.c.	5.81 c.c.	0.002242
0.1 per cent. 0.1 c.c. put into dialysing tube ...	85 c.c.	0.1480	0.0188	0.0334	2.49 c.c.	5.3 c.c.	0.002284
0.5 per cent. 0.5 c.c. put into dialysing tube ...	76 c.c.	0.1745	0.0268	0.0420	3.3 c.c.	7.2 c.c.	0.002967
1 per cent. 1.0 c.c. put into dialysing tube ...	88 c.c.	0.1168	0.0154	0.0343	2.35 c.c.	5.57 c.c.	0.0020207

In these tables it is shown that chloroform, ether, carbon dioxide, acetic acid and heat coagulation all influence the condition of the electrolytes in the corpuscles so that a larger amount dialyses out than when the dialysing fluid consists only of distilled water. That the results were not due to an action on a lipoid membrane was shown by the fact that the controls were completely laked by dialysis. Under the microscope the 'ghosts' were seen embedded in a red semi-solid mass. No. 3, Table II, is apparently an exception, and the results with lower percentages in Table III are not so striking as those in Table II; but in Experiment III the corpuscles were not so fresh as usual, thus comparing with the 'spontaneous' laking of Stewart.¹

1. *Journ. Physiol.*, Vol. XXIV, p. 230, 1899.

B. EXPERIMENTS WITH SERUM

The experiments under this section were performed under similar conditions to those in Section A except that, instead of using 50 c.c. of corpuscles, 50 c.c. of serum was in each case put into the dialysing tubes.

TABLE IV. PIG'S SERUM

Results of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P_2O_5 in grams	Chlorine in grams	$\frac{N}{10}H_2SO_4$ to Phenolphthalein	$\frac{N}{10}H_2SO_4$ to Dimethyl	Specific conductivity
1. Control ...	95 c.c.	0.2623	0.0079	0.1270	4.2 c.c.	6.8 c.c.	0.004853
2. Chloroform — 0.1 c.c. put into dialysing tube ...	94 c.c.	0.2254	0.0065	0.1170	3.19 c.c.	4.8 c.c.	0.04413
0.5 c.c. put into dialysing tube ...	95 c.c.	0.2284	0.0055	0.1121	3.2 c.c.	5.3 c.c.	0.004534
1 c.c. put into dialysing tube ...	96 c.c.	0.2416	0.0055	0.1146	2.08 c.c.	3.3 c.c.	0.00437
10 c.c. inside the dialysing tube ...	94 c.c.	0.2594	0.0075	0.1246	3.19 c.c.	5.3 c.c.	0.004574
3. Ether—10 c.c. inside, 20 c.c. outside, dialysing tube ...	108 c.c.	0.2204 (0.24 ¹)	0.0065	0.1085	2.7 c.c.	4.6 c.c.	0.004175 (0.0045 ¹)
4. Boiled ...	96 c.c.	0.2477	0.0073	0.1183	3.1 c.c.	5.2 c.c.	0.004477
5. ² CO_2 as before ...	97 c.c.	0.2318	—	—	—	—	—
6. $\frac{M}{50}$ Acetic acid ...	95 c.c.	0.2823	0.0092	0.1233	5.3 c.c.	10.5 c.c.	0.005017

TABLE V. PIG'S SERUM

Result of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P_2O_5 in grams	Chlorine in grams	$\frac{N}{10}H_2SO_4$ to Phenolphthalein	$\frac{N}{10}H_2SO_4$ to Dimethyl	Specific conductivity
1. Control ...	95 c.c.	0.2562	0.0084	0.1196	3.79 c.c.	6.31 c.c.	0.004937
2. Chloroform—10 c.c. inside dialysing tube ...	93 c.c.	0.2546	0.0096	0.1223	4.42 c.c.	6.6 c.c.	0.004949
3. Ether—10 c.c. inside, 20 c.c. outside, dialysing tube ...	103 c.c.	0.2704	0.0077	0.0966	2.91 c.c.	4.85 c.c.	0.004154 (0.0045 ³)
4. Boiled ...	96 c.c.	0.2852	0.0083	0.1246	4.17 c.c.	6.25 c.c.	0.004929
5. CO_2 passed through for an hour ...	95 c.c.	0.2686	0.0096	0.1196	5.26 c.c.	7.58 c.c.	0.005020
6. $\frac{M}{50}$ Acetic acid ...	95 c.c.	0.2867 ⁴	0.0084	0.1196	4.00 c.c.	6.00 c.c.	0.004795

1. See footnote, p. 417.

2. In No. 5 the porcelain dish broke during incineration, so that only the total salts could be estimated.

3. See footnote, p. 417.

4. All the carbon could not be properly burnt off in this experiment owing to the vessel cracking during the incineration.

The results in these cases were negative, thus contrasting with the experiments described by Moore and Roaf. In their experiments, however, the chloroform was added to the undiluted serum, whilst in the present experiments the dialysis would correspond to a two-thirds dilution with water. If the salts are so loosely united to the proteins that they are detached by mere dilution the negative results obtained by us would be easily explained. That the salts are detached from proteins by dilution has been shown by Stewart,¹ and more recently by Mellanby.²

C. EXPERIMENTS ON MUSCLE

The muscle used for these experiments was dissected as free as possible from fat, and it was then ground up in a mincing machine. Fifty grammes of this material was placed into each of the sausage tubes and they were then treated as in the preceding experiments. The rabbits' muscle was obtained from a couple of freshly-killed rabbits, but that of the ox was obtained from a butcher's shop, and hence was neither so fresh nor so sterile as the former.

TABLE VI. RABBITS' MUSCLE

Results of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P_2O_5 in grams	Chlorine in grams	$\frac{N}{10}$ H_2SO_4 to Phenolphthalein	$\frac{N}{10}$ H_2SO_4 to Dimethyl	Specific conductivity
1. Control ...	91 c.c.	0.2786	0.0243	0.0195	3.63 c.c.	14.29 c.c.	0.003143
2. $CHCl_3$ —10 c.c. put into the dialysing tube ...	89 c.c.	0.3239	0.0259	0.0248	5.62 c.c.	18.66 c.c.	0.00395
3. Ether—20 c.c. put inside dialysing tube, 10 c.c. into the dialysing water	91 c.c.	0.3356	0.0296	0.0195	5.82 c.c.	19.67 c.c.	0.00372
4. Boiled 10 mins. ...	89 c.c.	0.3868	0.0314	0.0248	7.76 c.c.	22.96 c.c.	0.0044

1. *Journ. Physiol.*, Vol. XXIV, p. 217, 1899.

2. *Journ. Physiol.*, Vol. XXXV, pp. 193-196, 1907.

TABLE VII. OX MUSCLE

Results of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P_2O_5 in grams	Chlorine in grams	$\frac{N}{10} H_2SO_4$ to Phenolphthalein	$\frac{N}{10} H_2SO_4$ to Dimethyl	Specific conductivity $K \times 10^3$
1. Control ...	88 c.c.	0.2207	0.0477	0.0141	5.68 c.c.	15.9 c.c.	0.003392
2. Chloroform—10 c.c. inside the dialysing tube ...	92 c.c.	0.2207	0.0469	0.0161	6.5 c.c.	15.2 c.c.	0.003205
3. Ether—10 c.c. inside 20 c.c. outside the dialysing tube ...	102 c.c.	0.3218	0.0523	0.0167	5.9 c.c.	15.7 c.c.	0.00342
4. $\frac{M}{50}$ Acetic acid ...	91 c.c.	0.2956	0.0529	0.0156	9.9 c.c.	21.4 c.c.	0.00373

These both show an increase above the control in all except No. 2, Table VII.

D. EXPERIMENTS ON LIVER

These experiments were performed similarly to those with muscle. Fifty grammes of the material minced and dissected free from visible fat was placed into each tube.

TABLE VIII. LIVER

Results of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P_2O_5 in grams	Chlorine in grams	$\frac{N}{10} H_2SO_4$ to Phenolphthalein	$\frac{N}{10} H_2SO_4$ to Dimethyl	Specific conductivity
1. Control ...	94 c.c.	0.1496	0.0307	0.0226	1.91 c.c.	6.7 c.c.	0.001656
3. Ether—20 c.c. inside, 10 c.c. outside, the dialysing tube ...	103 c.c.	0.1800	0.0383	0.0241	3.69 c.c.	9.5 c.c.	0.001858
4. Boiled ...	93 c.c.	0.1938	0.0452	0.0263	3.23 c.c.	8.6 c.c.	0.002609
6. $\frac{M}{50}$ Acetic acid ...	96 c.c.	0.2048	0.0456	0.0188	3.1 c.c.	10.63 c.c.	0.002438

TABLE IX. LIVER

Results of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P_2O_5 in grams	Chlorine in grams	$\frac{N}{10}$ - H_2SO_4 to Phenolphthalein	$\frac{N}{10}$ - H_2SO_4 to Dimethyl	Specific conductivity
1. Control ...	94 c.c.	0.1432	0.0340	0.0226	1.4 c.c.	6.4 c.c.	0.001804
2. Chloroform—5 c.c.	92 c.c.	0.1276	0.0326	0.0193	2.2 c.c.	6.85 c.c.	0.001900
1 c.c. ...	94 c.c.	0.1250	0.0276	0.0200	2.02 c.c.	5.64 c.c.	0.00175
10 c.c. inside dialysing tube ...	92 c.c.	0.1365	0.0409	0.0240	2.2 c.c.	6.2 c.c.	0.002017
3. Ether—10 c.c. inside, 10 c.c. outside, dialysing tube	103 c.c.	0.1324 (0.144 ¹)	0.0390	0.0197	1.94 c.c.	5.83 c.c.	0.0017000 0.00185 ¹
4. Boiled ...	93 c.c.	0.0553 (0.1553 ²)	0.0383	0.0267	2.15 c.c.	6.8 c.c.	0.002209

Another experiment on liver tissue shows an increase of electrolytes in the treated tubes, but, as all the analyses, etc., were not complete, the table is not shown.

Table VIII shows that the treatment to which the liver tissue was subjected caused a liberation of salts, but Table IX shows no such increase. A further experiment in which the analyses were not all complete, and hence not worth publishing, shows a detachment of salts by the treatment adopted.

E. EXPERIMENTS WITH KIDNEY

The kidney for this experiment was obtained from a pig, and cleared from visible fat in the usual way. After mincing, fifty grammes of the tissue was placed into each sausage tube and treated in the usual manner.

1. See footnote, p. 417.

TABLE X. KIDNEY

Results of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate.

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P_2O_5 in grams	Chlorine in grams	$\frac{N}{10}$ H_2SO_4 to Phenolphthalein	$\frac{N}{10}$ H_2SO_4 to Dimethyl	Specific conductivity
1. Control, as before	94 c.c.	0'1117	0'0121	0'0378	1'06 c.c.	4'26 c.c.	0'00224
2. Chloroform — 10 c.c. inside ...	95 c.c.	0'1406	0'0243	0'0374	2'10 c.c.	6'32 c.c.	0'002571
3. Ether—10 c.c. inside, and 10 c.c. 15 c.c. outside ...	105 c.c.	0'17105	0'0150	0'0345	1'90 c.c.	4'76 c.c.	0'001993 (0'00217) ¹
4. Boiled ...	93 c.c.	0'2447	0'0322	0'0791	3'25 c.c.	9'14 c.c.	0'004103
5. CO_2 ...	95 c.c.	0'1307	0'0166	0'0345	1'58 c.c.	4'74 c.c.	0'002568
6. $\frac{M}{50}$ Acetic acid, as before ...	96 c.c.	0'1242	0'0201	0'0370	1'98 c.c.	4'38 c.c.	0'002174

REMARKS

In this experiment the percentage of chlorine was found to be lower than the control in all except the boiled one, but this slight apparent lowering is due to the differences in volume of the dialysates, so lowering the percentages. The actual result of the analysis of the chlorine on taking 10 c.c. of each fluid was—1 c.c. of silver nitrate in each case except in the boiled.

In this experiment the chlorides showed no increase over the control, but all the methods of treatment showed an increase of total salts, phosphates and reactivity, and all save the ether and acetic acid tubes showed an increase of electrical conductivity.

F. EXPERIMENTS WITH SHEEP'S BRAINS

The heads of the sheep were brought up from the slaughter-house and the brains (cerebrum, cerebellum and medulla) dissected out. The larger blood vessels were removed and the whole of the brain matter minced. Fifty grammes of the minced material was put into each sausage tube and treated as usual. In Table XI the actual results of the analysis are given, and in Table XII the same experiment is shown corrected as in the other tables.

1. See footnote p. 417.

TABLE XI. SHEEP'S BRAIN

This table shows the actual figures of the quantities taken for titration, etc., obtained in the analysis of the incinerated dialysate after being made up to 100 c.c. with distilled water, before being corrected, so as to represent percentages of the original dialysate.

Result of the analysis of the incinerated dialysate made up to 100 c.c. with distilled water representing the actual figures for quantities taken.

Method of Treatment	Volume of dialysate	Total salts in grams	10 c.c. of dialysate taken to titrate for Chlorides AgNO ₃	20 c.c. of dialysate taken to titrate for the Phosphates ¹ Uran. Acet.	10 c.c. dialysate taken to titrate with $\frac{N}{10}$ H ₂ SO ₄ to Phenol-phthalein $\frac{N}{10}$ H ₂ SO ₄	10 c.c. dialysate taken to titrate $\frac{N}{10}$ H ₂ SO ₄ to dimethyl-amido-azole benzole $\frac{N}{10}$ H ₂ SO ₄	The resistance in ohms of the dialysate using a cell of a resistance capacity = 0.67196
1. Control ...	95 c.c.	0.0616	0.66 c.c.	0.4 c.c.	0.2 c.c.	0.35 c.c.	770 ohms
2. Chloroform — 10 c.c. put into dialysing tube ...	95 c.c.	0.1154	1.8 c.c.	0.72 c.c.	0.23 c.c.	0.70 c.c.	372 „
3. Ether—20 c.c. inside, 10 c.c. outside	109 c.c.	0.1234	2.20 c.c.	0.87 c.c.	0.38 c.c.	0.74 c.c.	358 „
4. Boiled ...	97 c.c.	0.2194	3.32 c.c.	1.60 c.c.	0.46 c.c.	1.08 c.c.	218 „
5. CO ₂ ...	95 c.c.	0.1298	1.98 c.c.	0.64 c.c.	0.3 c.c.	0.52 c.c.	357 „
6. $\frac{M}{50}$ Acetic acid ...	96 c.c.	0.1238	2.00 c.c.	0.65 c.c.	0.2 c.c.	0.6 c.c.	394 „

TABLE XII. SHEEP'S BRAIN

Results of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P ₂ O ₅ in grams	Chlorine in grams	$\frac{N}{10}$ H ₂ SO ₄ to Phenol-phthalein	$\frac{N}{10}$ H ₂ SO ₄ to Dimethyl	Specific conductivity
1. Control ...	95 c.c.	0.0648	0.0092	0.0123	2.10 c.c.	3.58 c.c.	0.000919
2. Chloroform—10 c.c. inside the dialysing tube ...	95 c.c.	0.1215	0.0166	0.0134	2.42 c.c.	7.37 c.c.	0.001902
3. Ether—20 c.c. inside the dialysing tube, but 10 c.c. outside ...	109 c.c.	0.1132	0.0175	0.0358	3.49 c.c.	6.79 c.c.	0.001603
4. Boiled ...	97 c.c.	0.2262	0.0361	0.0608	4.75 c.c.	11.13 c.c.	0.003179
5. CO ₂ passed through the 100 c.c. of distilled water for 1 hour ...	95 c.c.	0.1289	0.0136	0.0370	2.09 c.c.	5.42 c.c.	0.001766
6. $\frac{M}{50}$ Acetic acid ...	96 c.c.	0.1366	0.0150	0.0368	3.16 c.c.	6.32 c.c.	0.001981

1. Notice that 20 c.c. of dialysate was taken here instead of 25 c.c., which was taken in the other experiments. This method of illustrating the results shows well the marked difference between the control and the other bottles. Notice the large volume of the ether dialysate, the results of which have to be corrected to represent them in percentages, thus materially lowering the figures (see footnote, p. 417).

TABLE XIII. SHEEP'S BRAIN

Results of analysis of the dialysate after being incinerated and made up to 100 c.c. again, corrected so as to represent actual figures for 100 c.c. of original dialysate

Method of Treatment	Volume of dialysate	Total salts in grams	Phosphorus as P_2O_5 in grams	Chlorine in grams	$\frac{N}{10} H_2SO_4$ to Phenolphthalein	$\frac{N}{10} H_2SO_4$ to Dimethyl	Specific conductivity
1. Control ...	94 c.c.	0.1743	0.0343	0.0216	2.74 c.c.	9.47 c.c.	0.003463
2. Chloroform — 0.5 per cent. 0.5 c.c. put inside dialysing tube ...	95 c.c.	0.1690	0.0336	0.0463	4.42 c.c.	8.63 c.c.	0.003144
1 per cent. 1 c.c. put inside dialysing tube ...	96 c.c.	0.1800	0.0354	0.0481	4.17 c.c.	8.86 c.c.	0.003226
2 per cent. 2 c.c. put inside dialysing tube ...	93 c.c.	0.2090	0.0505	0.0657	6.45 c.c.	14.00 c.c.	0.00427
3. Ether—10 c.c. inside, 20 c.c. outside, dialysing tube	104 c.c.	0.2167	0.0679	0.0679	3.85 c.c.	10.58 c.c.	0.004038

In these experiments also there is an increase of inorganic salts in the dialysate after treatment. In the experiment with smaller quantities of chloroform it must be remembered that the large quantity of lipid material would dissolve the anaesthetic so that the vapour pressure would be lowered in the mixture, thus corresponding to a smaller active mass of chloroform acting on the protein.

DISCUSSION OF RESULTS AND THEORETICAL CONSIDERATIONS

The tables show that, in the great majority of the experiments, agents which diminish the activity of the cells cause a change in the physical condition of the contained electrolytes. This change consists in a setting free of the ions so that they are able to dialyse through parchment paper in greater numbers than in an untreated control.

The great interest of these results lies in the explanation they afford of the normal distribution of the inorganic constituents of the body to the different tissues and in the action of certain toxic substances on this distribution. The whole body is supplied by a common

nutrient medium, the blood plasma, and from this circulating medium the tissue cells pick out the particular constituents needful for their proper functioning. As the inorganic constituents of cells differ markedly in their percentage composition from the corresponding salts in the serum, there must be some mechanism which prevents the diffusion of salts from a position of higher concentration to that of lower concentration.

That this barrier to diffusion consists of an impermeable membrane is unlikely, as such a barrier would prevent fresh material being taken up for growth, and thus one would need to suppose that the ovum contains sufficient salts for the fully developed adult. A membrane possessing one-sided permeability is not known outside of biological processes, and thus the difference of composition between cells and their surrounding medium cannot, at present, be explained as a purely physical process. It is further probable that the salts are not held in the cell by a membrane, because in the experiments on red blood corpuscles the laking was complete, as was shown by examining the corpuscular mass under the microscope. The difference between the control and the treated specimens could not then be due to the destruction of an impermeable membrane¹.

It is either necessary to suppose that the cells transform energy from chemical processes into osmotic manifestations of energy, and thus they keep the concentration of certain salts higher inside their boundaries than in the lymph surrounding them; or that the proteins or other constituents of the cells adsorb or combine with particular ions. An equilibrium is thus maintained between the free ions in the cells and in the plasma, although the percentage of some ions is much higher inside the cells.

On such a view, that the selection of particular salts by cells is regulated by the affinity of some constituent for the desired ions, one would expect to find that the salts of the nutrient medium would be quite free or only loosely combined. Our experiments show that

1. Stewart, *Journ. Physiol.* Vol. XXIV, p. 230, 1899: he says that the salts are retained in the ghosts after blood corpuscles are laked but this does not necessarily mean that they are retained by a membrane. A membrane which permits the escape of haemoglobin would very likely be permeable to inorganic salts.

for serum this is the case, as dialysis into a comparatively small bulk of liquid separates nearly all the salts. It is difficult to say whether the detachment of electrolytes has anything to do with the causation of anaesthesia, but in view of the ion-protein hypothesis it is a very tempting deduction. The agents used in the present experiments were those that are known to cause narcosis. Chloroform, ether, and carbon dioxide all cause anaesthesia, whilst in diabetes, coma is due to an increased formation of organic acids which may act in a comparable manner to the acetic acid used in these experiments. In most cases the amount of narcotic used was greatly in excess of that necessary to cause anaesthesia, but with lesser amounts the action would probably be of the same nature only lesser in degree.

Heat coagulation is a more marked form of injury, and one from which recovery is not possible, as in the other modes of treatment employed, but it would be interesting if some of the symptoms of hyperpyrexia were due to a similar detachment of electrolytes.¹

The diminished activity due to anaesthetics, if caused by a redistribution of electrolytes, can easily be recovered from, as removal of the active agent would allow the normal relations to be re-established. This explanation would also hold for unicellular and multicellular plants and animals. To test this hypothesis on living tissues is extremely difficult. The salts of any perfusion fluid would mask small differences due to the narcotic, and even if salts are liberated from the protein² it is not likely that they would immediately pass into the blood vessels. The change in the condition of the salts would occur inside the cell, and the ions would have to diffuse into the surrounding lymph before they could pass into the blood vessels. For these reasons there is not much hope of being able to find any output of salt into a perfusion fluid as the result of the action of an anaesthetic on surviving tissues.

Considering the experimental conditions, it is remarkable that

1. Cf. Bayliss, *Bio-Chem. Journ.*, Vol. I, pp. 191 and 192, 1906, and J. Mellanby, *Journ. Physiol.*, Vol. XXXV, p. 492, 1907.

2. See Vernon, *Zeit. f. allg. Physiol.*, Vol. VI, p. 393, 1907, in which he shows that chloroform and ether, when perfused through the blood vessels of kidneys, cause an increased detachment of protein.

the results show such an increase of salts in the treated tubes above the amount diffusing out from the controls. The controls probably have a fair amount of salt detached by the dialysis lowering the concentration of the electrolytes in equilibrium with the combined salts, and any putrefactive change, to which the controls are more liable owing to the absence of preservatives such as chloroform and ether, would tend to liberate salts.¹

It is not intended to review the voluminous literature dealing with the relation of various salts to protein solutions, but there are one or two observations which are of value in connection with the present experiments.

The work of Macdonald has shown that injury and death of nerves liberates electrolytes.² He has also shown that nerves which are placed in saline containing chloroform tend to have the potassium salts limited in their distribution.³ Bayliss, in working at the adsorption of dyes by cellulose, found that electrolytes greatly affected the amount adsorbed,⁴ and Robertson has brought forward evidence that infusoria stain differently according to the nature of the salt in which they have been grown.⁵ Mayr showed that certain salts affect the staining of the Nissl granules by toluidin blue,⁶ and Hamilton Wright found that administration of chloroform and ether caused the Nissl granules to lose their affinity for methylene blue.⁷

From the evidence of these observers it would appear as if during anaesthesia there is some change in the condition of the electrolytes inside the cells, and it is not improbable that it is this change carried to an extreme degree which accounts for the results given in this paper.

1. Stewart, *Journ. Physiol.*, Vol. XXIV, p. 230, 1899.

2. *Loc. cit.*

3. *Journ. Physiol.*, Vol. XXXV., proc. p. 38, 1907.

4. *Bio-Chem. Journ.*, Vol. I, p. 175, 1906.

5. *Journ. Biolog. Chem.*, Vol. I, p. 279, 1906.

6. *Beit. z. Chem. Physiol. u. Path.*, Vol. VII, p. 548, 1906. See also Bethe, *ibid.*, Vol. VI, p. 399, 1905.

7. *Journ. Physiol.*, Vol. XXVI, pp. 30 and 362, 1901-1902.

SUMMARY

1. Chloroform, ether, carbon dioxide, acetic acid and heat coagulation cause a change in the physical condition of the inorganic constituents of tissues.

2. This change consists in a liberation of ions so that they are able to diffuse through parchment in greater number than before treatment with these reagents.

3. The tissues which detach salts under these conditions are red blood corpuscles, brain, liver, muscle and kidney, but blood serum does not show any appreciable difference when acted upon by the various reagents.

4. It is believed that the setting free of electrolytes is not due to the reagents acting on an impermeable membrane surrounding the cells, but is the result of an alteration in the relationship of the salts to the proteins.

5. The bearing of these changes on the activity of cells is briefly discussed.

We wish to thank Prof. Benjamin Moore for valuable advice during the course of our experiments.