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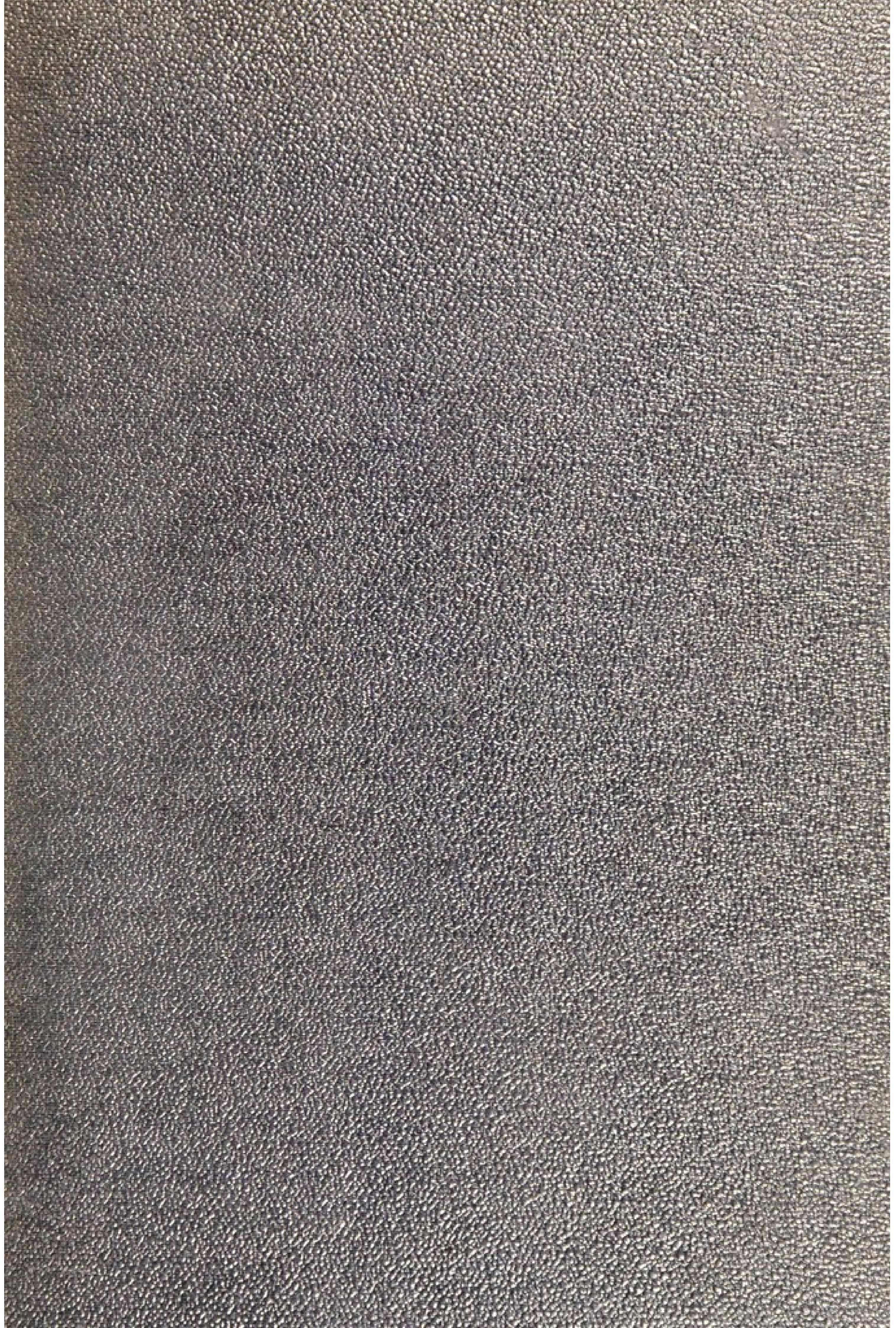

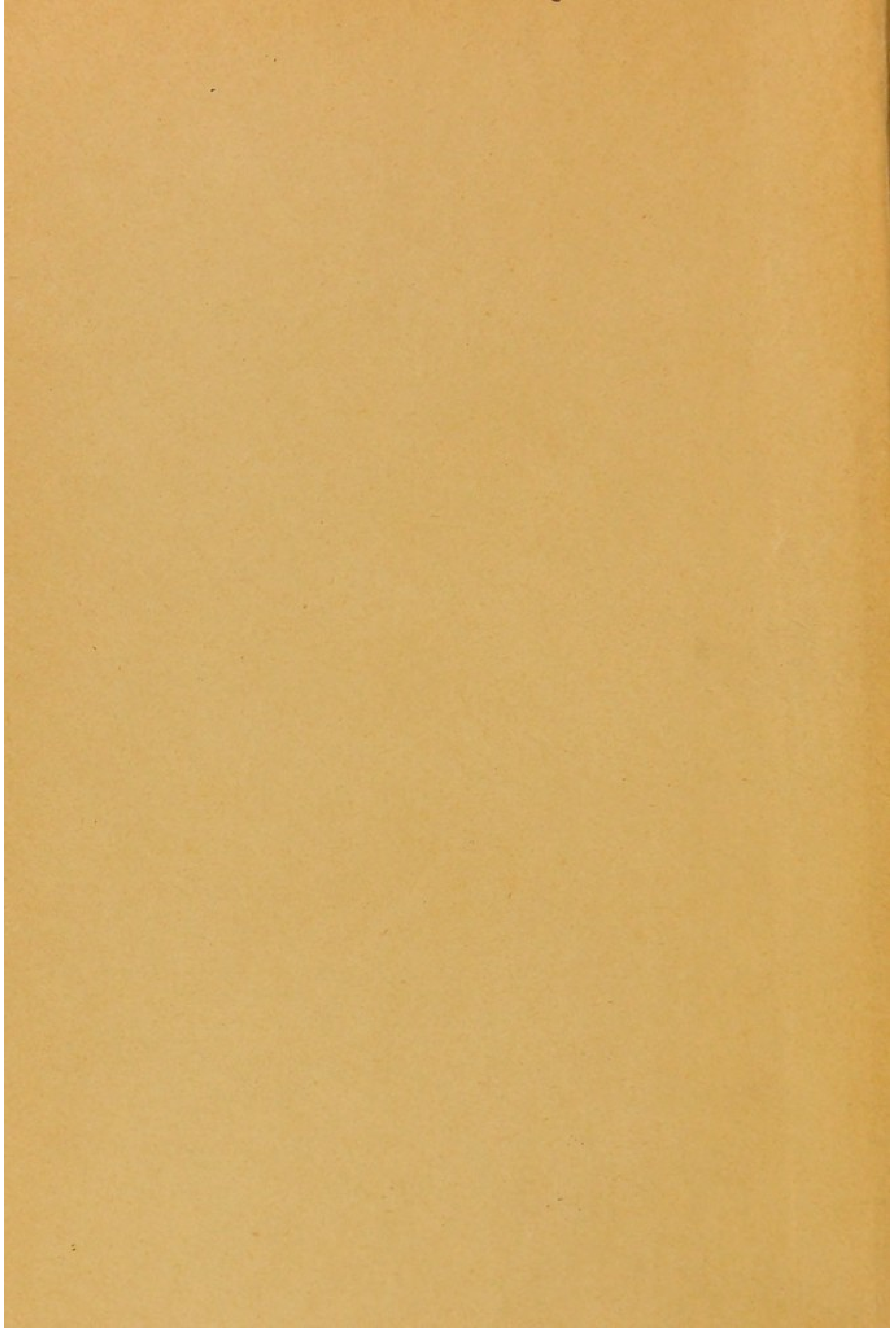

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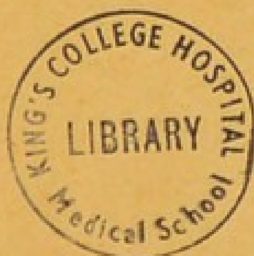

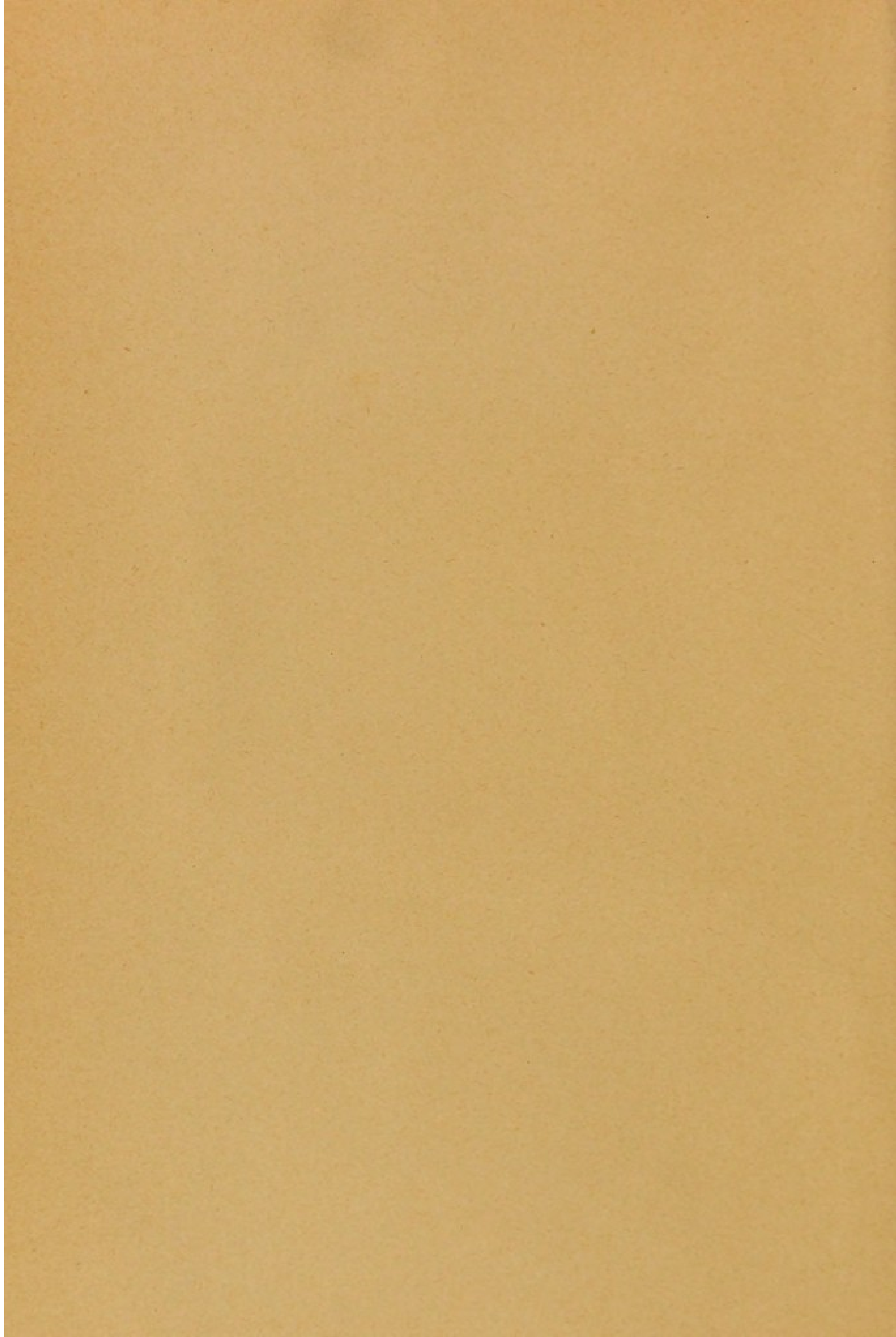

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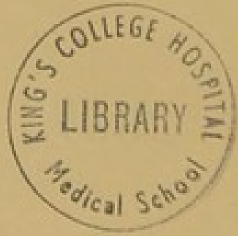

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[*From the Journal of Physiology. Vol. XVIII. Nos.* 1 & 2, 1895.]

SYNTHESISED COLLOIDS AND COAGULATION. By J. W. PICKERING, D.Sc. (*Lond.*), *George Henry Lewes Student.*

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

NOTWITHSTANDING the many and recent researches on the coagulation and clotting of proteids, but little attention has been paid to the solution of the problem from a synthetic standpoint. In a paper[1] published two years ago I pointed out that the experiments of Prof. Grimaux[2] would, if substantiated, throw considerable light on the conditions which characterise and control the heat-coagulation of proteids. The aim of Grimaux's researches has been to synthesise simple colloidal substances and follow in them the changes from the soluble to the insoluble condition. The principal substance synthesised was the "colloïde amidobenzoique," formed by the heating together in sealed tubes at 125° to 130° C. of meta-amidobenzoic acid and phosphorous pentachloride.

At a recent meeting[3] of the Physiological Society I announced that

[1] This *Journal*, XIV. 347. 1893.

[2] I. "Sur des colloïdes azotés." *Comptes Rendus*, XCIII. 771, 1881, and *Bulletin de la Société Chimique*, XXXVIII. 65. 1882.

II. "Sur la réaction de l'albumine et d'un colloïde azoté obtenu par la synthèse." *Comptes Rendus*, XCVIII. 231 et 1336. 1884. *Bulletin de Soc. Chim.* XLII. 74. 1886. *Comptes Rendus de la Soc. de Biologie*, XXXVI. 353. 1884.

III. "Sur l'éthylate ferrique et l'hydrate ferrique colloïdal." *Comptes Rendus*, XCVIII. 105. 1884. *Bulletin de la Soc. Chim.* XLII. 155. 1884.

IV. "Sur diverses substances colloïdales." *Comptes Rendus*, XCVIII. 1434. 1884. *Bulletin de la Soc. Chim.* XLII. 156. 1884.

V. "Sur quelques composés colloïdaux dérivés de l'hydrate ferrique." *Comptes Rendus*, XCVIII. 1485 et 1540. 1884. *Bulletin de la Soc. Chim.* XLII. 206. 1886.

VI. "Théorie de la coagulation." *Comptes Rendus*, XCVIII. 1578.

VII. "Sur les substances colloïdales." *Leçons de la Soc. Chim.* 1886; reprinted in *La Revue Scientifique*, 18 April, 1886. This is a very complete paper and gives a summary of the others.

VIII. "Sur un albuminoïde élémentaire obtenu avec la leucine." *Bulletin de la Soc. Chim.* XLII. 545. 1884. "Sur les albuminoïdes." *Ibid.* XLIV. 21. 1884.

[3] *Proc. Physiol. Soc.* No. II. 1895.

after experimenting with the three synthesised colloids kindly supplied me by Prof. Grimaux, not only was I able to confirm many of his observations, but that each of these bodies besides behaving chemically in a manner strikingly similar to proteids, would if intravenously injected into rabbits in a 1 to 2 p.c. solution, produce extensive intravascular clotting, which often involves the whole of the heart, all the larger veins, and extends along the aorta.

Further experiments have demonstrated that the three bodies under consideration show many chemical similarities to the globulins, and in their physiological action on the rabbit behave in a manner strikingly similar to nucleo-albumins.

BIBLIOGRAPHICAL.

The following is the method of preparation of the three bodies:

(1) The "colloïde amidobenzoique," hereinafter termed the colloid *A*, is made by heating in sealed tubes at 125° C. meta-amido-benzoic acid for one and a half hours with once and a half times its weight of phosphorous pentachloride. The product of the reaction, which is a white friable powder, should be washed repeatedly in boiling water so as to remove all the contaminating phosphoric acid. The remaining substance is supposed by Grimaux to be an intramolecular anhydride formed by the union of several molecules of the meta-amido-benzoic acid with the elimination of water. When ammonia is added it swells up and dissolves slowly and incompletely in the cold, rapidly and completely on warming. The solution obtained should be evaporated *in vacuo* at a low temperature. The resulting solid is a transparent jelly which dries into translucent yellowish plates, which in their physical properties resemble dried serum-albumin.

(2) The colloid *B* is prepared from the same substances in a similar manner; the temperature of the synthesis is allowed to rise to 135° C.

(3) The "colloïde aspartique," hereinafter termed the colloid *C*, is best prepared by the passage of a current of gaseous ammonia heated to 170° C. over solid aspartic anhydride. The product of the reaction should be washed in water. After evaporation in vacuo the solution yields a substance similar in appearance to the colloid *A*.

The chemical reactions of these substances are according to Grimaux best studied in 2 % solutions. From such a solution they

are precipitated by hydrochloric, nitric, acetic, oxalic or tartaric acids. The precipitate produced by acetic acid redissolves in excess and the solution produced gives a flocculent precipitate on the addition of potassium ferrocyanide. The colloid *A* gives a well-marked xanthoproteic reaction. With copper sulphate and potash the colloid *A* gives a blue-violet reaction and the colloid *C* a typical violet (like an albumin).

In the entire absence of salts they do not coagulate on heating; but the presence of soluble salts of barium, strontium, or calcium, or of a few drops of a saturated solution of sodium chloride, a 1 % solution of magnesium sulphate, or of a very dilute solution of ammonium chloride, determines the power of their heat-coagulation.

Sodium acetate, potassium acetate, or sodium sulphate hinder the power of heat coagulation. Thus if 1 c.c. of a saturated solution of sodium sulphate be added to 1 c.c. of a 2 % solution of the colloid *A*, in order to induce heat-coagulation, it is necessary to add four or five c.c. of a saturated solution of calcium sulphate, whereas if the sodium sulphate had been absent, five or six drops only would have been necessary. Moreover if the quantity of salt[1] present be insufficient to induce heat-coagulation, the passage of a current of CO_2 through the solution will often after subsequent heating induce coagulation. The coagulum like the precipitate formed by the passage of a current of CO_2 through a solution of a globulin redissolves when a current of air is passed through the solution. Further, the addition of a solution of calcium phosphate dissolved in carbonic acid coagulates the colloids in the cold. Carbonic acid in the cold determines in the presence of either sulphate or chloride of sodium the precipitation of the colloid *A*; this precipitate redissolves on the passage of a current of air through the solution in which it is suspended. If salts are absent from the solution carbonic acid will not alone either induce precipitation or determine heat-coagulation.

Using a 2 % solution of the colloid *A* with a trace of calcium sulphate, Grimaux states that opalescence can be induced by heating to 50° C., while coagulation takes place between 70° and 80° C.

Grimaux has also studied the heat-coagulation and spontaneous "clotting" of soluble hydroxides of iron and silicon, which he states obey the same physico-chemical laws that control the coagulation of organic nitrogenous bodies. For instance, the influence of carbonic acid and of salts in determining both heat-coagulation and precipitation is similar to that produced on both the colloid *A*, and on

[1] This term is used in its wider meaning.

albumin. The following are the salient points of Grimaux's researches on the colloids *A*, *B*, and *C*.

(1) The temperature of heat-coagulation of the colloid *A* lies between 70° C. and 80° C. Opalescence can be induced by heating to 50° C.

(2) The presence of certain neutral salts, or soluble salts of one of the alkaline earths (Ba, Sr or Ca,) is essential to the heat-coagulation of the colloid *A*.

(3) Carbonic acid favours the heat-coagulation of the colloid *A*. In noting this fact we may recall Hewlett's[1] observation that the acidity of dilute solution of albumin favours their heat-coagulation as well as Wright's[2] observation that the intravascular injection induced by the injection of nucleo-albumins occurs more readily in the veins than in the arteries, and Fano's statement that the passage of a stream of CO_2 through "peptone" plasma induces clotting.

(4) CO_2 and air have a reciprocal action in the precipitation and redissolving of the colloids *A* and *C*.

(5) Certain salts delay or prevent coagulation.

Physical and Chemical Characteristics of the Colloids.

In the solid state the colloids are light brown translucent plates, which are inodourless and tasteless. They are sparingly soluble in cold, readily soluble in warm distilled water, the resulting solutions being opalescent and pale straw coloured. Even a 5 °/₀ solution is only slightly viscid. In the absence of salts the solution may be boiled for several hours without the formation of a coagulum. Sterilised solutions exhibit no change in appearance after standing several weeks, although as will be shown hereinafter they either wholly or partially lose their physiological activity.

If the dry colloid is thrown into boiling water it is only partially soluble. The insoluble residuum is similar in appearance to the friable powder formed by the action of the PCl_5 on the meta-amidobenzoic acid in the preparation of the colloid. It, like that powder, is very soluble in ammonia, and gives a solution or after evaporation a substance similar in appearance and reaction to the original colloid from which it has been derived.

Pure solutions of the colloids are neutral.

[1] Hewlett. This *Journal*, XIII. 493. 1892.

[2] Wright. *Proc. Roy. Irish Acad.* 117. 1891. This *Journal*, XII. 184. 1891.

A. *Colour Reactions.* The following reactions were obtained with potash and the sulphates of copper cobalt, and nickel respectively.

Substance	Copper sulphate + Potash	Cobalt sulphate + Potash	Nickel sulphate + Potash
Colloid C	Typical violet solution	Red-brown solution	Pale yellow solution
Colloid B	Negative	Negative	Negative
Colloid A	Blue-violet solution	Brownish solution	Negative

Each of the colloids yields a typical xanthoproteic reaction. They are precipitated by Millon's reagent, but after boiling the coagulum becomes a dirty brown or remains almost unchanged. Negative results were obtained with the reactions of Liebermann and Adamkiewicz. These last two reactions are not characteristic of the non-aromatic proteid decomposition products, the former, according to Salkowski[1], not being given by any of the aromatic members of the putrefaction products of albumin, while I[2] can confirm Salkowski's statement that the latter test is only given by those putrefactive derivatives of albumin which belong to the indole group. In my paper cited above I have shown that the xanthoproteic reaction is given by substances which contain a benzene nucleus in which one hydrogen has been substituted by hydroxyl; while the violet reaction with copper sulphate and potash, together with the yellow given with nickel sulphate and potash, which were thought by Gnezda[3] in the case of albumin to be due to the presence of a (CN) group, are more probably due to the presence of a (CONH) group[4]. Meta-amidobenzoic acid itself will not give these reactions. It is therefore possible that each of the colloids may contain a hydroxybenzene group, while the colloid *C* probably also contains a (CONH) group. In its colour reactions the colloid *C* approaches the nearest to a proteid.

B. *Precipitation.* I can confirm Grimaux's statement that the colloids are precipitated by the mineral as well as by acetic, oxalic, and

[1] Salkowski. *Zeit. fur physiol. Chem.* XII. 215.
[2] Pickering. This *Journal*, XIV. 369. 1893.
[3] *Proc. Royal Soc.* XLVII. 208. 1889.
[4] Pickering. *op. cit.*

tartaric acids. They are also precipitated by metallic salts (e.g. lead, copper, iron, cobalt, nickel). The precipitates given by lead and copper salts pass into solution by the passage of a current of H_2S, like those given with proteids.

Each of the colloids is precipitated by a 10 % solution of a soluble salt of either barium, strontium, or calcium, the precipitates are insoluble in excess of water.

If a solution of either of the colloids be saturated with magnesium sulphate, ammonium sulphate, or sodium chloride, the whole of the colloid after standing rises to the top of the liquid and can be skimmed off. On placing the scum in excess of distilled water it rapidly dissolves, forming the typical opalescent solution, which gives all the chemical reactions of the original solution. Further, a single performance of the process does not interfere with its physiological activity, but repetition of the "purification" impairs and finally destroys its activity. In this respect the colloids behave in manner similar to that discovered by Halliburton[1] and Brodie to be characteristic of nucleo-albumins.

If the amount of neutral salt added be insufficient to induce precipitation, the passage of a current of either carbon dioxide or of sulphur dioxide through the liquid will induce precipitation. In the former case the passage of a current of air through the solution in which the precipitate is suspended will redissolve the precipitate.

Neither carbon dioxide nor sulphur dioxide will effect the precipitation in the entire absence of salts.

The colloids are also precipitated by the addition of trichloracetic acid. It is important to note in connection with the experiments on extra- and intravascular clotting recorded in later sections that the addition of the colloids *A*, *B*, or *C* to plasma or serum causes no precipitation. The colloidal hydrates of iron ("colloïde ferrique") precipitate the proteids of plasma and hence cannot be used in experiments in intravascular clotting.

C. *Heat-Coagulation.* In all the more important respects I can again confirm Grimaux's work cited in section I. The salts named by that writer are essential to the production of heat-coagulation. The action of salts is illustrated by that of calcium chloride. One drop of a 1 % solution of $CaCl_2$ will induce heat-coagulation in 4 c.c. of a 2 % solution of either of the colloids at about 75° C.

The addition of an equal volume of glycerine to the colloid inhibits the coagulability even though an amount of salt that would be sufficient

[1] Halliburton and Brodie. This *Journal*, XVII. 136. 1894.

in its absence is present. The passage of a current of either carbon dioxide or of sulphur dioxide will restore their coagulability. I have tried a parallel experiment with dilute egg-albumin and find that the addition of glycerine will not retard the heat-coagulation of that substance. I can confirm Grimaux's statement that the acetates of potassium and sodium, as well as sodium sulphate, hinder the coagulability of the three colloids.

To illustrate this I cite an experiment with the colloid *C*; 1 c.c. of a 1·5 % solution was taken which was coagulable after the addition of two drops of a saturated solution of sodium chloride. Another c.c. of the same solution had added to it 2 c.c. of a saturated solution of sodium sulphate. Heat-coagulation could not be induced even after the addition of 5 c.c. of a saturated solution of NaCl.

The colloids are precipitated by salicylsulphonic acid, and the precipitate coagulates on heating. I may mention, after a somewhat exhaustive study of the precipitation given by this reagent, that I have failed to find any other substance related to proteids or albuminoids that is precipitated by salicylsulphonic acid.

Digestion Experiments.

I have subjected each of the colloids to the action of pepsin and ·2 % hydrochloric acid in an incubator at 38° C. for fourteen days. The colloid *C* which, before digestion, exhibits with copper sulphate and potash a typical violet reaction, after two days' digestion gives the bright pink colouration with these reagents which is characteristic of peptones and proteoses. The digestion is however slower than that of the fibrin control experiments, and even after fourteen days was still incomplete. With cobalt sulphate + potash it gives a red-brown, with nickel sulphate + potash a pale orange. The colloid *B* is apparently not digestible. The colloid *A* digests much more slowly than the colloid *C*. After five days the pink reaction had not replaced the blue-violet of the unacted upon substance, but on the lapse of a week a faint pink had replaced the blue-violet, while after fourteen days, although the pink had somewhat deepened, the greater part of the colloid remained undigested.

Intravascular injection of the colloids.

The investigations of Halliburton[1] have shown that the various tissue extracts (known by different names by different observers) which

[1] Halliburton. This *Journal*, XIII. 806. 1892; *ibid.* vol. XV. 90.

induce intravascular clotting all consist of nuclein united to a proteid, and although small percentage variations occur in the extracts from different organs, yet their similarity and general reactions warrant the classing of all these bodies together as nucleo-albumins.

C. J. Martin[1] has found that snake venom which he showed to be free from nucleo-albumins produces intravascular clotting. He however advanced the hypothesis that the venom liberated a nucleo-albumin by disintegration of the cells in proximity to the blood stream. It should be borne in mind in examining this theory that the disintegration of leucocytes, which contain nucleo-albumin and which can be produced by the injection of distilled water, glycerine and other reagents does not cause intravascular clotting.

Löwit[2] states that the various reagents which produce leucolysis will, if intravenously injected together with a solution of calcium chloride, induce intravascular clotting. Halliburton[3] and Brodie have carefully repeated his experiments and have tried leech extract, curare, urea, uric acid, sodium urate, both Witte's and Grübler's "peptone," the injection in each case being accompanied by that of a solution of calcium chloride, which in some cases was as strong as 10 °/₀. In one case only was intravascular coagulation obtained, and in the isolated case the Witte's "peptone" used swarmed with bacteria, and probably contained a nucleo-albumin either in the bacteria themselves or as a product of their activity. They have further shown that no destruction of white blood corpuscles follows the admixture of "peptone" with a drop of freshly drawn blood on a slide. The present position of the question is that there are two classes of substances which can produce intravascular clotting: (1) nucleo-albumins, (2) snake venom. The first class is characterised by the phosphorus of its nuclein, the second by its absence. In this paper I shall restrict my remarks on the intravascular action of the colloids to the description of a few typical experiments and the recording of certain of the more characteristic of the results obtained. In collaboration with Prof. Halliburton I am further pursuing this question and a fuller account of our experiments will subsequently be published.

The following is the general method of procedure.

The colloids are dissolved in water, the strength being usually from

[1] Martin. *Ibid.* vol. xv. 380. 1894.

[2] Löwit. *Studien zur Physiol. u. Pathol. des Blutes und der Lymphe.* Jena. G. Fischer, 1892.

[3] Halliburton and Brodie. This *Journal*, XVII. 135.

1·5 % to 2 %. The opalescent straw coloured liquid is filtered, and on cooling is ready for injection. The animals used in these experiments were rabbits, all of which were anæsthetised by ether. All injections were made through the external jugular vein, and samples of blood when drawn were taken from the carotid of the opposite side. In those cases where the injection was not fatal the animal was killed by ligaturing the trachea. After the injection of from upwards of 5 c.c. of an active solution of the colloids the animal dies in a time varying from a few seconds to two or three minutes. There is no dyspnœa except where the trachea has been ligatured. The respirations suddenly cease, but there are usually stretching movements of the limbs, while pronounced exophthalmos and dilatation of the pupils are almost always present. The heart may continue beating or twitching some time after the respiratory stoppage. Post-mortem examination is immediately performed after death, and in a typical experiment thrombosis is found to extend throughout the venous system, the cavities of the heart (except sometimes the right auricle), and into the aorta. The vena portæ and vena cava inferior are characterised by enormous clots, and in this respect the clotting is often more pronounced than that produced by the injection of nucleo-albumins.

Each of the three colloids gives similar results, and are apparently equally active, but variations of the quantity of the colloid necessary to produce coagulation occur with different animals. Also the colloid loses its activity by being kept in solution, so that it is desirable not to keep it in solution for more than three or four days. In those cases where clotting is incomplete or where it has been necessary to kill the animal by asphyxia, the clots are found in the venous system and in the left side of the heart, the arterial blood remaining fluid. It however rapidly coagulates on being shed.

It is obvious that these three synthesised colloids, when injected into the vascular system, behave in a manner very similar to nucleo-albumins.

The following is a summary of six illustrative experiments; in all cases the animal was anæsthetised by ether.

1. Black rabbit. 5 c.c. of 2 % solution of colloid *A* injected into external jugular. Pronounced exophthalmos and dilatation of pupils. Sudden death owing to stoppage of respiration. Heart continued faintly beating some time after. Immediate post-mortem showed pronounced clots in all the cavities of the heart, in the aorta and in all the larger veins.

2. Brown rabbit. 7·5 c.c. of a 2 % solution of colloid *B* were intra-

venously injected and fatal. Pronounced clots in heart and vessels as above.

3. Brown and white rabbit. 42·5 c.c. of a 1 °/₀ solution of colloid *C* injected. Respiration failed after 30 c.c. but started again, 14·5 c.c. more proved fatal. A well marked clot was in left ventricle, a smaller clot in the right ventricle and in the inferior vena cava. The remaining blood was fluid but clotted rapidly on removal.

4. Brown rabbit. 40 c.c. of a freshly prepared 1 °/₀ solution of colloid *A* were injected. No exophthalmos, heart apparently beating typically; 100 c.c. of a 1 °/₀ solution of $CaCl_2$ were then injected followed by a further injection of 5 c.c. of the colloid *A*. No apparent change in the animal's condition. Trachea ligatured, animal apparently died in first stage of asphyxia. Well-marked clots were found in left side of heart, aorta, portal, inferior caval, jugular and pulmonary veins.

5. Brown rabbit. 25 c.c. of 1 °/₀ solution of colloid *C* injected, well marked clot in portal and inferior vena cava. Remainder of blood fluid.

6. Black rabbit. 30 c.c. of a 1 °/₀ solution of calcium chloride injected, heart stopped, no exophthalmos, nor intravascular coagulation.

The effects of the previous injection of "peptone," soap, potassium oxalate, on the clotting produced by the subsequent injection of the colloids is in general similar to the results obtained with nucleo-albumins. Full details and discussion of these experiments will be given in the conjoint paper with Prof. Halliburton.

Experiments on Extravascular Plasma.

Having found that the three organic colloids under consideration each produce after injection into the circulation intravascular coagulation, it became of interest to determine if these synthesised substances would induce coagulation in extravascular plasma. Each of the colloids were used in a 2 °/₀ solution, and experiments made on the same afternoon proved their activity. The plasma selected was that kept fluid by the influence of sodium sulphate. A number of test tubes were taken and kept at the temperature of the laboratory, viz. 9° C.

No. 1. Contained 2 c.c. of plasma + 10 c.c. of distilled water and was found firmly coagulated after 16 hours.

No. 2. Contained 2 c.c. of plasma + 10 c.c. of a solution of fibrin ferment + 2 drops of a 1 °/₀ solution of $CaCl_2$. Coagulation was complete after 20 minutes.

No. 3. Contained 2 c.c. of plasma + 10 c.c. of distilled water + 2 drops of a 1 °/₀ solution of $CaCl_2$. Coagulation complete after 14 hours.

No. 4. Contained 2 c.c. of plasma + 2·5 c.c. of the colloid B + 7·5 c.c. of distilled water. Remained fluid indefinitely.

No. 5. Contained 2 c.c. of plasma + 2·5 c.c. of the colloid B + 7·5 c.c. of distilled water + 2 drops of 1 % solution of $CaCl_2$. Remained fluid indefinitely.

No. 6. Contained 2 c.c. of the colloid B + 2 c.c. of fibrin ferment. Remained fluid indefinitely.

Similar results were obtained with the colloids A and C.

The experiments detailed above show that these colloids will not induce coagulation in sodium sulphate plasma, neither will a further addition of a 1 % solution of $CaCl_2$ determine the clotting. In these respects they behave in a similar manner to nucleo-albumins, which unless warmed to 40° C. with a 1 % $CaCl_2$ will not induce coagulation in extravascular plasma.

Each of the colloids are like albumin, and unlike fibrin ferment coagulated by the addition of alcohol. Further it is almost unnecessary to remind the reader that fibrin ferment will not induce intravascular clotting. Experiment No. 6 illustrates the fact that fibrin ferment fails to induce coagulation of the colloids, neither will the addition of two drops of a 1 % solution of $CaCl_2$ produce this result. I have, up to the present, failed to induce spontaneous coagulation of these substances, although their temperature of heat-coagulation can be considerably lowered by the passage through them of a stream of carbonic dioxide.

It is however essential for heat-coagulation that an alkaline earth (Ba, Sr or Ca) or some of the salts enumerated in Section I. should be present. If salts are requisite for the intravascular coagulation produced by the colloids they are probably furnished by the blood itself.

Summary and Remarks.

The following is a brief summary of the principal points of interest:

(1) The researches of Prof. Grimaux, made nearly ten years ago, show that it is possible to synthesise organic colloids, which exhibit many of the chemical reactions which have hitherto been deemed as characteristic of either proteids or of their decomposition products.

(2) Of the three colloids here considered, the first two—the colloids A and B are prepared by the interaction of PCl_5 and meta-amido-benzoic acid; the colloid C by the action of a current of gaseous ammonia at 170° C. on aspartic anhydride. Each of these substances in the solid form is not unlike dried serum albumin; they yield

opalescent pale straw-coloured solutions when dissolved in distilled water.

(3) A typical xanthoproteic reaction is given by the three colloids. With copper sulphate and potash the colloid *A* gives a blue violet colour, the colloid *B* a negative result, but the colloid *C* a typical violet colouration. With cobalt sulphate and potash the colloid *C* gives a red-brown solution, while with nickel sulphate and potash a pale yellow solution. Another colloid which I have not yet had the opportunity of examining is said by Grimaux to yield the xanthoproteic, "biuret" and Millon's reactions.

(4) These colloids will not in the absence of salts coagulate on heating. If, however, a trace of a soluble salt of Ba, Sr, or Ca be present opalescence can be induced by heating to 50° C., coagulation about 75° C. Certain other salts will play the *rôle* of the alkaline earths in heat-coagulation.

(5) Their heat-coagulation is retarded by addition of potassium acetate, sodium sulphate or excess of glycerine. The passage of a current either of CO_2 or SO_2 restores the coagulability destroyed by excess of glycerine.

(6) The colloids are removed from solution (rising to the surface of the fluid) by saturation with either $Mg.SO_4$, $(NH_4)_2SO_4$, or NaCl. The separated solid readily redissolves in excess of distilled water.

(7) Boiling water renders the colloids insoluble. Treatment of the insoluble product with ammonia and evaporation *in vacuo* regenerates the colloid, forming a soluble body which yields all the reactions of the original substance.

(8) Carbonic acid in the presence of salts will cause precipitation of the colloids. The passage of a current of air through the solution will redissolve the precipitate. The colloids in this reaction behave like globulins.

(9) The colloid *B* is apparently not digested by pepsin and ·2% HCl. Evidence of slight digestion was obtained with the colloid *A*. Considerable digestion of the colloid *C* was obtained with pepsin + ·2% HCl, the typical violet reaction with $CuSO_4$ + KHO of the undigested colloid being changed to a brilliant pink after two days' digestion. An orange reaction was obtained with $NiSO_4$ + KHO after the same lapse of time.

(10) Each of the colloids when intravenously injected causes extensive intravascular clotting of the blood of black and brown rabbits. In a typical experiment death is due to respiratory failure, which usually

results after the injection of 5 to 20 c.c. of a 1·5% solution. Pronounced exophthalmos and dilatation of the pupil has been observed in each of 15 experiments performed. In those cases where the injection of larger quantities of the colloids did not produce death, the animals were killed by asphyxia, and an immediate post-mortem showed clots in one or more of the larger veins. Albino rabbits are very resistant to the action of the colloids.

(11) The action of potassium oxalate and "peptone" in conjunction with the colloids is not unlike its action in conjunction with nucleo-albumins. Details are deferred.

(12) The colloids lose their physiological activity if kept for a long time in solution.

(13) The "regenerated" colloid mentioned in paragraph 7 gives when intravenously injected typical blood-coagulation.

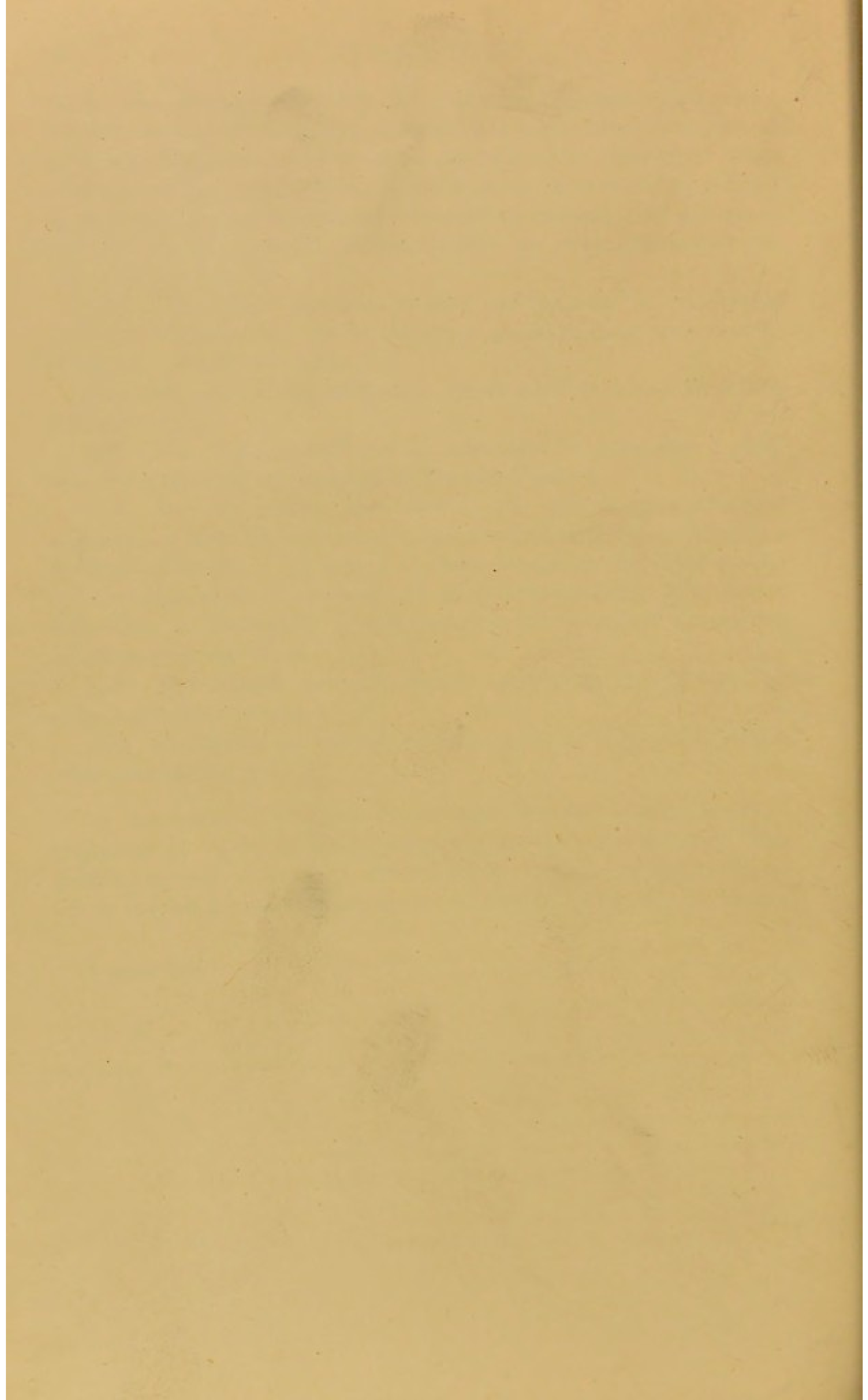
(14) The experiments here recorded as well as more extended observations made in collaboration with Prof. Halliburton point to a striking similarity in the action of these colloids on the intravascular blood to that which is characteristic of nucleo-albumins. It is almost impossible to distinguish a difference in the action of these two substances on the blood.

(15) The colloids will not induce coagulation in extravascular sodium sulphate plasma.

(16) Fibrin ferment will not induce coagulation of the colloids even after the addition of $CaCl_2$.

In conclusion I wish to record my warmest thanks to Prof. Grimaux of Paris for kindly supplying me with specimens of these colloids, as well as to Prof. Halliburton, whose constant help and advice renders it most difficult for me to adequately express my thanks.

9 *April*, 1895.



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THE INTRAVASCULAR COAGULATION PRODUCED BY SYNTHESISED COLLOIDS.

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Introduction. Recent investigations have shown that there are two classes of substances, both metabolic products of the organism, which when intravenously injected will introduce intravascular clotting. The first class, termed nucleo-albumins[1], consists of various tissue extracts all of which are characterised by containing nuclein, and consequently phosphorus; while the second class, which includes the proteoses of the venom of certain snakes[2], is distinguished by the absence of phosphorus from its molecule.

More recently one of us (Pickering[3]) has published an account of the effect produced by the intravenous injection of certain synthesised colloids, which in their characteristic reactions are strikingly similar to proteids. The following is a summary of the chief points contained in that paper: (1) Three synthesised colloids, hereinafter termed the colloids *A*, *B*, and *C*, respectively were investigated. The colloids *A* and *B* are formed by the interaction of phosphorous pentachloride and meta-amidobenzoic acid in sealed tubes at a temperature of 125° and 135° C. respectively. The resulting products are according to their discoverer, Prof. Grimaux[4] of Paris, intramolecular anhydrides formed by the union of several molecules of meta-amidobenzoic acid with the

[1] Halliburton and Brodie. This *Journal*, XVII. 135. Pekelharing, *Centralblatt für Physiologie*. 4 Mai, 1895. Hft. 3.

[2] Martin and McGarvie-Smith. *Proc. Roy. Soc. N.S.W.* (1892), and this *Journal*, XV. 380 (1894).

[3] Pickering. This *Journal*, XVIII. 54 (1895). *Comptes Rendus de l'Académie des Sciences* (Paris), 17 Juin, 1895, T. CXX. p. 1348, and *Comptes Rendus de la Société de Biologie*, 14 Juin, 1895.

[4] Full bibliographical notice of Grimaux's researches will be found in Pickering's paper in this *Journal*.

elimination of water. They are repeatedly washed in warm water, until all traces of the contaminating phosphoric acid is removed, and finally dissolved in ammonia and evaporated down at the temperature of the laboratory. Analysis shows that they contain no phosphorus when pure. (2) The colloid *C* is formed by the action of a current of gaseous ammonia heated to 170° C. on solid aspartic anhydride. The product of the reaction after solution in water should be evaporated down *in vacuo.* (3) Each of these substances resembles albumin, in (*a*) its physical properties, (*b*) in the temperature necessary to determine its heat-coagulation, (*c*) in the salts and physical conditions which influence (retard or accelerate as the case may be) its heat coagulation, (*d*) in its yielding many of the colour reactions hitherto deemed diagnostic of either proteids or of their katabolic products. (*e*) In their behaviour with the precipitants of proteids (*f*) when intravenously injected in a 1°/₀ to 2°/₀ solution they produce extensive intravascular clotting. Further they do not precipitate the proteids of white of egg, of serum, or of extravascular plasma.

The general conclusion of the author was that these substances in their chemical reactions are strikingly similar to globulins and in their physiological action on the rabbit to nucleo-proteids[1].

The present paper consists of a further account of the phenomena produced by the intravascular injection of these substances into various animals.

Method. The procedure followed in each case, except where otherwise stated, is the same as adopted by the authors in their previous papers on blood -coagulation. Intravenous injection was performed through the external jugular vein, samples of blood when taken were withdrawn from the carotid on the opposite side. In all cases an anæsthetic was used, usually ether. The figures in brackets refer to the protocols of experiments at the end of the paper. In all cases post-mortem examination was performed immediately after death.

The solutions used varied in strength from 0·5°/₀ to 2°/₀ and were dissolved in distilled water except where otherwise stated. The solutions of the pure material are colourless, or pale straw-coloured, faintly opalescent and neutral. Even a very strong solution (e.g. a 5°/₀) is only slightly viscid. Each of the colloids apparently acts in a similar manner. The colloid *A* will generally be referred to throughout the text, confirmatory experiments with the colloids *B* and *C* will be found

[1] We have throughout this paper adopted the name nucleo-proteid instead of the term nucleo-albumin formerly used by one of us (H).

in the protocols of the experiments (Exps. 2, 4, 9, 11, 12, 19, 21, 22, 23, 24, 28, 30, 31, 43, 44, 46, 47, 49, 50.)[1].

Physiological action on the animal other than blood coagulation. Intravenous injection of small doses of the colloids, say 5 to 10 c.c. of a 1·5 % solution, often causes temporary respiratory stoppage, which if the dose be not too large, passes off after an interval varying from a few seconds to one or two minutes. Death is apparently always due to failure of the respiratory centre, and occurs typically after the section of both vagi (Exp. 11). In this connection we may remark that the fatal action of nucleo-proteids is probably due to their action on the respiratory centre.

Concomitant with the respiratory failure is marked dilatation of the pupils and exophthalmos. That these last two phenomena are not a specific action of the colloids is shown by the fact that intravascular injection of substances which cause precipitation of the proteids of the plasma like the "colloïde ferrique" cause both marked exophthalmos and dilatation of the pupils. It is however noteworthy that injection of large doses of the colloid into albino rabbits produces these three symptoms while the blood still remains fluid (Exps. 8, 15).

The colloids are apparently not so toxic to the heart as to the respiratory centre, the rhythmic action of the former continuing after the failure of the latter. Another characteristic of the action of the colloids is the pronounced stretching movements of the fore and hind-limbs which occur immediately before the death of the animal. The movements are very similar to those observed in death due to the intravascular injection of nucleo-proteids.

In dogs the injections of larger doses sometimes provokes hypernœa or dyspnœa which accompanies the stretching movements (Exps. 54, 59).

The action of the colloids alone on rabbits. Solutions varying in strength from 1 to 2 % were employed. The action on the animal varies in degree but not qualitatively. When the solution was freshly prepared and the animal not an albino, intravascular coagulation was always obtained.

In those cases where large doses did not produce the usual respiratory failure the animal was killed by asphyxia and post-mortem examination revealed clots in one or other of the larger veins or in the cavities of the heart.

In a typical experiment the cavities of the heart, subclavian, portal,

[1] The preparations of these colloids were in some cases obtained from Prof. Grimaux; most of the colloid *A* used in this paper was however prepared by one of us (P).

inferior vena cava and pulmonary artery contain extensive clots, while the blood in the aorta and pulmonary veins is often clotted.

Reference to the protocols in the Appendix will show that there is considerable variation in the amount that is toxic to different animals. In a like manner to the coagulation produced by nucleo-proteid the clotting occurs more readily in the veins than in the arteries, and the blood in the portal and inferior vena cava seems the most susceptible to its action (Exps. 1, 2, 9, 11, 12, 19, 23, 24, 35, 36).

The colloids that have been kept in solution behave differently. After a few days in solution their physiological activity decreases, till after a period varying from ten to twenty-one days they become inactive. Thus in one experiment where the colloids were kept in solution for fifteen days, 172 c.c. of a 1·5% solution was required before the typical result was obtained (Exp. 5), while the wholly inactive condition is exemplified by Experiments 28, 29, 30, 31, and 32. In these cases the production of asphyxia will not induce the coagulation. The colloids after remaining in solution become slightly acid, possibly due to the absorption of carbon dioxide from the atmosphere. Neutralization before injection will not however restore their activity. It is well known that nucleo-proteids similarly lose their activity when kept in solution. The inactive colloid does not induce a "negative phase" in the animal in which it fails to produce clotting, since the blood coagulates very rapidly on withdrawal.

Action on albinos. In a manner strikingly similar to nucleo-proteids the colloids even in very large doses fail to induce intravascular clotting in albino rabbits. Respiratory failure, exophthalmos, dilatation of the pupils result, but the intravascular blood remains fluid. The coagulability of samples of blood after they have been withdrawn from the carotid is accelerated by previous injection of the colloids. Thus in an albino whose normal carotid blood coagulated in six minutes after withdrawal; after the injection of 20 c.c. of a 1·5% solution of the colloid *A* coagulated in two minutes after withdrawal.

An animal that is nearly all white does not react as rapidly to the injection of the colloids as a dark or black animal (Exps. 8, 15, 21, 22, 56).

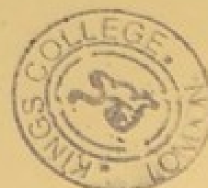
The "regenerated" colloid. If the colloids be thrown into boiling water they become partially insoluble. The insoluble portion resembles in appearance the white friable powder formed by the interaction of phosphorous pentachloride on meta-amidobenzoic acid. If this substance is dissolved in ammonia and evaporated down *in vacuo* and the

product redissolved in water, and subsequently intravenously injected into a rabbit, intravascular coagulation results (Exp. 14). This shows that the ammonia plays an important part in the synthesis of the colloids, which may possibly contain a hydroxyl group. The "regenerated" colloid gives the same chemical reactions as the original colloid from which it has been derived.

Action of the colloids on dogs. Large doses of the colloids, that is more than 10 c.c. of a 1 % solution, act in a manner similar to that described as characteristic of the rabbit. The coagulation produced is very marked, clots being found in the heart, in all the larger veins, and in the aorta, while the portal system is characterised by enormous clots. The dyspnœa, exophthalmos, dilatation of the pupils and stretching movements are even more marked than in the rabbit (Exps. 41, 51, 52, 54, 59).

Negative phase. Working with snake venom as far back as 1854 Brainard[1] found that some time elapsed between the biting and death of the animal bitten, while the intravascular blood of the poisoned animal remained fluid. A similar "negative phase" was discovered by Wooldridge to be characteristic of the intravascular blood after injection of minute doses of tissue fibrinogens (nucleo-proteids). It therefore became of interest to determine whether a negative phase could be obtained by the injection of small doses of the colloids. Reference to the protocol of Exp. 60 will show that with a dog weighing 30 lbs. a dose of ·000083 gram per kilo weight, that is after the injection of 1 c.c. of ·5 % solution of the colloid *A*, there was a retardation of 2 minutes 30 seconds in the time of commencement of coagulation of the carotid blood. After a second dose of ·000083 gram per kilo weight (the actual dose being ·005 gram), an interval of seven minutes having elapsed, there was a retardation of four minutes in the time of commencement of coagulation of the carotid blood. Three other samples taken during the ensuing sixteen minutes showed a retardation of three, five and four minutes respectively. Immediately following the injection of another c.c. (·005 gram) into the animal the blood withdrawn from the carotid coagulated in the normal time of four minutes, but three minutes subsequently there was a retardation of three minutes in the time of coagulation of the withdrawn blood. Even after 18 c.c. had been injected (·09 gram) there was still a retardation in the coagulation of the carotid blood of one minute and a

[1] Brainard. *Smithsonian Reports*, 1854. See also Martin, this *Journal*, xv. 380.



half. An increase of the dose however gave typical intravascular clotting.

Experiment 54 shows a similar result. Thus small doses of the colloids retard the coagulability of dogs' blood, while larger doses hasten the phenomenon.

The retardation is certainly not so marked as that described as characteristic of the negative phase in experiments with snake venom and nucleo-proteid, yet the parallel is a very striking one.

The theoretical explanation advanced by Wright[1] and Pekelharing[2] to explain the negative phase produced by small doses of nucleo-albumin was that the retardation of coagulation was due to the splitting off of a proteose moiety from the nucleo-proteid molecule. The colloid *A*, which gives this retardation of coagulability in small doses, will not peptonise when treated with pepsin and ·2 °/₀ HCl for many days at 38° C. There can therefore be no splitting off of a peptone moiety in this instance.

Effect of colloid on other animals. We have injected 1 °/₀ solutions of the colloid *A* into the jugular vein of the guinea-pig, rat and cat, and in each case death ensued, apparently owing to the failure of the respiratory centre with the typical symptoms as previously described. Post-mortem examination revealed clots in the larger veins (Exps. 53, 55 and 56).

Effect of the colloid that had been precipitated by acetic acid. The method adopted by Wooldridge of separating tissue fibrinogens (nucleo-proteids) from tissue extracts was precipitation by weak acetic acid, and redissolving in sodium carbonate. Since this method yields a substance active in the production of intravascular clotting, it became desirable to ascertain if precipitation by acetic acid modified the physiological action of the colloids. Experiments 58 and 59 show that precipitation by acetic acid and redissolving the precipitate in sodium carbonate does not destroy the action of the colloids on rabbits.

Action of the colloids on blood corpuscles and on mesentery. If a solution of normal saline containing a 0·1 °/₀ solution of snake venom be mixed with frog's blood disintegration of the red corpuscles occurs within a quarter of an hour. The white corpuscles, though more resistant, become first intensely granular, with very prominent nuclei, and finally disintegrate (Martin)[3]. The same writer also records

[1] Wright. *Proc. Roy. Irish Acad.* 3rd series, No. 11, 1892, 117.

[2] Pekelharing. *Verhand. d. Konink. Akad. Wetenschappen te Amsterdam*, Tw. Sc. Deel 1, No. 3.

[3] Martin. *Op. cit.*

stoppage of amœboid movements of the white corpuscles of rabbits, dogs and man, as resulting from the admixture with 0·1 % of the venom. It is therefore probably not due to leucolysis and liberation of nucleo-proteid that the viper's venom produces intravascular clotting. Martin has however found that the venom causes extensive disintegration of the endothelial lining of the vascular wall, and has advanced tentatively the hypothesis that the intravascular clotting was due to a nucleo-proteid liberated from the disintegrated cells.

Consequently we have tried the action of the colloids first on the corpuscles themselves, and subsequently on the endothelial cells of the capillary wall.

Experiment 42, when frog's blood was mixed with an equal volume of a 1·5 % solution of the colloid *A*, showed after five minutes' action no change in the white corpuscles, while the red corpuscles appeared rounder. After 20 minutes' action no further change was observed in the red corpuscles, but the white corpuscles appeared more granular than in the normal condition. It was only after one hour's action that disintegration of the white corpuscles had partially taken place (see also Exps. 43 and 44). With human blood on a warm stage the red corpuscles only appeared slightly rounder, while disintegration of the white corpuscles did not commence until 30 minutes had elapsed. Similar results were obtained with rabbit's blood (Exps. 56, 57, 58, 59, 60).

Experiment 40 shows that if a rabbit's mesentery is bathed in a 1·5 % solution of the colloid *B* (precautions having been taken to avoid cooling) neither stasis nor disintegration of the endothelial lining of the vascular wall results.

Injection into the dorsal lymph sac and at the same time bathing the web of a frog's foot in a 1·5 % solution of the colloid also failed to induce disintegration of the vascular wall (Exp. 38). The action of the colloid is therefore not due to disintegration of the endothelial lining of the vascular wall. As regards the destruction of the corpuscles, that takes place too slowly to account for the action of the colloids, which will induce clotting in from one to two minutes, while disintegration does not commence till after ten or fifteen minutes. These points will be returned to in the discussion on the significance of our observations.

Injection of soap. It has been shown by Munk[1] that intravascular injection of soaps hinders coagulation, and in extreme cases is fatal. Experiment 17 confirms his statement.

Injection of soap + colloid. If however colloid is injected before

[1] Munk. *Archiv für Anat. u. Physiol. Physiol. Abth.* Supp. Bd. 1890, 116.

the fatal effect of the soap ensues, the retardation of coagulability caused by the soap can be antagonised by the action of the colloid. A further injection of the colloid causes death with the typical symptoms and results (Exp. 16).

Injection of colloid + "peptone." The inhibitory action over the processes of coagulation that follows the intravascular injection of peptone being well known, it became of interest to observe the influence of the colloids on animals into whose circulation "peptone" had been previously injected. The antagonistic action of the two substances is well illustrated by Experiments 7 and 25. The retardation of coagulability produced by the "peptone" is first reduced, finally annulled, and then the colloid asserts its typical action.

Pekelharing[1] advanced the hypothesis that the inhibitory action of "peptone" is due to its affinity for the calcium salts of the plasma. Our experiments hardly support this view; at least they show that the action of "peptone" is annulled by other substances like the colloid *A*, which contains no calcium at all, and it is difficult to assume that the colloid injected causes the peptone to give up the calcium salt it had taken up.

Potassium oxalate + colloid. The similar antagonistic action of these substances is illustrated by Experiment 10, from which it is evident that the action which potassium oxalate possesses of delaying the coagulation is antagonised by the subsequent injection of colloid; and further it is noteworthy that a not much larger dose of colloid was required to induce intravascular clotting than is effective under ordinary circumstances.

Glycerine + colloid. It has been pointed out by Grimaux[1] that glycerine exerted a retarding influence on the heat-coagulation of the colloids, this influence is however not extended to the heat-coagulation of proteids (Pickering)[2]. The injection of 20 c.c. of a 5 % solution of glycerine instead of retarding the coagulability of the blood, accelerated that withdrawn from the carotid by one minute. A subsequent injection of 50 c.c. of 1·5 % solution of the colloid *A* produced typical death and intravascular coagulation (Exp. 6).

Calcium chloride. The injection of 30 c.c. of a 1 % solution of this substance, although fatal did not induce intravascular clotting (Exp. 3).

Calcium chloride + colloid. Large quantities of calcium chloride precipitate solutions of the colloid, and consequently the clotting which

[1] *Op. cit.* [2] *Op. cit.*

results from their injection is probably due to the formation of a precipitate in the vessels.

In the case of the injection of smaller quantities the precipitation does not occur, and the injection of the calcium chloride does not determine the coagulation when the amount of the colloid is too small to effect it alone.

Calcium chloride + colloid + CO_2. In this case where neither the colloid nor a small addition of $CaCl_2$ will induce the coagulation, the carbonic dioxide in the vessels which accumulates during asphyxia almost immediately induces extensive intravascular clotting (Exps. 4, 13).

Colloid + CO_2. If the amount of colloid be too small to produce intravascular clotting the production of asphyxia will often determine the coagulation. The clotting under these circumstances is apparently not so marked as that produced by a larger dose of the colloid (Exp. 12).

Other substances. Grimaux has shown that certain colloid alhydrates of iron coagulate spontaneously when diluted with an equal volume of water. We have injected his "colloïde ferrique" into a rabbit (Exp. 18) and intravascular clotting resulted. The clot was however very brittle, and not long and coherent like the clots given by colloids and by nucleo-albumin. Since the "colloïde ferrique" is a precipitant of the proteids of the plasma it is probable that the intravascular coagulation is due to this cause and is of no significance. The clot moreover has a brownish tint similar to that produced by an iron salt.

We have also endeavoured to ascertain whether the injection of certain proteid decomposition products and other substances synthesised from either proteid decomposition products or from allied bodies would induce intravascular clotting.

Skatole. Exps. 26 and 27 yielded with large doses of this body negative results, a similar result was obtained with a body formed by the interaction of PCl_5 on skatole at 125° C. (Exp. 20).

Negative results were also obtained with the substances formed by the action of PCl_5 on a mixture of alloxan and biuret, and PCl_5 on hippuric acid (Exps. 33, 34, 39). The last two bodies were colloidal in nature but did not give the proteid colour reactions. Soap and glycerine, both colloidal substances, do not cause intravascular coagulation. This phenomenon is not therefore characteristic of the injection of a substance in the colloidal state.

Significance of the experiments. The results we have described were most unexpected, and they certainly increase the difficulties already surrounding the coagulation question. The view most gene-

rally held at present with regard to the causes of blood coagulation is that the determinant factors in the process are nucleo-proteids, together with salts of calcium and possibly carbon dioxide (Green, Pekelharing, Halliburton, Arthus and Pagès and Wright). A variation of this view (Lilienfeld, Löwit) is that leucolysis liberates the nucleo-proteid or nucleo-histon. In the case of coagulation produced by the intravascular injection of snake-venom it has been advanced (Martin) that the coagulation produced is due to liberation of a nucleo-proteid by destruction of the endothelial lining of the vascular wall.

Following the train of thought suggested by these views a possible explanation of our experiments might be found in the hypothesis that the coagulation caused was due to leucolysis, the colloids breaking down the white corpuscles and liberating a nucleo-proteid. Direct experiments on the corpuscles and on the endothelial lining of the vascular wall do not however favour this view. The following are alternative hypotheses that might be advanced in explanation. (*a*) The colloids act by breaking up one or more of the chemical constituents of the plasma and the disintegration products are the cause of intra-vascular coagulation. (*b*) The colloids themselves act specifically, causing coagulation. The first of these hypotheses though not impossible is we think not probable, because of the failure of the colloids to induce coagulation in extravascular (salted) plasma, and we are more inclined to accept tentatively the second. It is now necessary to emphasise the points of resemblance in the action of the colloids to those characteristic of nucleo-proteid.

(1) The symptoms of death are the same, respiratory stoppage and exophthalmos, and dilatation of the pupils, stretching movements, &c.

(2) The negative phase characterises both substances.

(3) Carbonic dioxide favours the action of both substances.

(4) Neither substance induces intravascular clotting in albino rabbits.

(5) Neither substance induces coagulation in extravascular plasma.

(6) The influence of oxalates on the physiological action of both substances is similar.

The chief differences are—

(1) The absence of phosphorus from the colloid molecule.

(2) The colloid *A* (which will give a "negative phase") will not peptonise.

This difference does not apply to the colloids *B* and *C*.

A study of the colour reactions of these substances made by one of us[1] tends to show that the colloid molecule contains a molecular group or groups which are very similar, if not identical to the molecular groups in proteids. Indeed in chemical behaviour these synthesised substances, and more especially the colloid *C*, satisfy the leading tests and reactions which have been deemed diagnostic of proteids. In this connection it is important to note that Grimaux has shown these substances give coagula on heating in a manner indistinguishable from proteids. That author considers the phenomena of heat-coagulation both of organic (colloids and albumin) and inorganic substances (colloidal hydrates of iron and silicon) to be strictly comparable to etherification, and further that heat-coagulation and spontaneous coagulation are the same class of phenomena.

Speaking of the continual condensations (with elimination of water) which characterise the passage of certain soluble hydroxides of iron from a colloidal to a solid state he says, "Arrivé à un certain point de condensation le corps n'est plus soluble et la coagulation commence." He attributes heat-coagulation to increase of molecular weight owing to the loss of water and polymerisation.

Bearing in mind the curious chemical analogy between these synthesised colloids and proteids, and the similarity of conditions which control the phenomena of coagulation in both the inorganic and organic series, we are faced with the question whether the cause which produces intravascular clotting is not the same in nucleo-proteids and in the proteid-like synthesised colloids, and to carry the question further whether in like manner as each substance contains molecular groups which give the same colour reactions, so they may contain a similar molecular group which is the determinant factor in coagulation?

In support of the hypothesis that the determination of coagulation is not a mere result of injecting a heavy colloidal substance into the blood, but rather the consequence of the chemical action of a definite molecular group, we have the fact that the introduction of large quantities of other colloidal substances[2] like glycerine and soap does not cause intravascular clotting.

Incidentally it may here be remarked that both these substances are very destructive to white blood corpuscles, and if intravascular coagulation was the result of leucolysis we might expect extensive clotting to result from their intravascular injection.

[1] Pickering, *op. cit.*

[2] Vide section on the effect of introduction of skatole derivates, etc.

The next question that presents itself is what molecular group is there common to the three colloids and nucleo-albumins?

Since the colloids *A* and *B* are as active in the production of intravascular coagulation as the colloid *C*, and if the determinant factor be a molecular group it should be one that is common to the three substances. The first two are intramolecular anhydrides formed by the union of several molecules of meta-amidobenzoic acid, the third (the colloid *C*) an intramolecular anhydride formed by the action of ammonia on aspartic anhydride, the common molecular group or more probably similar group being an intramolecular molecular anhydride of an amido fatty acid. Although similar in constitution they are owing to their difference of colour reaction and preparation probably not identical in nature.

It is very probable that proteids contain (Pickering[1]) a similar molecular group in association with their benzene nucleus[2]. We would therefore tentatively suggest the following as a possible explanation of our experiments:

(1) The colloids *A*, *B* and *C* are the nearest substances at present known to proteids, and probably may be termed elementary proteids. The colloid *C* is the most worthy of this designation.

(2) The coagulation induced by the intravascular injection of these substances is possibly due to a molecular group of similar nature.

(3) That it is not impossible the determinant cause in the production of intravascular coagulation by nucleo-proteids may be a molecular group of a similar nature. Such a group might chemically be of the nature indicated in the foregoing paragraphs.

(4) The phenomena of heat coagulation and spontaneous coagulation may possibly be one of the same series, the determinant cause only differing. The similar *rôle* played by calcium salts and carbon dioxide in both cases tends to favour this view.

(5) The general outline of the phenomenon of coagulation is a molecular condensation, possibly comparable to etherification, polymerisation being accompanied with loss of water.

(6) That the negative phase is not a subsidiary phenomenon due to disintegration of the material intravenously injected, but is rather a result characteristic of the action of minute doses of the material which in larger doses induces intravascular clotting.

(7) Such an "inhibitory phase" as we would call it may be similar

[1] Pickering. This *Journal*, XIV. 347.

[2] The word is used in the sense in which it is used by Kekulé.

to the inhibitory action of many small doses which act contrary to the action of larger doses, such as the physiological immunity produced by small doses of alexines.

General Summary.

The following briefly emphasises the leading points made out in this paper, other than the theoretical considerations summarised in the preceding section:

(1) The colloids *A* and *B*, synthesised by dehydration and condensation of meta-amidobenzoic acid, and the colloid *C*, formed by the action of gaseous ammonia on aspartic anhydride, when intravenously injected produce extensive intravascular coagulation in rabbits, dogs, cats, guinea-pigs, and rats.

(2) The colloids do not apparently cause destruction of the endothelial lining of the vascular wall; neither apparently are they destructive to the blood corpuscles.

(3) Small doses of the colloid induce a 'negative phase' in dogs.

(4) The colloids will not induce intravascular clotting in albino rabbits; in all other animals examined, a large dose of the freshly prepared colloid has never failed to induce coagulation.

(5) The colloids apparently kill by their action on the respiratory centre. Doses that are too small to be fatal induce temporary respiratory stoppages. Section of the vagi does not modify the result. The action of the heart usually continues after the respiratory failure. Death is typically preceded by stretching movements, which in dogs are accompanied by hypernœa. Exophthalmos and dilatation of the pupils is almost always present.

(6) If the colloids are kept in aqueous solution they lose their physiological activity and fail to produce intravascular clotting.

(7) Carbonic dioxide and soluble calcium salts favour the action of the colloids. A dose of the colloid that is too small to produce alone intravascular clotting will induce intravascular coagulation during the first stage of asphyxia.

(8) If the colloid is precipitated by acetic acid and redissolved in sodium carbonate, the fresh solution acts physiologically like the original solution.

(9) The parallel between the results produced by the intravascular injection of nucleo-proteid and the phenomena described in the six preceding paragraphs is worthy of note.

(10) The "regenerated colloid" formed by the action of ammonia upon colloid that has been changed by boiling water acts like the normal colloid.

(11) The retarding influence of soap and potassium oxalate on the coagulation of blood withdrawn from the carotid is antagonised by intravascular injection of the colloid.

(12) The intravascular injection of glycerine and of soap although producing leucolysis will not induce intravascular coagulation.

(13) The introduction of glycerine into the circulation does not prevent the action of the colloids.

(14) Various synthesised substances other than those named were injected, and although from their chemical nature they were allied to proteids they failed to cause any intravascular coagulation.

(15) In connection with the intravascular coagulation resulting from the injection of the colloids *A*, *B*, *C*, it should be noted that each of these substances behaves chemically in a manner strikingly similar to proteids and that the colloid *C* is so close to these substances as to give nearly all the reactions hitherto deemed diagnostic of them.

July 9, 1895.

APPENDIX.

The following are protocols of experiments, all of which were performed during anæsthesia of the animal. Except where otherwise stated ether was used.

EXP. I. Black rabbit. 5 c.c. of 2 % solution of the colloid *A* injected into jugular. Death accompanied by pronounced exophthalmos and dilatation of the pupil. Post-mortem showed large clots in all the cavities of the heart, the aorta, jugular, sup. vena cava, and inf. vena cava.

EXP. II. Brown rabbit. Injection of 7·5 c.c. colloid *B* into jugular with same symptoms as above, clots in all cavities of heart, aorta, portal, jugular, vena cava and subclavian.

EXP. III. Black rabbit. Intravascular injection of 30 c.c. of a 1 % solution of calcium chloride. Death apparently due to cardiac stoppage. No intravascular coagulation.

EXP. IV. Black and white rabbit. Injection of 30 c.c. of 1 % solution of colloid *B*. Condition of animal apparently unchanged; 30 c.c. of 1 % solution of $CaCl_2$ then injected, animal showed no change, and was after a lapse of 10 minutes killed by asphyxia. Post-mortem showed well-marked clots in all the cavities of the heart, jugular, subclavian, vena cava inferior, portal and small skin veins. The exophthalmos was slight.

EXP. V. Brown and white rabbit. The 1·5 % solution of the colloid *A* that was used had been kept in solution fifteen days; 172 c.c. were injected before death resulted, which was apparently due to respiratory stoppage. Post-mortem showed well-marked clots in right heart and pulmonary artery, portal, inferior vena cava, subclavian, and in the small gastric veins; the left side of the heart and aorta were empty.

EXP. VI. Large black rabbit. Sample of carotid blood coagulated in 2·5 minutes; 20 c.c. of a 5 % solution of glycerine injected, sample of carotid blood coagulated in 1·5 minutes; 50 c.c. of 1·5 % solution of colloid *A* injected. Complete clots formed throughout heart, aorta, subclavian and portal vein and in the inferior vena cava.

EXP. VII. Black rabbit. Sample of carotid blood coagulated in 2 minutes. After 10 c.c. of a 2 % solution of "peptone" had been injected the carotid blood coagulated in 3½ minutes. Injection of 20 c.c. of 1·5 % solution of colloid *A* killed the animal. There was a small clot in the left ventricle, a pronounced clot in the aorta and inferior vena cava, but the remainder of the blood was fluid.

EXP. VIII. Albino rabbit. 38 c.c of 1·5 % of colloid *A* injected. Animal died apparently due to respiratory stoppage. There were no clots in blood vessels.

EXP. IX. Black rabbit. Injection of 30 c.c. of 1 % solution of colloid *C* fatal. Well-marked clots throughout heart, and in jugular, portal and superior vena cava. There was only a small clot in the inferior vena cava and the blood was fluid in the aorta and in the pulmonary veins.

EXP. X. Brown and white rabbit. Sample of carotid blood coagulated in 7 minutes; 2 c.c. of a 6 % solution of potassium oxalate injected; second carotid sample coagulated after 18 minutes. 3 c.c. more of potassium oxalate were then injected. Third carotid sample coagulated after 21 minutes. 10 c.c. of 1·5 % of colloid *A* was then injected. Carotid blood coagulated after three minutes. After a second injection of 10 c.c. of 1·5 % solution of colloid *A* the carotid blood clotted in 1½ minutes. 10 more c.c. of colloid *A* were then injected. Symptoms similar to dyspnœa ensued followed by death. Post-mortem showed clots in left heart, in aorta, subclavian, portal veins and inferior vena cava. The right heart was empty.

EXP. XI. Both vagi cut in a black rabbit. 38 c.c. of 1 % solution of colloid *B* injected. Death (with extreme exophthalmos and dilatation of pupil) due to respiratory stoppage. A small clot in the right auricle, left heart empty, right ventricle full of fluid blood. The aorta, inferior vena cava mesenteric, portal, pulmonary, and subclavian veins contained well-marked clots.

EXP. XII. Brown rabbit. 25 c.c. of 1·5 % solution of colloid *B* injected. Animal killed by asphyxia. A clot found in portal and inferior vena cava. Remainder of blood fluid.

Exp. XIII. Brown rabbit. 40 c.c. of 1 % solution of colloid *A* injected. No change in animal observed. 100 c.c. of 1 % solution of $CaCl_2$ then injected, followed by 5 c.c. of colloid *A*. The animal was killed by ligature of the trachea, and died in the first stage of asphyxia. Well-marked clots were found in left heart, aorta, jugular, subclavian, vena cava, portal and pulmonary veins. The heart continued beating after death and there was no exophthalmos.

Exp. XIV. Very large black rabbit. 120 c.c. of ·75 % of "regenerated colloid" injected. Animal killed by asphyxia. Well-marked clots in left heart, the subclavians and inferior vena cava. The portal vein and aorta were empty.

Exp. XV. Albino rabbit, weight 2 lbs. Sample of carotid blood coagulated in 6 minutes 10 c.c. of 1·5 % solution of colloid *A* injected. Sample of carotid blood coagulated in 5 minutes. 10 c.c. more of colloid *A* injected. Carotid blood clotted in 2 minutes. 10 c.c. of colloid injected. Carotid blood clotted in 2 minutes. After 20 c.c. more of the colloid the carotid blood still clotted in 2 minutes. Injection of 12 c.c. more of the colloid caused death, apparently due to respiratory failure with pronounced exophthalmos and dilatation of the pupils as symptoms. Blood throughout the vessels fluid.

Exp. XVI. Brown rabbit. 8 c.c. of 1·25 % solution of soap injected. Before injection carotid blood coagulated in 4 minutes, after 5 c.c. of the soap solution in 6½ minutes 8 c.c. more of the soap was injected, followed by 20 c.c. of 1 % colloid *A*. Death due to respiratory stoppage. Pronounced clots in inferior vena cava. A small clot in the left ventricle and in the hepatic veins. Remainder of blood fluid.

Exp. XVII. 20 c.c. of 1·25 % solution of soap injected into brown rabbit. Death apparently due to respiratory stoppage. All blood remained fluid.

Exp. XVIII. Black rabbit. 10 c.c. of 1·5 % solution of the "colloïde ferrique" (of Grimaux) injected. Complete clotting throughout heart, aorta and larger veins. Pronounced exophthalmos and dilatation of the pupils. The clots were very brittle.

Exp. XIX. Black rabbit. 25 c.c. of 1 % solution of colloid *C* injected. Death with pronounced exophthalmos and dilatation of pupil. Clots in left heart, inferior vena cava, portal, subclavian, jugular and pulmonary veins, aorta empty.

Exp. XX. Brown rabbit. 27 c.c. of substance found by action of PCl_5 on skatole (all H_3PO_4 and HCl being removed by washing). Death apparently due to respiratory stoppage. No clots in blood vessels, but blood rapidly clotted after withdrawal.

Exp. XXI. Albino rabbit. 125 c.c. of 1·5 % solution of colloid *C* injected. Animal showed no change. Killed by ligature of trachea. No intravascular clotting, blood clotted rapidly after withdrawal.

Exp. XXII. Albino rabbit. 140 c.c. of 1·5 % solution colloid *B* injected. Animal killed by asphyxia. No intravascular clotting.

Exp. XXIII. Brown rabbit (small). 28 c.c. of 1·5 % solution of colloid *C* injected. Death with pronounced exophthalmos, and clots in all larger veins and in cavities of heart. (Control Exp. for Exp. XXI.)

Exp. XXIV. Brown rabbit. 30 c.c. of 1·5 % of colloid *B*. Death with pronounced exophthalmos. Clots in left heart, vena cava inferior, jugular, subclavian and portal veins. (Control Exp. for Exp. XXII.)

Exp. XXV. Black rabbit. Sample of carotid blood coagulated two minutes after withdrawal. After 5 c.c. of a 3 % solution of Witte's "peptone" had been injected the carotid blood coagulated 4 min. after withdrawal. After 20 c.c. clotted 10 minutes after withdrawal. 10 c.c. of a 1 % solution of colloid *A* were then injected, the carotid blood on withdrawal clotted after 5 minutes. 10 more c.c. of the colloid were then injected and the carotid blood coagulated 2 minutes after withdrawal. 5 c.c. more of the colloid were fatal—and post-mortem showed clots in left ventricle, jugular, vena cava inf., and portal veins. The remainder of the blood was fluid but coagulated rapidly after withdrawal. There was pronounced exophthalmos.

Exp. XXVI. Brown rabbit. 150 c.c. of 1 % solution of skatole injected. Animal killed by asphyxia, no intravascular clotting.

Exp. XXVII. Repeat with same result.

Exp. XXVIII. Brown rabbit. Solution of colloid *B* that had been kept 15 days used. 120 c.c. injected. Animal killed by asphyxia, no intravascular clotting.

Exp. XXIX. Black rabbit. Repeat experiment with colloid *A*. Same result.

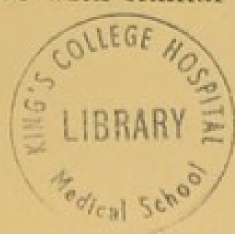
Exp. XXX. Black rabbit (small). Neutralized solution that had stood 16 days of colloid *B*. 130 c.c. injected. Animal killed by asphyxia, no intravascular clotting.

Exp. XXXI. Black rabbit. Similar result with colloid *C* that had stood in solution 18 days. 150 c.c. injected.

Exp. XXXII. Black rabbit. 120 c.c. of colloid *A* that had stood in solution 16 days injected. No coagulation; neutralised solution gave similar result.

Exp. XXXIII. Black rabbit. Alloxan, skatole and biuret heated together with PCl_5; product or products dissolved in ammonia, which was evaporated off and resulting substance dried *in vacuo*. Animal died after injection of 35 c.c., but there was no intravascular clotting.

Exp. XXXIV. Repeat above with similar result.



Exp. XXXV. Black rabbit (small). 65 c.c. of colloid *A* (1 °/₀ solution) injected. Clots in left ventricle, aorta and portal vein. Remainder of blood fluid, but rapidly coagulated on withdrawal.

Exp. XXXVI. Small brown rabbit. 16 c.c. of same solution as used in previous experiment fatal. Clots in left ventricle, portal and inf. vena cava. Aorta and remaining veins contained fluid blood, which coagulated rapidly on withdrawal.

Exp. XXXVII. Frog (anæsthetic ether). Exposed mesentery bathed in 1·5 °/₀ solution of colloid *B*. Slight stasis in larger veins and in capillaries, no disintegration of endothelial wall observed.

Exp. XXXVIII. Frog (anæsthetic ether). Injection of 1·5 °/₀ solution in both dorsal lymph sacs. Web of foot showed neither stasis nor disintegration although also bathed in the colloid.

Exp. XXXIX. Brown rabbit. Substance formed by action of PCl_5 on hippuric acid (the product dissolved in ammonia, evaporated down *in vacuo* and redissolved in distilled water). 30 c.c. injected and fatal, pronounced exophthalmos, but no intravascular clotting.

Exp. XL. Brown rabbit. Mesentery exposed and bathed in 1·5 °/₀ solution of colloid *B*, neither stasis nor disintegration observed.

Exp. XLI. Brown and white dog. Weight 3 kilos. 30 c.c. of 1·5 °/₀ of colloid *A* injected. Death apparently due to respiratory failure, pronounced exophthalmos and dilatation of pupils. Clot in portal and inferior vena cava. Remainder of blood fluid, but coagulated rapidly on withdrawal.

Exp. XLII. Frog's blood placed on slide mixed with equal volume of 1·5 °/₀ solution of colloid *A*. After five minutes red blood corpuscles became rounder and their nuclei more distinct. The white corpuscles apparently were unchanged. After 20 minutes no further change was observed in red corpuscles, but white corpuscles appeared more granular. After 30 minutes no further change, but after an hour had elapsed the white corpuscles had partially disintegrated.

Exp. XLIII. Similar experiment with frog's blood and colloid *B*, same result.

Exp. XLIV. Same result with frog's blood and colloid *C*.

Exp. XLV. Human blood mixed with equal volume of colloid *A*. After five minutes' action the red corpuscles appear slightly swollen and the white corpuscles more granular than the normal. After 20 minutes no further change was observed, but disintegration of the white corpuscles commenced after 30 minutes and was complete in 40 minutes. The blood was throughout the experiment kept at about 37° C.

Exp. XLVI. Similar result with human blood and colloid *B*.

Exp. XLVII. Similar result with human blood and colloid *C*.

Exps. XLVIII., XLIX. and L. gave similar results with rabbit's blood and the colloids *A*, *B* and *C*. The disintegration of the white corpuscles commencing in from 15 to 20 minutes after mixture with equal volumes of a 1·5 % solution and being complete in from 30 to 40 minutes.

Exp. LI. Fox terrier (weight 14¾ lbs.). Carotid blood before injection coagulated in four minutes. 10 c.c. of a 1 % solution of colloid *A* was injected, after which carotid blood coagulated in four minutes. There was temporary cessation of breathing. 10·5 c.c. more of colloid injected and carotid blood coagulated nine minutes after withdrawal. After two minutes animal died, apparently owing to respiratory failure. Post-mortem showed clot in right subclavian, very large and firm clots in right heart, in portal, inferior vena cava and pulmonary veins. Remainder of blood fluid, but coagulated rapidly after shedding. Anæsthetic mixture of ether and chloroform.

Exp. LII. Small mongrel (weight 8 kilos). Carotid blood before injection coagulated in 3 minutes. After injection of 10 c.c. of ·75 % colloid *A* in 3 minutes. Temporary cessation of breathing after injection. After 20 c.c. more carotid blood coagulated in four minutes. Killed by asphyxia. Clot in portal and small clot in inferior vena cava. Remainder of blood fluid, but coagulated in 3 to 4 minutes. Anæsthetic ether.

Exp. LIII. Large brown rat. 20 c.c. of 1 % colloid *A* injected. Respiratory failure and death. Clots in both ventricles, in inferior vena cava and portal. Remainder of blood fluid, but coagulated rapidly on withdrawal.

Exp. LIV. Large pug dog, weight 18lbs. 8oz. ·5 % solution of colloid *A* used. Samples of blood drawn from carotid.

No. of sample of blood.	No. of c.c. injected into circulation at time of taking sample.	Time of commencing of sample to clot.	Time of completion of coagulation.	Remarks as to condition of animal.
1	none	3½ min.	5½ min.	Normal.
2	5	5½ ,,	9 ,,	,,
3	10	3½ ,,	8 ,,	,,
4	15	6 ,,	9 ,,	Dyspnœa.
5	25	1½ ,,	2 ,,	Pronounced dyspnœa.
6	35	1½ ,,	2 ,,	Temporary stoppage of respiration, resumption after interval of 30 sec.
7	35	1½ ,,	2 ,,	Respiration irregular.
8	45	1½ ,,	2 ,,	Stoppage of breathing for 30 sec.
9	45	1 ,,	2 ,,	Intermittent respirations, struggles and stretching movements. Breathing stopped and resumed after 2 min.

After a few minutes the animal died. A sample of blood withdrawn by a

hypodermic syringe from the right ventricle completely clotted in 1 min. 30 sec. and another from the left ventricle in 30 sec. P.m. showed a pronounced clot in right auricle and along the length of the aorta. The remainder of the blood was fluid but coagulated rapidly on withdrawal.

EXP. LV. Guinea-pig. 11 c.c. of a 1 °/₀ solution of colloid *A* injected. Death due to sudden stoppage of respiration. P.m. showed clots in inferior vena cava, and pulmonary veins and a very large clot in the portal vein.

EXP. LVI. White rabbit (with small black patches on back). 20 c.c. of colloid *A* fatal, with extreme dilatation of pupil and exophthalmos. Small clot in aorta, and inferior vena cava. Remainder of fluid clotted rapidly on withdrawal.

EXP. LVII. Cat. 15 c.c. of a 1 °/₀ colloid *A* injected. Sudden respiratory stoppage, exophthalmos and dilatation of pupils. P.m. clot in aorta and vena azygos. Remainder of blood fluid but clotted rapidly on withdrawal.

EXP. LVIII. Black rabbit (6 lbs. 5 oz.). 50 c.c. of 1 °/₀ solution of colloid *A* there had been precipitated by excess of acetic acid and redissolved in ½ °/₀ sod. carbonate. Typical symptoms. Clots in portal, left heart, inferior vena cava.

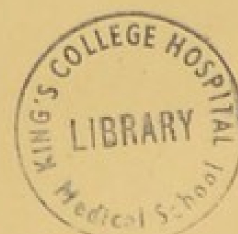
EXP. LIX. Black rabbit (8 lbs. 9 oz.). 53 c.c. 1 °/₀ solution of above injected. Typical results. Clots in portal, left auricle and ventricle, inferior vena cava.

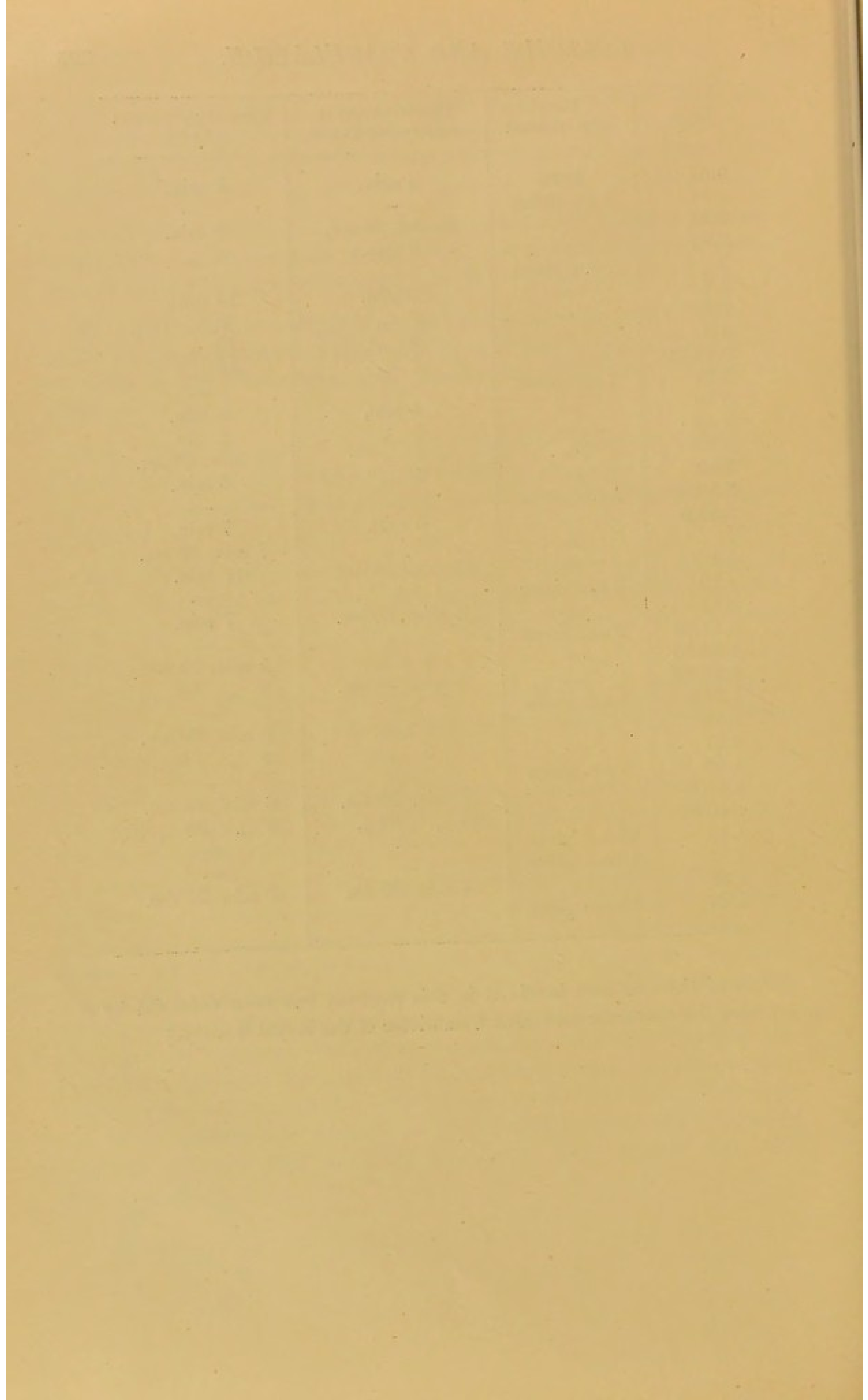
EXP. LX. Large black dog (weight 30 lbs.). Anæsthetics used were ether, chloroform and morphia. Samples of blood taken from carotid. A 5 °/₀ solution of colloid *A* injected.

At 4.44 after 8 c.c. had been injected there was temporary stoppage of respiration, at 4·51 after 38 c.c. had been injected there was a prolonged respiratory stoppage accompanied by exophthalmos, stretching movements and spasms. At 4·57 hypernœa ensued, while the exophthalmos and stretching movements became more pronounced. At 5 o'clock 10 more c.c. were injected, which resulted in extreme stretching movements and prolonged respiratory stoppage. A temporary resumption of breathing occurred, followed by final respiratory stoppage. Post-mortem showed a large clot in right auricle, small clot in right ventricle, blood in left heart very viscid but fluid. Very large clot in the inferior vena cava and aorta, and smaller clots in the pulmonary veins and arteries. The remaining fluid blood clotted rapidly after withdrawal.

Time.	Dose administered	Time occupied in commencing to clot.	Time of completion of clot.
2.51	none	4 min.	7 min.
2 54	1 c.c. given	—	—
2.56	—	6 min. 30 sec.	9 min.
2.59	—	6 min.	8 „
3	1 c.c. given	—	—
3.2	—	8 min.	10 min.
3.5	—	7 „	8 „
3.9	—	9 „	10 „
3.18.30	—	8 „	10 „
3.27	1 c.c. given	—	—
3.30	—	4 min.	5 min.
3.32	—	7 „	8 „
3.40	—	7 „	8 min. 30 sec.
3.50	—	5 „	6 min.
3.50.30	1 c.c. given	—	—
3.53.0	—	6 min.	7 min.
4	—	6 „	7 min. 30 sec.
4.4	—	5 min. 30 sec.	7 min.
4.10	2 c.c. given	—	—
4.13	—	5 min. 30 sec.	7 min.
4.21.30	2 c.c. given	—	—
4.25.30	—	5 min.	5 min. 30 sec.
4.31.30	—	5 min. 30 sec.	6 „ 30 „
4.35.0	5 c.c. given	—	—
4.36	—	5 min.	5 min. 30 sec.
4.40	—	5 „	6 „ 30 „
4.40	5 c.c. given	—	—
4.41.30	—	5 min. 30 sec.	6 min. 30 sec.
4.46.30	—	4 „ 30 „	5 „ 30 „
4.48	10 c.c. given	—	—
4.51	10 c.c. given	—	—
4.53	—	4 min. 30 sec.	5 min. 30 sec.
4.55	15 c.c. given		

[Part of the expenses involved in this research has been defrayed by a grant from the Government Grant Committee of the Royal Society.]





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NUCLEO-PROTEIDS. Supplementary Paper. By W. D. HALLIBURTON, M.D., F.R.S., *Professor of Physiology, King's College, London.*

In a paper recently published in this *Journal*[1] by T. Gregor Brodie and myself, attention was called to the preparation and properties of nucleo-albumins[2], and especially to the part played by these substances in the causation of blood-coagulation. A reconsideration of our experiments and conclusions having shown me that several points still required further elucidation, I have just concluded a fresh series of experiments. I have to gratefully acknowledge valuable assistance from Dr Brodie, and also from Mr P. C. Colls, Assistant Demonstrator of Physiology at King's College.

The subjects of which I have to treat are somewhat various, and may be best arranged under the following heads:—

1. The preparation of nucleo-proteids.
2. The nucleo-proteid of red marrow.
3. The stromata of the red corpuscles.
4. Cell-globulins.
5. Schmidt's fibrin ferment.
6. Summary.

1. The Preparation of Nucleo-proteids.

The first point I wish to consider is a theoretical one. In the paper just referred to the following sentence occurs[3]:—"We therefore conclude that while repeated alternate treatment with sodium chloride and water (our method of obtaining the material), and repeated alternate treatment with acetic acid and sodium carbonate (Wooldridge's method of obtaining the material), both destroy the physiological activity of nucleo-albumin, and both alter the composition of the material, the alteration is different in the two cases; the former method causing a loss of nuclein (as indicated by a fall in the percentage of phosphorus);

[1] Vol. xvii. 135—173. 1894. Here references to previous literature will be found.

[2] Through this paper I shall use the term nucleo-proteid instead of nucleo-albumin. This term originally suggested by Hammarsten (*Zeitsch. f. physiol. Chem.* xix. p. 37) has already been adopted by Pekelharing (*Centr. f. Physiol.* ix. 102).

[3] p. 155.

the latter causing a loss of proteid, and consequently a rise in the proportion of nuclein (as indicated by a rise in the percentage of phosphorus)."

This result somewhat surprised us, for if the action of sodium chloride and water is to displace nuclein, we expected that the same action would be produced by acid even more readily, since the essential constituent of nuclein is a weak organic acid called nucleic acid. It has, however, since occurred to us that the action of acetic acid is probably after all the same as that produced by repetition of the sodium chloride method, and that the rise in the percentage of phosphorus does not really indicate that the chemical decomposition is different. We are now inclined to regard it as extremely probable that the chemical decomposition is in the two cases identical, namely, a cleavage more or less complete, of the nucleo-proteid into its two constituents nuclein (nucleic acid) and proteid. As the sodium chloride process is repeated, more and more of the nuclein passes into solution and so is lost, whereas the proteid, the solubilities of which resemble those of a globulin, is repeatedly being subjected to a process which tends to keep it precipitated. No doubt some of the proteid is lost as well, but the results of analysis indicate that a greater relative proportion of the nuclein disappears. In the other case, treatment with acid and alkali will lead to a continual loss of the proteid by conversion into soluble acid- and alkali-albuminates, whereas the nuclein remains precipitated by each addition of acid.

2. The Nucleo-proteid of Red Marrow.

The proteids of red marrow were investigated by J. R. Forrest[1] in this laboratory. The most important of these was found to be a nucleo-proteid. Forrest's research was incomplete, as he did not make any estimations of the amount of phosphorus present in the nucleo-proteid. This omission I have now filled up.

The nucleo-proteid was prepared from horse's ribs by Wooldridge's method. This method was selected, because in preliminary experiments in which the sodium chloride process was employed it was found extremely difficult to prevent mechanical admixture with minute spicules of bone, the calcium phosphate from which was not dissolved out by the subsequent washing with dilute acid.

Twelve ribs taken from one horse gave only a scanty yield of nucleo-proteid; the horse was an old one, and the marrow in the ribs

[1] This *Journal*, xvii. 174. 1894.

was largely of the yellow variety. In the case of two other horses, both younger and more vigorous, the material yielded from a dozen ribs was plentiful.

The nucleo-proteid from the three horses was mixed, and the product thoroughly washed, and subjected to the process for estimating phosphorus already described[1]. The two estimations made agree very well. The result was as follows:—

Weight of nucleo-proteid taken	Yield of magnesium pyrophosphate	Percentage of phosphorus		Percentage of ash
		in material	in ash-free material	
A 0·918 gramme	0·0552	1·6793	1·7581	
B 1·049 „	0·0560	1·4909	1·5608	
Mean of A and B		1·5851	1·6594	
C				4·481

If now we compare these results with those previously published[2] we get the following table:—

Nucleo-proteid from	thymus	contains	0·8 %	of phosphorus.	
„ „	kidney	„	0·37	„	„
„ „	liver	„	1·45	„	„
„ „	brain	„	0·5	„	„
„ „	red marrow	„	1·6	„	„

Here we see that the nucleo-proteid of red marrow contains the highest percentage of any.

3. The Stromata of Red Corpuscles.

This result led me once more to take up the question of the red corpuscles, which have their origin in the red marrow. Some years ago Dr W. M. Friend and myself published a paper on this subject[3]; our main conclusion was that the principal proteid obtainable from the stromata was one identical with that termed cell-globulin obtained from lymphoid structures; and that nucleo-proteid was absent. This conclusion was arrived at, during a stage in my researches in which one's knowledge regarding the properties and preparation of nucleo-proteids was much more scanty than it is now; and on looking through my notes of these experiments I found evidence that the question would bear a fresh investigation. The main result of these new experiments is that the so-called cell-globulin obtained from the stromata is really a nucleo-proteid.

[1] This *Journal*, xvii. 152. 1894. [2] *Ibid.* p. 142.

[3] The Stromata of the Red Corpuscles. This *Journal*, x. 532–549. 1889.

Pekelharing was the first to discover my mistake, and I now propose to give an account of the experiments which I have myself performed on this subject.

The animals I have used have been cats and rabbits, principally the former. The animal having been anæsthetised with a mixture of chloroform and ether, the jugular vein of one side, and the carotid artery of the other was exposed, and a cannula inserted into each. A few c.c. of a solution of Witte's peptone was then injected into the jugular vein, the amount varying with the size of the animal[1]. The object of this procedure was to cause the disappearance of leucocytes, so that when the blood was subsequently collected it was almost free from these elements[2]. The blood was collected from the carotid artery and whipped. Clotting was found to be only slightly delayed by the previous injection of 'peptone.' The whipped blood was diluted with a large volume of normal saline solution, and the red corpuscles allowed to settle. The supernatant liquid was after a day or two siphoned off and the red corpuscles thoroughly washed with salt solution, the centrifugal machine being employed to hasten the process[3].

The mass of red corpuscles was then dissolved in water, and a few drops of ether added to complete the laking. Then a few drops of sodium bisulphate (1 °/₀ solution) were added and the stromata so precipitated were collected in a filter, and thoroughly and rapidly washed (a filter pump being used) with water. The precipitate was placed under alcohol for subsequent analysis; the material so obtained from two rabbits in one vessel, and from five cats in another was then thoroughly washed with hot and cold alcohol and ether to remove lecithin, protagon, fat, and cholesterin, and with acidulated water to remove inorganic phosphates. The residue was dried at 110° C. divided into separate portions for analysis and weighed.

In other cases the stromata were dissolved in 1 °/₀ sodium carbonate, and the solution filtered and injected into the circulation of living animals to test its physiological activity.

Some of the material was subjected to artificial gastric digestion, and found to yield a residue of nuclein. Phosphorus estimation led to a positive result; the numbers obtained will be given immediately. Injection of the sodium carbonate solution produced intra-vascular coagulation in rabbits; details will be given immediately.

[1] The amount given was 0·2 gr. per kilo. of body weight.

[2] See Wright. *Proc. Royal Society*, LII. 564. 1893. Bruce, *ibid.* LV. 295. 1894.

[3] This was the method used in the winter. In the summer when rapidity of work was essential the centrifugal machine was employed throughout.

In other words the stromata contain nucleo-proteid similar to that obtained from other tissues and organs.

The method just described for the preparation of the stromata was the one formerly used by Dr Friend and myself and originally employed by Wooldridge[1].

I considered it necessary next to see whether the result could be accounted for in any other way than that just stated; the nucleo-proteid could not have originated from the few white corpuscles remaining after the injection of 'peptone'; its amount was too great. It could not have come from the 'peptone' injected; for though the Witte's peptone used did contain a trace of phosphorus the amount injected was extremely small, and the subsequent washing of the red corpuscles with salt solution very thorough. Moreover it could not have originated from the small amount of hæmoglobin with which the stromata remained contaminated after washing, for hæmoglobin in mammals contains no phosphorus.

I then tried whether nucleo-proteid can be prepared from the red corpuscles by the usual methods; the answer was found to be in the affirmative. Wooldridge's method (precipitation of an aqueous solution of the corpuscles by dilute acetic acid) gave a small yield. The sodium chloride method was very successful, and an abundant white preparation of nucleo-proteid is easily obtained, provided that the previous centrifugalisation is so efficiently performed that the corpuscles form an adherent mass. If the centrifugal machine is not efficient enough for this, the sodium chloride process generally leads to failure, as it did in the previous experiments performed by Dr Friend and myself. Some of the nucleo-proteid prepared by this method was dissolved in 1 $^0/_0$ solution of sodium carbonate for injection experiments, some was subjected to artificial gastric digestion with the result that a residue of nuclein occurred, and the rest (from seven cats) was collected under alcohol, washed as before described, dried, weighed, and subjected to analysis.

Injection experiments. Wooldridge[2] was the first to show that the stromata of the red corpuscles contain a substance which produces intravascular clotting. The following brief protocols of the experiments I have performed confirm this statement. The experiments were made on rabbits anæsthetised with ether. If the injection did not kill the animal, it was killed by ligaturing the trachea. The method of the experiment was exactly the same as previously described by Brodie and myself[3].

[1] "Zur Chemie der Blutkörperchen." *Du Bois Reymond's Archiv*, p. 387. 1881.

[2] *Chemistry of the Blood*, p. 137. [3] *loc. cit.*

Rabbit 1. 15 c.c. of 1 % sodium carbonate solution of nucleo-proteid prepared by the sodium chloride method from cats' red blood-corpuscles. This small quantity (all that was available) did not kill. On killing the R. by asphyxia, no intra-vascular coagulation was found, but the blood was extremely viscid, and clotted rapidly on removal.

R. 2. 40 c.c of 1 % sodium carbonate solution of stromata prepared by Wooldridge's sodium bisulphate method injected. This killed the animal. The blood was extraordinarily viscid, and in some vessels it was difficult to say if it had clotted or not. The blood would hardly flow, but when it did it clotted instantaneously and firmly. A well-marked clot was found in the inferior vena cava, the most frequent situation for clotting.

R. 3. Similar experiment to R. 1. 40 c.c. fatal. Result same as in R. 1.

R. 4. ditto. 15 c.c. only available. R. killed by asphyxia. Same result.

R. 5. ditto. 30 c.c. injected. Fatal. Blood viscid throughout; clotting within right jugular, and inferior vena cava above entrance of hepatic veins, and in the hepatic veins themselves.

R. 6. ditto. 12 c.c. injected. R. killed by asphyxia. Blood viscid and adherent to vessel walls. Distinct clots in subclavian veins and in aorta.

Results of analysis. The following table gives the results of phosphorus estimation in the preparations already alluded to:—

Material	Weight in grammes taken for analysis	Yield of magnesium pyrophosphate	Percentage of phosphorus in material	Percentage of phosphorus in ash-free material	Percentage of ash
Rabbit's stromata washed free from lecithin, etc. Prepared by Wooldridge's method					
A	0·155	0·00599	1·0793	1·1609	
B					7·027
Cat's stromata ditto					
A	0·463	0·01069	0·64482	0·65278	
B	0·2745	0·00719	0·72984	0·73885	
Mean of A & B			0·68733	0·69581	
C					1·22
Nucleo-proteid prepared by NaCl method from cat's red blood-corpuscles					
A	0·241	0·00559	0·64779	0·66803	
B					3·03

I am not inclined to place much reliance on the results from the rabbits' stromata; the quantity available was only sufficient for a single analysis and was even then so small that minute experimental errors would tell largely in the final result. The close agreement of the three remaining analyses is very satisfactory.

4. Cell-globulins.

The term cell-globulin was originally introduced by me as a convenient designation for certain proteids obtainable from the cells of lymphoid structures like lymphatic glands and thymus. In my papers on the subject[1] I recognised the existence of a nucleo-proteid which became viscid on admixture with strong solutions of sodium chloride or magnesium sulphate. An extract made with sodium sulphate did not however exhibit the same viscidity, and contained two proteids, one of which coagulated at about 50° C., the other at 75° C. The first I called cell-globulin α; the second cell-globulin β. Cell-globulin α is really a globulin[2]. Cell-globulin β is like a globulin in its solubilities, but as Pekelharing pointed out is a nucleo-proteid. This I admitted in the paper just quoted (p. 846). My words were "the proteid which I formerly called cell-globulin β is a nucleo-albumin. Whether it is a different one from that already mentioned (i.e. the nucleo-proteid which can be prepared by Wooldridge's acetic acid method, or my sodium chloride process), or part of the same in a somewhat modified form must be the subject of renewed research. I assume for the present that they are identical."

The necessity for renewed research on this point was pressed home when I had found that the cell-globulin of the stromata of red corpuscles is also a nucleo-proteid.

The cell-globulins were extracted from a calf's thymus by a five per cent. solution of sodium sulphate. The extraction was twice repeated with large quantities of the same solution. The residue was then taken, and divided into two parts. From one of these parts an attempt was made to prepare nucleo-proteid by the sodium chloride process; from the other by Wooldridge's method. The yield in each case was very scanty. The converse experiment gave corresponding results; after nucleo-proteid had been removed by either method, attempts to prepare it by another were unsuccessful or only yielded minimal quantities. This was strong evidence that it is the same nucleo-proteid

[1] *Proc. Roy. Soc.* XLIV. (1888), p. 255; this *Journal*, vol. IX. 229. 1888.

[2] "Proteids of Kidney and Liver Cells"; this *Journal*, XIII. 806. 1892.

with which one has to deal in the three cases, though the method of preparation involves certain physical differences in the product.

I sought to confirm this by the method of phosphorus estimation. The extracts just referred to were slightly acidified and heated to 50° C. and the cell-globulin α thus precipitated was filtered off. After washing the precipitate and performing the analysis in the usual way, phosphorus was found to be absent in all but unponderable traces. The filtrate was heated to 80°, a small amount more of acetic acid being added, and the precipitated cell-globulin β was collected, washed, dried, weighed and subjected to analysis. A second supply was mixed with this from another thymus. The results of the analysis were as follows:—

Weight in grammes of cell-globulin β taken for analysis	Yield of magnesium pyrophosphate	Percentage of phosphorus		Percentage of ash
		in material	in ash-free material	
A 0·98	0·06819	1·9433	2·0351	
B 1·238	0·06969	1·5721	1·6464	
Mean of A and B		1·7577	1·8407	
C				4·511

Let us next compare these results with those previously published as the result of the analysis of the thymus nucleo-albumins[1]. This is shown in the following table:—

Nucleo-albumin from thymus.

	Mode of preparation	Percentage of phosphorus
1.	Sodium chloride method	0·8
2.	The same method, only repeated several times	0·006 to 0·38
3.	Acetic acid method	0·8
4.	The same method, only repeated several times	1·1 to 1·32
5.	The present method; the substance prepared being that previously termed cell-globulin β	1·7

From this table we conclude that the percentage of phosphorus in the material prepared by the two first methods is very close; compare analyses 1 and 3. The table also illustrates the gradual loss of phosphorus, occasioned by the repetition of the sodium chloride process (analysis 2), and the rise occasioned by the repetition of the acetic acid method (analysis 4). Analysis 5 appears to be discordant for it is not

[1] Halliburton and Brodie, *loc. cit.* pp. 141, 153, 155.

identical with any of the preceding; it is however nearest to analysis 4, and consideration will show that this is what one would expect; the rise in the percentage of phosphorus in analysis 4 has already been explained (Section 1 of the present paper) as due to cleavage of the nucleo-proteid by the acid in the cold and loss of proteid by conversion into acid-albumin. This would occur to even a greater extent in an acid liquid at 80° C., which was the very severe treatment necessary for the isolation of the substance in the condition in which it was previously called cell-globulin β.

The results of phosphorus estimation though at first sight disappointing really confirm in a very striking way the opinion hitherto expressed, that in all cases one is dealing with the same material.

5. Schmidt's Fibrin-ferment.

Cell-globulin β being a nucleo-proteid, it next became necessary to investigate the question whether the fibrin-ferment, which I formerly considered to be identical with cell-globulin β, is also a nucleo-proteid. Pekelharing considers that it is; he looks upon nucleo-proteid as being the zymogen of fibrin-ferment, and the fibrin-ferment itself as being a calcium compound of the nucleo-proteid. Lilienfeld[1], who has also recently taken up this matter, thinks on the other hand that fibrin-ferment is the product, not the cause of blood coagulation, and confirms my original position that it is a globulin, free from phosphorus.

In the paper already cited by Brodie and myself, we adopted a cautious attitude and were unable from the evidence before us to accept Pekelharing's inclusion of the fibrin-ferment among the nucleo-proteids. The differences between nucleo-proteids and the ferment appeared to be sufficiently striking to warrant the inference that they are different substances. We enumerated the principal differences between them as follows:—

1. Fibrin-ferment is not readily coagulated by alcohol; nucleo-albumin is.

2. Fibrin-ferment causes coagulation in extra-vascular (salted) plasma; nucleo-albumin does not.

3. Nucleo-albumin causes coagulation in intra-vascular plasma; fibrin-ferment does not.

Such a statement, however, did not finally settle the question, but suggested fresh work, and a preliminary communication was made to

[1] *Zeitsch. f. physiol. Chem.* xx. 163. 1894.

the Physiological Society in the early part of this year[1]. Our criticism also has produced a fresh paper from Pekelharing[2] in which he confirms with fresh experiments his original statements. I am glad to be able to anounce that my own experiments, and the fresh ones recorded by Pekelharing are in substantial agreement, and that working independently we have removed some of the difficulties surrounding this question, and that we may now safely include the fibrin-ferment among the nucleo-proteids.

Pekelharing's recent paper, so far as it relates to my work and criticisms, may be briefly summarised as follows:—

1. Fibrin-ferment yields on gastric digestion, an insoluble residue of nuclein.

2. The difference in the behaviour of alcohol to fibrin-ferment and nucleo-proteid is only a matter of degree. I am perfectly willing to admit that the difference is not so important as the two others just mentioned, and in fact reference to my previous papers will show that I have always insisted that the difference is not one of kind. The fact that Pekelharing has worked chiefly with one kind of nucleo-proteid (from blood) and I with the nucleo-proteids from various tissues still further explains any differences between us on this head. He also states that he has obtained fibrin-ferment by Schmidt's method from nucleo-proteid, and thus his answer to this objection is fairly complete.

3. Fibrin-ferment causes coagulation in extra-vascular (salted) plasma; nucleo-proteid does not. This is a more serious difference, and Pekelharing admits that dilute salted plasma does not coagulate on the addition of nucleo-proteid (unless calcium chloride is added as well) but it does coagulate on the addition of fibrin-ferment. If now the ferment is a calcium compound of nucleo-proteid, why does this compound not form in extra-vascular plasma when nucleo-albumin is added, for the amount of calcium salt present is presumably the same as in intra-vascular plasma? This question which I asked is answered by Pekelharing as follows:—That the magnesium sulphate present in the salted plasma prevents or hinders the union of calcium and nucleo-proteid, and he quotes some very striking experiments in which he shows that in a mixture of fibrinogen and nucleo-proteid, it is necessary to add more calcium chloride in order to bring about coagulation when magnesium sulphate is present as well, than when it is absent.

4. His answer to my last objection appears equally satisfactory; I

[1] *Proc. physiol. Soc.* Feb. 16, 1895. See also Schäfer, *ibid.* March 16, 1895.

[2] *Centralbl. f. Physiol.* IX. 102. 1895.

ask why fibrin-ferment does not produce intra-vascular coagulation? He replies because the use of alcohol in the preparation of fibrin-ferment renders the material so insoluble that only a very weak solution is obtainable, and that this, like all weak solutions of nucleo-proteids, produces not intra-vascular clotting, but the reverse, namely, a hindering influence on coagulation (Wooldridge's negative phase). I can now proceed to the description of my own experiments, most of which were completed before the appearance of Pekelharing's paper, but some of which were suggested by his very interesting replies to my criticisms.

In the first place I sought to ascertain by the usual process of gastric digestion whether fibrin-ferment prepared by Schmidt's method from serum is a nucleo-proteid. Finding that it gave like other nucleo-proteids an insoluble residue of nuclein, I proceeded to supplement this by estimations of phosphorus (see Experiments 1 to 5). I further investigated the action of nucleo-proteid on extra-vascular plasma without the addition of extraneous substances like magnesium sulphate, and found that the accelerating influence on the rate of coagulation was very marked (Exp. 7); this entirely confirms Pekelharing's contention that salted plasma is not a fair test plasma. Lastly, I proceeded to repeat Pekelharing's experiment in which he found that injection of fibrin-ferment into the circulation produces Wooldridge's negative phase. This is such an important point that I did not like to accept it without first verifying it for myself. The single experiment I have done entirely confirms Pekelharing's observation. (Exp. 6.)

The details of the experiments are as follows:—

EXPERIMENT 1. 10 litres of ox serum were mixed with many times their volume of alcohol, and allowed to stand for six weeks. The precipitate was collected, dried at 40° C. and powdered. A small quantity of the powder was extracted with water, and its activity tested on rabbit's sodium sulphate plasma. The diluted plasma coagulated on addition of the ferment solution in four minutes; without such addition, it did not coagulate for two hours.

EXP. 2. The remainder of the powder was thoroughly extracted with distilled water. The collected aqueous extracts amounted to about 50 litres. This was concentrated on a water bath to a small bulk. The concentrated liquid was slightly alkaline; it coagulated on heating after faint acidification at about 75° C., and in another portion the proteid in it was found to be entirely precipitable by saturation with magnesium sulphate. This confirmed my earlier statement that the solubilities of the proteid present in solutions of fibrin-ferment are those of a globulin.

EXP. 3. The remainder of the concentrated extract was mixed with

about six times its volume of alcohol, a little acid being added to assist precipitation. The precipitate was collected, washed free from lecithin, fat, protagon, cholesterin, and inorganic phosphates in the usual way, dried, divided into portions for analysis, and weighed. The total weight of the product from about 10 litres of serum was nearly 1·8 grammes.

Exp. 4. Some of this powder subjected to artificial gastric digestion, gave an insoluble residue of nuclein.

Exp. 5. The results of phosphorus and ash determinations made in the usual way gave the following results:—

Weight of material taken for analysis	Yield of magnesium pyrophosphate	Percentage of phosphorus in material	Percentage of phosphorus in ash-free material	Percentage of ash
A 0·5475	0·02299	1·1727	1·2692	
B 0·6898	0·02819	1·1413	1·2352	
Mean of A and B		1·157	1·2522	
C				7·6

The ash (the high percentage of which is very noticeable) resembled in composition that previously described in nucleo-proteids [1], but differed in that it was very rich in calcium; this confirms Pekelharing's contention that fibrin-ferment is a calcium compound of nucleo-proteid.

Exp. 6. A solution of fibrin-ferment was prepared by the usual method from another quantity of ox serum; on salted plasma it was found to be active in promoting coagulation.

A dog was anæsthetised with ether and morphia; the jugular vein of one side and the carotid artery of the other were exposed, and cannulæ inserted into them. The fibrin-ferment solution was injected into the vein, and samples of blood withdrawn from the artery. The following table gives the rate of clotting in various specimens.

i. Before injection of fibrin-ferment, normal blood clotted firmly in 9·5 minutes.

ii. 10 c.c. of fibrin-ferment solution run in. A loose clot formed in 55 minutes.

iii. 10 c.c. more run in. Loose clot in 87 minutes.

iv. 20 c.c. more run in. Two specimens of blood withdrawn within an interval of 3 minutes. The first clotted firmly in 10, the second in 10·5 minutes.

v. 20 c.c. more run in. Blood clotted firmly in 14 minutes.

vi. 30 c.c. more run in. Blood clotted firmly in 9·5 minutes.

vii. 50 c.c. more run in. Blood clotted firmly in 4 minutes.

viii. 50 c.c. more run in. Blood clotted firmly in 4 minutes.

This experiment shows the negative phase after the injection of a small

[1] Halliburton and Brodie, *loc. cit.* p. 154.

quantity of fibrin-ferment. Injection of more hastened the rate of coagulation very considerably. The total amount injected, however, produced no symptoms and no intra-vascular coagulation. The dog was finally killed by asphyxia.

Exp. 7. This experiment was also performed on a dog, anæsthetised in the same way. Nucleo-proteid was prepared from cat's kidneys by the sodium chloride process, dissolved in 1 per cent. solution of sodium carbonate and filtered. The blood of a dog collected from the carotid artery was mixed with this solution, and in a control experiment with the corresponding volume of 1 per cent. solution of sodium carbonate. The specimens were watched for two hours, and the expression no clot in the following table means that no coagulation occurred during that time. The following were the results obtained.

	Proportion of blood to solution	Solution of 1 °/₀ sodium carbonate (control specimens)	Solution of nucleo-proteid in 1 °/₀ sodium carbonate
i	Equal parts	no clot	no clot
ii	Blood + $\frac{1}{3}$ its volume of solution	no clot	clot in 10 minutes
iii	Blood + $\frac{1}{4}$ its volume of solution	clot in 19 minutes	clot in 5 ,,
iv	Blood + a few drops of solution	clot in 25 ,,	clot in 2 ,,
v	Ditto	clot in 17 ,,	clot in 2 ,,

This experiment shows the marked accelerating influence that nucleo-proteid has on the extra-vascular blood especially when added in small quantities; if a large volume of the solution is added (as in i) the influence of the diluting solution inhibits the action of the nucleo-proteid.

6. Summary.

The principal points to which this paper calls attention are:—

1. That the proteid formerly called cell-globulin β whether it is obtained from lymphoid structures like thymus, or from the stromata of the red corpuscles is a nucleo-proteid.

2. Schmidt's fibrin-ferment comes also under the same category. Those interested in the coagulation question will find in section 5 of this paper a review of Pekelharing's most recent contribution to the controversy, and an account of those experiments I have performed which bear out in a striking way his principal contentions.

3. Section 1 relates to a theoretical subject in relation to the mode of preparation of nucleo-proteids; and in section 2 will be found analytical details showing the high percentage of phosphorus in the nucleo-proteid of red marrow.

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FURTHER EXPERIMENTS ON THE EMBRYONIC HEART. By J. W. PICKERING, D.Sc. (Lond.), *George Henry Lewes Student.*

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My attention has been directed to certain critical remarks written by Dr W. His[1] on my paper published in This *Journal*[2] on the Embryonic Heart; and consequently I have repeated some of my observations, and made further ones in order to throw light on the questions raised. Dr His thinks that some of the variations of cardiac rhythm, which I have recorded as resulting from the action of drugs, may possibly be due to variations of the temperature of the embryos during the experiments, and he calls especial attention to the experiments with ammonia.

In answer to this suggestion, I must point out that precautions for the maintenance of a constant temperature during the experiments were taken[3]; and all the observations published were made in an incubator enclosed on five sides by a water-jacket and roofed in by glass. A thermometer inside the incubator indicated the temperature at which the experiments were conducted.

I have repeated the experiments on ammonia, taking additional precautions for the maintenance of a constant temperature throughout the experiments. The incubator was heated by a small gas flame controlled by a gas-regulator and five of the sides of the incubator were covered by a jacket of silicate of "cotton" to reduce heat radiation to a minimum. Embryo-chicks aged 75 hours were used, and the temperature remained constant during the observations. The following is an account of a typical experiment selected from a number.

Embryo under conditions described above. The normal frequency of the embryo's cardiac rhythm at the temp. of 38° C. was found to be constant at 60 beats per minute during the half-hour immediately preceding the time when the observations were recorded.

[1] His. *Centralblatt für Physiologie*, VIII. 11. 1894.

[2] Pickering. This *Journal*, XIV. 383. 1893. See also *Proc. Roy. Soc.* 52. 461; *Trans. Odont. Soc. of Great Brit.* XXVI. 42, and This *Journal*, XVII. 495. 1895.

[3] Under the conditions of the experiments, the cardiac rhythm of embryos kept at 38° C. was constant for four hours.

12.0.0 1 c.c. of normal saline (·65 °/₀ NaCl) containing ·00075 of a c.c. of concentrated liquor ammoniae directly applied to the embryo's heart.

12.1.0 No change observed in the cardiac rhythm.

12.2 to 12.15 " "

12.16 A second c.c. containing ·00075 of a c.c. of ammonia given.

12.17 to 30 No change observed in cardiac rhythm.

12.30.30 A c.c. of normal saline containing ·001 of a c.c. of ammonia given.

12.31.30 Cardiac frequency fell to 58 beats per minute.

12.32 to 12.36 Frequency constant at 56 beats per minute.

12.37.0 Another c.c. containing ·001 of c.c. of ammonia given.

12.38.30 Frequency rose to 75 beats per minute.

12.40.0 Frequency 80 per minute, beats very strong.

12.42.0 Frequency 86 per minute " "

12.45.0 Frequency 86 " " "

12.48.0 Frequency 86 " " "

12.48 to 1.10 The frequency was maintained at 86 beats per minute and the beating was very strong.

In my former work (loc. cit. pp. 436--7), in the experiment cited on the action of ammonia, similar doses at a temperature of 38° C. gave an increase of the embryo's cardiac frequency of 30 beats per minute, in the experiment recorded above the increase was 26 beats per minute; the stimulant result of the ammonia being typically shown in both cases.

I also emphasised the influence of variations of temperature on the action of chemical stimuli on the embryonic heart and in the experiment alluded to I recorded the effect of raising the temperature to 42° C. while in the case of my observations on the action of morphine acetate, barium chloride, strychnine and veratrine special stress was laid on the importance of the temperature of administration as affecting the results obtained.

The effect of variations of temperature on the physiological action of a drug on the embryonic heart is well illustrated by the following experiments (selected from a number) where alcohol was the chemical stimulus used.

Exp. I. Embryo-chick aged 75 hours, observed with precautions previously mentioned. Temperature throughout the experiment 20° C. The constant frequency of the embryo's cardiac rhythm at this temperature was 60 beats per minute.

Time	Dose administered	Cardiac frequency	Remarks
10.30	·0001 of a c.c. of pure alcohol dissolved in 1 c.c. of normal saline	60	Condition normal
10.31.30	—	64	do.
10.33	—	58	Beats slightly weaker
10.34	—	54	do.
10.35	—	50	do.
10.38	—	46	No change noted
10.40.0	—	44	do.
10.40.30	Second c.c. containing ·0001 of a c.c. of alcohol given	44	
10.41		42	
10.42		38	Beats irregular, systoles drawn out
10.44		30	Frequent diastolic stoppages
10.46		—	Cardiac stoppage

On raising the temperature to 35° C. the heart resumed beating, and while the temperature was kept at 38° C. the heart maintained a constant rhythm of 70 beats per minute.

Exp. II. Embryo aged 75 hours, conditions as above. Temperature throughout experiment 30° C. The normal rhythm at this temperature was 70 beats per minute.

Time	Dose administered	Cardiac frequency	Remarks
3.10.0	·0001 c.c. of pure alcohol dissolved in 1 c.c. of normal saline given	70	Rhythm normal
3.10.30	—	76	do.
3.11.30	—	78	Beats apparently a little stronger
3.13.0	—	84	—
3.14.0	—	86	Rhythm shows no change
3.15.0	—	90	Rhythm apparently normal in characteristics
3.18.0	—	96	do.
3.20.0	—	96	do.
3.25.0	—	94	do.
3.30.0	—	92	do.
3.35.0	—	92	do.
3.35.30	·0001 c.c. of alcohol (dissolved as before) given	92	do.
3.36	—	96	do.
3.38.30	—	96	do.
3.40	—	92	Beats weaker than normal
3.44.30	—	80	do.
3.46	—	76	do.
3.48	—	72	do.

Time	Dose administered	Cardiac frequency	Remarks
3.55	—	68	Beats much weaker than normal
3.58	·0002 c.c. of alcohol given	68	do.
4.0	—	56	Beats very weak
4.10	—	50	do.
4.20	—	48	do.

Exp. III. Embryo aged 75 hours, conditions of experiment unvaried; except the temperature which was maintained at 38° C. The cardiac frequency was constant during the half hour preceding the observations recorded, at 86 beats per minute.

Time	Dose administered	Cardiac frequency	Remarks
12.15	·0001 c.c. of pure alcohol dissolved in 1 c.c. of normal saline	86	Rhythm normal
12.15.30	—	90	No change in characteristics observed
12.16.30	—	94	do.
12.18	—	100	do.
12.20.0	—	108	do.
12.23.0	—	116	Beats apparently a little weaker than normal. The difference was not marked
12.28.0	—	118	No change observed
12.32.0	—	116	do.
12.40.0	—	116	do.
12.40.30	·0001 c.c. alcohol given (dissolved as above)	116	do.
12.41.0	—	120	Rhythm apparently normal
12.42.30	—	120	do.
12.44.30	—	126	do.
12.45.30	—	126	do.
12.49.30	—	126	do.
12.50	·0002 c.c. alcohol given	126	do.
12.50.30	—	128	do.
12.52.0	—	120	Beats slightly weaker than normal
12.55.0	—	110	do.
1.0.0	—	100	Beats weaker than normal
1.5.0	—	92	do.
1.7.0	—	90	do.
1.10.0	—	90	do
1.10.30	·0003 c.c. alcohol given	90	do.
1.11.0	—	84	do.
1.12.0	—	80	Beats irregular
1.15.0	—	60	Frequent diastolic stoppages
1.16.0	—	—	Heart stopped in diastole

On raising the temperature to 42° C. the heart recommenced to beat at a frequency of 100, but the beats were weaker than normal.

Exp. IV. Embryo aged 75 hours. Conditions of experiment unvaried except the temperature which was maintained at 42° C. during the experiment. Normal cardiac frequency during the half hour preceding the observations recorded was 100 beats per minute.

Time	Dose administered	Cardiac frequency	Remarks
12.0.0	·0001 c.c. of alcohol dissolved in 1 c.c. of normal saline	100	Rhythm normal
12.0.30	—	106	do.
12.1.30	—	120	Beating irregular and weak
12.2.30	—	130	do.
12.3.0	—	142	Beats fluttering and irregular
12.5.0	—	148	do.
12.8.0	—	160	—
12.10.0	—	cannot count frequency	Rhythm reversed, very irregular and too rapid to record
12.12.0 to 12.16.0	—	do.	do.
12.17.0	—	150	Irregularities not so marked, rhythm normal in direction, beats weak
12.19.0	—	140	Beats fluttering, weak but in normal direction
12.21.0	—	136	Beats show more normal characteristics
12.28.0	—	120	do.
12.35.0	—	110	Beats apparently normal
12.36.0	—	110	do.
12.42.0	—	108	do.
12.50	—	104	do.
1.15	—	104	do.
1.20	—	104	do.
1.20.30	·0001 c.c. of alcohol as before	109	Rhythm apparently normal
1.22.30	—	130	do.
1.25.0	—	144	Beating irregular and weak
1.26.30	—	158	Rhythm fluttering and irregular
1.29.0	—	160	do.
1.40.0	—	beats too rapid to record	—
1.41.0	—		Heart stopped in diastole

On applying a weak interrupted current the heart recommenced beating with an irregular rhythm, which gradually after discontinuance of the electrical stimulation became more regular and finally remained constant at a frequency of eighty beats per minute.

The following table shows the different effects produced by ·0001 of a c.c. of alcohol on the cardiac frequency of 75-hour chick-embryos at various temperatures. The other conditions of the experiments were constant.

TABLE A.

Time after administration of dose		1st embryo kept at temp. of 20° C.		2nd embryo kept at temp. of 30° C.		3rd embryo kept at temp. of 38° C.		4th embryo kept at temp. of 42° C.	
min.	sec.	Increase or decrease of cardiac frequency in beats per min.	Percentage variation of cardiac frequency	Increase or decrease of cardiac frequency in beats per min.	Percentage variation of cardiac frequency	Increase or decrease of cardiac frequency in beats per min.	Percentage variation of cardiac frequency	Increase or decrease of cardiac frequency in beats per min.	Percentage variation of cardiac frequency
1	30	+ 6	+ 10 %	+ 8	+ 11·4 %	+ 8	+ 9·3 %	+ 20	+ 20 %
3		− 2	− 3·3 ,,	+ 14	+ 20 ,,	+ 14	+ 16·2 ,,	+ 42	+ 42 ,,
5		− 10	− 16·6 ,,	+ 20	+ 28·5 ,,	+ 22	+ 25·5 ,,	+ 48	+ 48 ,,
8		− 14	− 23·3 ,,	+ 26	+ 37 ,,	+ 30	+ 34·8 ,,	+ 60	+ 60 ,,
12		− 22	− 36 ,,	—	—	—	—	frequency too rapid to count	—
13		—	—	—	—	+ 32	+ 37·2 ,,	do.	—
15		—	—	+ 24	+ 34·2 ,,	+ 30	+ 34·8 ,,	do.	—

The next table shows similar variations of effect produced by ·0002 of a c.c. of alcohol when given at different temperatures.

TABLE B.

Time after administration of dose		Embryo kept at 30° C.		Embryo kept at 38° C.		Embryo kept at 42° C.	
min.	sec.	Increase or decrease of cardiac frequency	Percentage variation of cardiac frequency	Increase or decrease of cardiac frequency	Percentage variation of cardiac frequency	Increase or decrease of cardiac frequency	Percentage variation of cardiac frequency
2		+ 4	+ 4·3 %	+ 4	+ 3·4 %	+ 21	+ 19·2 %
4	30	0	0	+ 10	+ 8·6 ,,	+ 45	+ 41·2 ,,
9		− 8	− 8·6 ,,	+ 10	+ 8·6 ,,	+ 51	+ 46·7 ,,
10	30	− 16	− 17·3 ,,	—	—	too rapid to count	—
12	30	− 20	− 21·7 ,,	—	—	do.	—

The variations of cardiac rhythm of five 75-hr. embryos produced by ·0001 of a c.c. of alcohol is summarised in Table C. In each of the experiments the embryos were kept at 20° C.

TABLE C.

Number of embryo	Cardiac frequency before giving alcohol	Maximum increase of cardiac frequency	Time elapsed from administration to maximum increase	Maximum percentage increase of cardiac frequency
1	50	8	2 min.	16 %
2	48	6	1 min. 30 sec.	12 „
3	52	8	1 min. 40 sec.	15 „
4	46	4	1 min. 30 sec.	8·6 „
5	48	5	2 min.	10·4 „

In the five experiments recorded in the next table the same dose was used (·0001 of a c.c.) but the temperature was maintained throughout the experiment at 30° C.

TABLE D.

Number of embryo	Cardiac frequency before giving alcohol	Maximum increase of cardiac frequency	Time elapsed from administration to maximum increase	Maximum percentage increase of cardiac frequency
1	80	20	7 min.	25 %
2	84	22	8 min.	26 „
3	78	24	6 min.	30 „
4	86	20	7 min. 30 sec.	23 „
5	90	26	6 min. 30 sec.	28 „

In the five experiments summarised in the table below the conditions and dose administered were unchanged. The temperature was maintained throughout the experiments at 38° C.

TABLE E.

Number of embryo	Cardiac frequency before giving alcohol	Maximum increase of cardiac frequency	Time elapsed from administration to maximum increase	Maximum percentage increase of cardiac frequency
1	86	30	8 min. 30 sec.	34·8 %
2	90	32	7 min. 30 sec.	35·5 „
3	84	26	6 min.	30·9 „
4	92	30	8 min.	32·6 „
5	88	28	7 min.	31·8 „

A similar series of experiments to those recorded in the foregoing tables with 75-hour embryos kept at 42° C. in each case resulted in a cardiac rhythm characterised by a frequency too rapid to be counted; and at the same time irregular and weak.

TABLE F. Shows the average maximum increase of cardiac frequency produced in twenty experiments with ·0001 of a c.c. of alcohol at the temperatures as below stated on 75-hour chick-embryos.

Embryos kept at 20° C.	Embryos kept at 30° C.	Embryos kept at 38° C.	Embryos kept at 42° C.
6·2	22·4	29·2	Too frequent to be counted

TABLE G. Is the average record obtained from twenty experiments on 75-hour embryos made with veratrine (·0001 gram dissolved in 1 c.c. of normal saline).

	Embryos at 20° C.	Embryos at 30° C.	Embryos at 38° C.	Embryos at 42° C.
Average maximum variation of cardiac frequency	− 4	+ 2	+ 5	+ 16

TABLE H. Is the average record obtained from ten experiments on 75-hour embryos where ·0003 gram of veratrine dissolved in 1 c.c. of normal saline was used.

	Embryos at 20° C.	Embryos at 30° C.	Embryos at 38° C.	Embryos at 42° C.
Average maximum variation of cardiac frequency	fatal	− 20	− 10	− 2

The cardiac rhythm at 42° C. was much weaker than the normal.

Remarks on the Preceding Experiments.

In considering the observations here recorded two important factors must be borne in mind. 1st. The experiments were conducted on a heart prior to the development of a nervous mechanism. 2ndly. At this stage of development Pembrey[1] has shown that the embryo-chick has not developed its "mechanism" for heat regulation. Consequently we have a direct action of drug and heat on the heart *without* the complications of either of these two sources of variation.

A study of my experiments here recorded, has convinced me that my previous conclusions, cited in the opening of this paper as regards the *rôle* played by temperature variations on the action of drugs on the

[1] This *Journal*, XVII. 331. 1895.

hearts of embryo-chicks do not require modification. Besides this statement I wish to emphasise the following points.

(1) The combined accelerator and augmentor action of small doses of alcohol on the embryonic heart reaches its maximum at about 38° C.

(2) At low temperatures even small doses of alcohol rapidly depress the rhythm of the embryonic heart (see Table A).

(3) A temperature of 40° C. and upwards has a marked influence on the action of alcohol on the embryonic heart. The frequency becomes too rapid to record, while the force of the beats is apparently greatly diminished and in case of higher temperatures becomes reduced to mere spasmodic twitching (see Exp. IV).

(4) A dose of alcohol which at a low temperature (20° C.) is a depressant has at higher temperatures an accelerator action (vide Tables A and B).

(5) The maximum accelerator action of alcohol on the embryonic heart is attained more rapidly at lower than at higher temperatures.

(6) Similarly the depressant action also comes on more rapidly at a lower than at a higher temperature.

(7) The cardiac stoppage produced by moderate doses of alcohol at a low temperature can be usually removed by heating or by the application of electrical stimuli.

(8) Similarly the cardiac stoppage produced by small doses of alcohol at a high temperature can sometimes be removed by cooling. Electrical stimulation however induces a condition not unlike tetanus.

(9) A dose of ·1 mg. of veratrine (dissolved in ·65 % NaCl) acts as a depressant to the embryonic heart at 20° C.; but at a temperature above 30° C. produces a marked acceleration of the cardiac rhythm.

(10) A dose of ·3 mg. of veratrine is fatal at 20° C. while at 42° C. the average reduction of cardiac frequency produced by this dose was two beats per minute (see Table H).

(11) Ammonia acting at 38° C. has a marked accelerating action on the embryonic heart.

(12) The preceding observations render it probable that the metabolic changes produced by drugs on the embryonic heart are dependent on the temperature of administration.

Action of Muscarin nitrate and Atropin sulphate.

Dr His also suggests that my result with muscarin, viz. that it is inactive on embryo chicks' hearts from the 70th to 100th hour, was due

to my preparation of that substance being inactive. Reference to my paper will show that I performed control experiments on frogs' hearts to make sure of its activity before testing it on those of embryo chicks. Further my observations are entirely in accord with those of both Krukenberg[1] and Kobert[2], who have also found that muscarin had no action on the hearts of early chick-embryos.

I have since found that active samples of muscarin nitrate which fail to induce stoppage in 75-hour embryos will at the normal temperature of 38° C. produce a typical stoppage in 200-hour embryos.

The following experiment illustrates the action of muscarin nitrate on the hearts of 200-hour chick embryos. It also records the antagonistic action of muscarin nitrate and atropin sulphate.

The temperature throughout the experiment was constant at 38° C. Precautions observed as in preceding observations. Normal cardiac frequency of the embryo at this temperature 102 beats per minute.

Time	Dose administered	Cardiac frequency	Remarks
11.30	·15 mg. of muscarin nitrate dissolved in 1 c.c. of ·75 % NaCl	102	Rhythm normal
11.31	—	96	Rhythm apparently unchanged
11.33	—	94	Beats slightly weaker
11.36	—	80	Beats much weaker
11.40	—	62	do.
11.44	—	54	Rhythm very weak
11.50	—	26	do.
11.56	—	22	do.
11.57	—	14	Rhythm very difficult to observe
11.58	—	0	Heart stopped
11.59	·15 mg. of atropin sulphate dissolved in 1 c.c. of ·75 % NaCl	0	do.
12.1	—	2	Heart gave 2 feeble twitches during minute
12.2	—	4	Rhythm very feeble and with long diastolic pauses
12.3	—	8	
12.8	—	22	Beating slightly stronger
12.10	—	26	do.
12.15	—	26	Characteristics unchanged

[1] Krukenberg. *Vergleichend-physiologische Studien*, Dritte Abtheil., Heidelberg, 1880. S. 151.

[2] Kobert. *Archiv für exper. Path. u. Pharm.* xx. 110. 1886.

Time	Dose administered	Cardiac frequency	Remarks
12.15	·2 mg. of atropin sulphate dissolved in 1 c.c. of ·75 °/₀ NaCl	26	Characteristics unchanged
12.17	—	34	Rhythm stronger than before
12.22	—	38	do.
12.30	—	46	Rhythm fairly strong
12·35 to 1.10	—	46	do.

In those cases where muscarin nitrate had caused complete cardiac stoppage I failed to restore the embryos' rhythm by means of atropin sulphate to its normal frequency.

The following table summarises the results of nine experiments on embryos varying in age from 195 to 210 hours.

A study of this table shows that the average dose of muscarin nitrate requisite to induce cardiac stoppage was ·173 mg.; and partial restoration of the rhythm resulted from the subsequent administration on the average of ·2 mg. of atropin sulphate. The average frequency of the restored rhythm was 40·9 °/₀ of the original frequency before administration of the muscarin nitrate.

Table I.

All the experiments recorded in this table were performed at 38° C. and the muscarin nitrate used was of Martindale's preparation.

No. of embryo	Age of embryo	Cardiac frequency at 38° C.	Dose of muscarin nitrate to induce cardiac stoppage	Dose of atropin sulphate to antagonise same	Frequency per min. of restored cardiac rhythm
I	210 hrs.	86	·15 mg.	·2 mg.	40
II	200 ,,	106	·2 ,,	·25 ,,	44
III	195 ,,	92	·175 ,,	·2 ,,	40
IV	206 ,,	88	·2 ,,	·25 ,,	36
V	200 ,,	60	·15 ,,	·2 ,,	30
VI	208 ,,	75	·1 ,,	·15 ,,	26
VII	200 ,,	100	·15 ,,	·25 ,,	38
VIII	200 ,,	80	·2 ,,	·25 ,,	30
IX	198 ,,	86	·25 ,,	·3 ,,	42

A record of the antagonistic action between muscarin nitrate and atropin sulphate, when the dose of muscarin nitrate is too small to induce complete stoppage is shown in the following table (Temperature 38° C.).

TABLE J.

No. of embryo	Age of embryo	Dose of muscarin nitrate given	Frequency before date of muscarin nitrate	Frequency after dose of muscarin nitrate	Frequency after muscarin nitrate + ·2 mg. of atropin sulphate
1	200 hrs.	·075 mg.	110	40	86
2	250 "	·075 "	136	100	130
3	200 "	·015 "	88	76	47*
4	210 "	·025 "	90	66	88
5	218 "	·025 "	88	63	88
6	220 "	·03 "	100	58	90
7	210 "	·1 "	100	17	26
8	215 "	·05 "	88	56	84

* Probably the excess of atropin sulphate exhibited its own depressing effect.

In the following table the action of muscarin nitrate at 38° C. on chick-embryos of various ages is recorded.

TABLE K.

No. of embryo	Age of embryo	Dose of muscarin nitrate given	Reduction of cardiac frequency
I	120 hrs.	·75 mg.	0
II	130 "	·75 "	0
III	150 "	·9 "	0
IV	160 "	·9 "	4
V	170 "	·9 "	50
VI	170 "	·5 "	30
VII	180 "	·5 "	60
VIII	185 "	·5 "	stoppage
IX	190 "	·25 "	do.
X	195 "	·25 "	do.
XI	198 "	·15 "	do.
XII	200 "	·1 "	do.
XIII	250 "	·075 "	do.
XIV	280 "	·075 "	do.

The next table records the action of muscarin nitrate, and the attempts to antagonise the same with atropin sulphate on embryo chicks kept at 20° C.

TABLE L.

No. of embryo	Age of embryo	Dose of muscarin nitrate given to induce stoppage	Dose of atropin sulphate subsequently given	Restored cardiac rhythm in beats per minute
1	170 hrs.	·75 mg.	·85 mg.	0
2	175 "	·75 "	1·5 "	0
3	180 "	·5 "	·6 "	4
4	185 "	·5 "	·75 "	10
5	200 "	·25 "	·35 "	5
6	200 "	·2 "	·3 "	8
7	200 "	·2 "	·3 "	9
8	250 "	·15 "	·2 "	4
9	200 "	·15 "	·15 "	8
10	220 "	·2 "	·275 "	15

The diastolic stoppage produced by muscarin nitrate on the hearts of chick-embryos aged 175 hours and upwards is often removable by the application of heat. In this respect the hearts of the older chick-embryos behave in a similar manner to that described by Petri[1] as characteristic of frogs' hearts. In the table below is recorded the action of muscarin nitrate on five embryos kept at 25° C., and in the third column the degree of temperature is noted at which the hearts recommenced beating.

TABLE M.

No. of embryo	Dose of muscarin nitrate to produce stoppage	Temperature at which rhythm recommenced	Cardiac frequency before muscarin	Cardiac frequency after heating the stopped heart
1	·15 mg.	36°	60	28
2	·15 "	39°	70	32
3	·175 "	40°	65	34
4	·2 "	38°	80	41
5	·175 "	39°	72	36

The following points are evident from a study of the statistics recorded in the last five tables (I to M):

(1) Muscarin nitrate depresses the cardiac rhythm of chick-embryos which are older than 200 hours. In larger doses its action culminates in diastolic stoppage; which in many cases can be removed by the

[1] Petri. Dissert. Bern, 1881.

subsequent application of atropin sulphate. In this respect the hearts of older embryos behave in a similar manner to frogs' hearts.

(2) The depressing action of muscarin nitrate on hearts of chick-embryos is typically exhibited when the age of the embryo is over 200 hours. It is shown to a lesser degree in embryos aged from 170 to 190 hours; and in one case a slight cardiac depression (4 beats per minute) was observed in an embryo aged 160 hours. On younger embryos this drug has no depressing action on the heart rhythm.

(3) The dose requisite to induce a "muscarin stoppage" decreases with the age of the embryo. Thus ·5 mg. was necessary for a 185 hr. embryo, ·1 mg. for a 200 hr. embryo, and only ·075 mg. for a 250 hr. embryo. With a 150 hr. embryo ·9 mg. had no action (see Table K).

(4) Atropin sulphate will only partially restore the cardiac rhythm of embryos whose hearts have been stopped by muscarin (see Table I), but if the depressing action of the muscarin has not gone so far as to induce stoppage, a subsequent application of atropin sulphate may almost completely restore the cardiac rhythm (see Table J).

(5) Muscarin nitrate has a much more powerful action on the hearts of embryo-chicks kept at a subnormal temperature than at the normal temperature of incubation. The frequency of the restored rhythm of hearts kept at 20° C. averaged in ten experiments 6·3 beats per minute, while the average in the case of embryos kept at 38° was 36 beats per minute (see Tables I and L).

(6) The stoppage produced by muscarin nitrate at a low temperature can often be removed by heat (see Table M).

(7) There is therefore a marked contrast between the absence of action of muscarin nitrate and atropin sulphate on the hearts of embryo-chicks aged from 50 to 140 hours, and the typical action of these substances on older embryos.

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NOTES ON RENNET AND ON THE COAGULATION OF MILK. By ARTHUR EDMUNDS, B.Sc. (Lond.)

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I. *Rennin in the Testis.*

IN his *Text-book of Chemical Physiology*, p. 580, Prof. Halliburton states that pieces of testis possess the power of curdling milk. This statement has been recently contradicted by Dr R. Peters[1] who writes "A testis removed from a dog immediately after death was divided into small pieces and extracted for 24 hours with glycerine; the extract was filtered, but after several hours at 40·5° C. no coagulating action on milk could be observed. Further the fluid squeezed out from a fresh bull's testis when added to milk after neutralisation, produced a similar negative result." From this he concludes that the testis does not contain a milk curdling ferment.

Prof. Halliburton tells me, that the statement was inserted in his book on the authority of a traveller in Italy, who informed him, that the peasants in certain districts were in the habit of using pieces of testis, instead of rennet, in cheese making. Recognising however the necessity of putting the matter on a surer basis, he suggested to me that I should investigate the subject. The two experiments of Peters are inconclusive, because he used fresh tissues; for the extraction of ferments, especially when they occur as zymogens, it is well known that some means such as treatment with dilute acid is necessary, to convert the zymogen into the ferment.

The following is an account of the experiments I have performed. The first testis that I used was from a ram. It had been kept on ice for four days and was without any trace of putrefaction. Portions of this were dried in the sun, fragments of it (and of epididymis), added to milk did not cause clotting. But I obtained a clot by adding to the milk a few drops of an extract of the dried testis made with saturated

[1] *Unters. ü. das Lab, und die labähnlichen Fermente; Preisschrift.* Rostock, 1894.

salt solution. The clot however, took twenty-four hours to form, but was perfectly typical; when thrown into a vessel of water it remained coherent. In many of the experiments subsequently to be described, but not in all, the clots took quite as long to form; it was necessary therefore by control experiments to guard against the error of mistaking for a true clot, the precipitation of the caseinogen caused by the milk turning sour.

Part of the same ram's testis was cut up and extracted with glycerine; the extract alone added to milk caused either no curdling at all or only after twenty-four hours, but the addition of a few drops of a two per cent. solution of calcium or barium chloride as well as the extract, produced a clot within a couple of hours; in control experiments it was found that the same quantity of the calcium or barium salt alone, produced no clotting.

The next experiments I carried out were with rats' testes. The fresh testis added to milk, or the glycerine extract of fresh testis gave negative results; but a glycerine extract of rat's testis which had been treated previously for twenty-four hours with 0·1 per cent. hydrochloric acid gave typical clots, though often only after twenty-four hours had elapsed even if calcium chloride was added as well; the same was true for the 0·1 per cent. hydrochloric acid extract.

Other specimens of the same milk were curdled in a few minutes by commercial specimens of rennet or glycerine extract of stomach. These experiments I take to mean that the testis sometimes contains a very minute trace of rennin or a rennin-like ferment, sufficient however to cause curdling in the presence of excess of calcium.

II. *Rennin in other Organs.*

The preceding experiments naturally led me to investigate whether this property is confined to the testis, and consequently I examined a number of other organs. In each case the organ was divided into small pieces, then treated for twenty-four hours with 0·1 per cent. hydrochloric acid, and then with glycerine. Both the glycerine and the acid extracts were examined, the acid extracts being first neutralised; and in many cases I have succeeded in obtaining extracts active in curdling milk, though as already stated the action was usually very slow. The acid extracts and the glycerine extracts generally gave the same results. In the first series of experiments, extracts were prepared from the liver, lung, muscle, kidney, spleen and ovary, all of which coagulated milk;

but in these experiments the curious and unexpected result was obtained that while the extracts which were used without any calcium salt almost all caused coagulation within fifteen hours, most of those specimens which contained calcium chloride had undergone no change. The extract of muscle however seems, like the testis extracts, to have been markedly helped by the calcium, a good clot forming in less than two hours. It is somewhat remarkable that the calcium chloride should in the case of the testis and muscle accelerate the coagulation, but in that of the other organs retard it.

The dog from which the above-named organs were obtained had been killed in an experiment on intravascular coagulation, but this could not be the cause of the curious result just referred to. I have since then made extracts of various organs from normal mammals (dogs and cats) and in all cases the extracts of numerous organs caused milk to coagulate; and the action of calcium chloride was most erratic; in some cases it appeared to make no difference in coagulation time, in others it accelerated and in others still it retarded the clotting. It is just possible that this is in part due to the differences in amount of the ferment present, the phenomenon having a parallel in the varied effects produced by nucleo-proteids on blood, according to the amount injected.

I also made an experiment with sheep's thymus. This was cut up and extracted with 0·1 per cent. hydrochloric acid. A few drops of this extract together with 0·5 c.c. of a 10 per cent. solution of calcium chloride caused a well-marked curd to form in about ninety minutes. This is the shortest time in which I have been able to obtain a clot from an extract of any organ other than the stomach.

I will give the details of only one other experiment. A cat was killed by chloroform; ligatures were placed around the left half of the liver, and the left renal artery. A stream of salt solution followed by one of tap-water was then injected through the aorta so as to thoroughly wash out the blood from those organs not protected by the ligatures. Extracts were then made from various organs in the usual way, and portions of the extracts added to specimens of milk in test-tubes. In each test-tube were 10 c.c. of milk, and to some 0·5 c.c. of a 10 per cent. solution of calcium chloride was added as well as the tissue extract. The result of the experiment is given in the table. It will be seen that those extracts which were used with calcium chloride, caused on the whole more marked clotting than those which were used without the calcium salt; and that those extracts from tissues freed from blood, were on the whole more active than those from tissues in which the blood had been

allowed to remain. It will also be noted that the extract of blood clot was the least active of any, a fact which I noticed in all my experiments.

Organs	HCl Extracts neutralised		Glycerine Extracts			
	Examined after 24 hours		Examined after 24 hours		Examined after 48 hours	
	No $CaCl_2$	with $CaCl_2$	No $NaCl_2$	with $CaCl_2$	No $CaCl_2$	with $CaCl_2$
Liver, with blood vessels washed out	weak clot	firm clot	contracted clot	contracted clot	contracted clot	contracted clot
Liver, vessels ligatured	nil	clot	nil	nil	contracted clot	contracted clot
Kidney, with vessels washed out	clot	clot	weak clot	nil	contracted clot	firm clot
Kidney, vessels ligatured	firm clot	clot	nil	nil	contracted clot	contracted clot
Brain	firm clot	firm clot	no clot	weak clot	contracted clot	contracted clot
Lymph Glands [mesenteric]	firm clot	clot	weak clot	nil	contracted clot	firm clot
Thyroid	clot	clot	weak clot	nil	contracted clot	firm clot
Small Intestine	weak clot	clot	nil	nil	contracted clot	firm clot
Blood Clot	weak clot	weak clot	weak clot	nil	contracted clot	firm clot

These results are the reverse of what would be expected on the theory that the rennin, like pepsin, had been re-absorbed from the alimentary canal. It may be, however, that rennin though it reaches the tissues *via* the blood and lymph is more or less "fixed" by them, in much the same way as pepsin is absorbed by fibrin from fluids like urine which contain only minute traces of it[1]. The fact that Holovotscheiner and Helwes[2] have obtained from urine a small quantity of a rennin-like ferment appears to me in favour of the re-absorption theory. Why the tissues containing blood yielded less active extracts than those freed from blood, I can only answer by suggesting that the alkalinity of the blood partly neutralised the acid employed and so retarded the extraction, or that glycerine dissolves out from blood other substances besides rennin which inhibit the action of that ferment.

[1] Leo. *Centralblatt med. Wiss.* 1875. 933.

[2] *Pflüger's Archiv*, XLIII. 384. 1888.

The only other alternative to the re-absorption theory as far as I can see is that rennin, or a substance which acts in the same way on milk, is formed in minute quantities in the metabolism of many tissues. In favour of this view it must be admitted that rennin is undoubtedly formed in some situations where it cannot possibly serve the purpose of curdling milk, *e.g.* in certain plants, Ficus etc. Here it must either be formed for some hitherto unknown use, or merely be an accidental product of metabolism. The presence of a milk-curdling ferment in the pancreatic juice can also be of little service to the economy because the milk upon which it would act has already been acted upon by the gastric juice.

In connection with this point it is interesting to note that none of the ferments of the animal body, with the possible exception of trypsin, are absolutely confined to the organ of which they are a characteristic product. Brücke for example has observed the presence of pepsin in muscle and most other tissues, also in the urine. Holovotscheiner[1] has reported a diastatic ferment resembling ptyalin from the urine, while a sugar inverting ferment has been detected by Robertson[2] in a large number of organs, including the whole of the alimentary canal, even the œsophagus, the liver, pancreas, spleen, thyroid, salivary glands, muscle and kidney. To these, as I have pointed out above, rennin must be added as an occasional constituent of practically all tissues and organs.

III. *Solubility of casein.*

In a paper on the Proteids of Milk which appeared some years ago, Prof. Halliburton[3] makes the following statement, "The similarity of caseinogen and casein to myosinogen and myosin appears to me to be exceedingly close." Prof. Halliburton allows me to state what were the experiments that led him to that conclusion.

Caseinogen was prepared by a modification of Hammarsten's method which is described in full in the paper just quoted[4], and dissolved in lime water. On adding rennet to this solution no coagulation occurs; on adding rennet and a few drops of dilute phosphoric acid (to form calcium phosphate), a clot of casein is produced. On taking this clot, washing it free from calcium salts and once more

[1] *Chem. Centralb.* 1886. 327.
[2] *Edinburgh Med. Journ.* Sept. 1894.
[3] This *Journal*, XI. 464. 1890.
[4] This *Journal*, XI. 449. 1890.

dissolving it in lime water, the addition of rennet causes no coagulation; but the addition of rennet *plus* phosphoric acid caused the formation of a curdy precipitate. This may be again dissolved, and the process repeated indefinitely. From this it appeared that re-coagulation of casein could occur in a similar way to that in which the re-coagulation of myosin may be made to take place. Subsequently and after a paper by Dr Ringer[1] on the subject, Dr Halliburton made a further experiment which modified his previous conclusion. He took the solution of casein, and added phosphoric acid alone without any rennet; the calcium phosphate so formed caused an immediate precipitation of the casein. The apparent re-coagulation of the casein by rennet and a calcium salt was really only the precipitation of the casein by the calcium salt; other calcium salts like the chloride will do equally well. Dr Ringer showed that one of the most striking differences between caseinogen and casein is that the solutions of the former are not, but of the latter are readily precipitated by soluble salts of calcium. Hence it is, by such methods, absolutely impossible to investigate whether rennin is capable of re-coagulating casein, because the ferment solution demands the presence of a calcium salt which of itself causes a precipitate. Peters[2] has also taken up this question, and while he adopts Halliburton's nomenclature of caseinogen and casein, he nevertheless maintains that caseinogen cannot be distinguished from casein (as Hammarsten originally stated) by the action of rennin, for solutions of both are, he states, curdled by that ferment. He took the curd produced in milk by the addition of rennet, washed it and dissolved it in lime water. On adding rennet to this solution a heavy precipitate (Fällung) took place. This was again dissolved in lime water, and again gave a precipitate; he repeated this process four times more.

He further states that the preparation of rennet he employed (Witte's Labessenz) gave a clot when added to a solution of caseinogen prepared by Hammarsten's method, without any addition of phosphoric acid, a result never obtained with preparations of rennet used in this laboratory. It therefore seemed advisable to examine Witte's preparation to see if it contained some soluble salt of calcium. So far however I have been unable to obtain any of this preparation.

The most recent paper dealing with the question is one by Dr D. Harris[3]. Though he admits the many and important differences,

[1] This *Journal*, XI. 450. 1890. [2] *loc. cit.*
[3] *Journ. of Anat. and Physiol.* XXIX. 195. 1895.

of solubility etc., between caseinogen and casein, he like Peters, though by a different series of experiments, contends that casein resembles myosin in its capability of being re-dissolved and re-coagulated. He finds that when casein is pounded with water, a creamy fluid is obtained which, when put into the water bath with rennet, sets into a solid; this solid can in its turn be treated in the same way and with the same result, and this can be repeated many times.

I disagree with the deduction drawn from this experiment, on the basis of the following experiments.

(i) A clot of casein was pounded with distilled water, and the creamy fluid thus obtained was divided into two parts. To one part rennet was added, to the other nothing. Both were put into the water bath at 40° C. and in a few minutes both were solid. Harris seems to have performed this experiment also, but he has apparently missed the point of it. In fact pounding a curd with water does not dissolve the casein at all; it merely breaks it up and hastens the separation of the whey; if the mixture is kept quiet the particles simply stick together again, whether rennet is added or not.

(ii) Casein was pounded up as before and the resulting creamy fluid centrifugalised. The fluid which separated out contained only a trace of proteid matter (lactalbumin and whey proteid from the whey) and no clot formed on the addition of rennet.

(iii) Ringer's method for preparing caseinogen is as follows:—The precipitate produced by adding acetic acid to milk is washed with water and ground up in a mortar with calcium carbonate. The mixture is then thrown into excess of distilled water; the fat rises to the surface; the chalk falls to the bottom and the intermediate fluid contains the caseinogen in solution. This coagulates on the addition of rennet and calcium chloride. Dr Ringer[1] states that casein similarly treated with calcium carbonate is almost insoluble in distilled water. This statement I have confirmed. Instead however of pouring the mixture of casein and chalk into a tall vessel of distilled water, I added a small quantity of water and centrifugalised. The fluid obtained was slightly opalescent, owing to some fat which had not risen, but it was practically proteid free and gave no clot on adding rennet.

(iv) The following experiment I have performed at the suggestion of Mr Colls. It consisted in pounding a clot of casein not with distilled water but with a few crystals of ammonium oxalate. The clot

[1] *loc. cit.* p. 477.

broke down into a fluid of the consistency of ordinary milk which does not show the slightest tendency to set once more into a coherent clot. I then filtered the fluid thus obtained and found that the fat was almost entirely retained on the filter while the filtrate was a slightly opalescent liquid which gave strong proteid reactions. It was also readily precipitated by a trace of a calcium salt and by acetic acid. I took another specimen of the dissolved casein clot and centrifugalised it, and here again found that the fat separated from the rest of the fluid. The whole divided into three layers; the fat floated at the surface; a sediment, probably calcium oxalate, sank to the bottom; there was an opalescent liquid between the two.

Microscopical investigation revealed the total disappearance of the casein granules visible in a preparation of a small piece of crushed clot, and also the fact that the fat globules had slightly run together, although on the whole most of them were as free as in normal milk.

This peculiarity of separation into these three layers may be also observed in milk to which rennet has been added after the addition of sufficient ammonium oxalate to prevent coagulation. After standing for twenty-four hours practically all the fat will have risen, leaving the casein in solution as a slightly opalescent liquid.

These experiments I take to mean, that casein is soluble in a solution of ammonium oxalate and that it is merely rendered soluble, probably by the removal of calcium salts, and not reconverted into caseinogen, since it is precipitable by calcium chloride—some of this precipitate would of course be calcium oxalate, but as only a small quantity of ammonium oxalate was originally used, this would only account for a small fraction of the precipitate. They show furthermore that casein is inferior to caseinogen as an emulsifier.

Thus calcium salts can be removed from casein either mechanically, as for example by prolonged washing, or chemically, by the addition of ammonium oxalate, the effect being the same in both cases, viz. the casein is rendered soluble.

IV. *The Relation of Alkali Albumin to Caseinogen.*

The exact relation of these two proteids has given rise to a good deal of discussion. One of the chief reasons for holding that they are different bodies is, that while caseinogen can be curdled by rennin in the presence of a small quantity of calcium salt, alkali albumin is incapable of being so acted upon.

Dr Peters, however (*op. cit.*), states that this distinction does not exist. He prepared alkali albumin (Lieberkühn's jelly) from white of egg; and after a certain amount of washing, dissolved it in boiling water, filtered, precipitated the alkali albumin with acetic acid, and re-dissolved in lime water. This he found gave a flocculent precipitate (Fällung) with neutral rennet, a firm clot with rennet to which a trace of acetic acid had been added, and was unaffected by boiled rennet. We may pass by the fact that Lieberkühn's jelly if thoroughly washed from alkali becomes opaque and tough, somewhat resembling fibrin as obtained from whipped blood, and is not soluble in boiling water; and we take the solution of alkali albumin as prepared by Dr Peters. This solution is in fact precipitated by most of the commercial rennet extracts, but it is also precipitated by the extracts when boiled. In this point it will be noticed I do not agree with Dr Peters. I find further that these extracts contain calcium salts. And it has been shown by Dr Ringer[1] that alkali albumin is precipitable by a trace of a calcium salt, and that the more the reaction of the solution approaches to neutrality, the less calcium is required to precipitate it.

The results obtained by Dr Peters are then, I think, to be explained by the presence of calcium salt in the rennet preparation used by him.

V. *The effect of Peptone on Milk Coagulation.*

If a solution of commercial "peptone" such as Witte's or Grübler's be injected into the circulation and the animal killed by bleeding it is found that the blood has lost the power of clotting and this property can be restored to the blood by the addition of a calcium salt provided that a small dose only of peptone has been injected.

At Prof. Halliburton's suggestion I have undertaken a series of experiments to determine whether "peptone"[2] has any effect on the coagulation of milk by rennin at all comparable to that which it has in the case of blood. The method adopted was to dissolve a known weight of peptone in a known volume of milk and then to dilute this with normal milk to the desired extent, thus obviating the error which would have been introduced by diluting the milk if peptone dissolved in water had been used.

[1] *Proc. Physiol. Soc.* 1891. This *Journal*, XII.

[2] Where peptone is mentioned it should be understood to be in all cases "commercial peptone," which consists mainly of albumoses containing little peptone in the strict sense of the word.

In all cases I found that the action of rennet was retarded by the presence of peptone and those specimens of milk which contained a relatively large percentage of peptone clotted after those specimens which contained a smaller percentage. I give the details of one experiment which illustrates this point. Each tube contained 10 c.c. of

Temperature of bath 40° C.

Number of grams of "Peptone" per 100 c.c. of milk	5	2·5	1·25	·625	0
Exp. commenced at 5.13 p.m.					
5.23 ,,					clotting commenced
5.27 ,,					clotting finished
5.33 ,,				clotting commenced	
5.37 ,,			clotting commenced		
5.39 ,,				clotting finished	
5.41 ,,			clotting finished		
5.57 ,,		clotting commenced			
5.63 ,,		clotting finished			
7.10 ,,	Still fluid. It was however clotted by next morning at 11 a.m.				

milk, a varying quantity of Witte's peptone, and 150 cubic millimetres of rennet (Martindale's).

The next point that naturally occurred was to determine whether the coagulability of milk which has been destroyed by peptone can be restored by calcium in the same way as the action of a small dose of peptone can be neutralised in the case of blood. I find that 0·5 c.c. of a 10 per cent. solution of calcium chloride in 10 c.c. of milk to which the usual quantity, 150 cm. of rennet, had been added will cause rapid clotting even in the presence of 2·5 grms. of peptone per 100 c.c.; with a larger percentage, 5 grms. per 100 c.c., the action of the calcium is slower and more unsatisfactory. At present I can offer no explanation of this peculiar action. I think, however, that the theory that peptone removes or throws out of action the calcium salts which

has been advanced in the case of blood is equally untenable here. I have pounded a clot of casein with peptone, and, although as stated above, ammonium oxalate readily removes the calcium and causes liquefaction, peptone does not cause the slightest alteration in the consistency of the clot.

VI. *Synthesised Colloids and Milk.*

The remarkable colloid substances prepared by Prof. Grimaux, which resemble proteids in so many of their reactions, having been found by Dr Pickering[1] to possess the power of causing intravascular clotting, it was desirable to ascertain if they had any effect on milk. I have experimented with a 0·5 per cent. solution of Grimaux's "colloïde aspartique" prepared by passing a current of gaseous ammonia heated to 170° C. over solid aspartic anhydride, but have only obtained negative results, the milk remaining unaltered at the end of 18 hours.

CONCLUSIONS.

(i) A small quantity of milk-curdling ferment can be obtained from other tissues and organs besides the stomach, viz. Testis, Liver, Lung, Muscle, Kidney, Spleen, Thymus, Thyroid, Brain, Blood, Small Intestine, Ovary.

(ii) There is no evidence that casein can be converted into caseinogen and re-coagulated by rennin, the apparent re-coagulation obtained by Peters being probably due to calcium salts present in the rennet extract.

(iii) The statement made by Peters as to the coagulability of alkali albumin by rennin is inaccurate, the coagula he obtained being probably due to the presence of calcium salts in the rennet essence.

(iv) Peptone has a marked retarding effect on coagulation which may be partly, at least, neutralised by the addition of calcium chloride.

(v) Casein is soluble in ammonium oxalate without being re-converted into caseinogen.

(vi) Grimaux's colloïde aspartique has no action on milk coagulation.

[1] This *Journal*, XVIII. 54. 1895.

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ACTION OF PANCREATIC JUICE ON MILK. By W. D. HALLIBURTON, M.D., F.R.S., and T. GREGOR BRODIE, M.D.

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KÜHNE[1] was the first to point out that extracts made from the pancreas of the dog cause milk to coagulate; this action was described in some detail by Sir William Roberts[2], various conditions which influence the clotting were observed by Edkins[3], and the occurrence of the action in the extracts from a number of animals determined by Harris and Gow[4].

Our attention was drawn to the subject by a sentence in Prof. Gamgee's[5] Physiological Chemistry, in which he points out that it does not necessarily follow that because extracts of the organ have a clotting action, the pancreatic juice possesses it also.

We have accordingly performed experiments with the actual pancreatic secretion.

We obtained the juice from a temporary fistula in dogs; the animal was anæsthetised with a mixture of chloroform and ether; a cannula was inserted into the main pancreatic duct, the abdominal wound was sewn up, and the animal kept warm by hot fomentations. In many cases the flow of juice was very sluggish and scanty, and the readiest way of obtaining more juice was found to be Claude Bernard's plan of injecting a small quantity of ether into the stomach.

The operative part of the proceedings was performed rapidly, and care taken to injure the pancreas as little as possible. The juice was collected for about two hours, but never more than one or two cubic centimetres was obtained. The animal was kept under the anæsthetic all this time and then killed by asphyxiation. We have done nine experiments altogether.

[1] *Verhandl. Naturhist. Med. Ver.* (N. S.) Heidelberg. III. 3.
[2] *Proc. Roy. Soc.* 1879 and 1881.
[3] This *Journal*, XII. 193. 1891. [4] *Ibid.* XIII. 469. 1892. [5] Vol. II. p. 446.

The juice is perfectly clear and colourless, looking very like saliva. On keeping it for twenty-four hours or more we did not observe any spontaneous coagulation as described by some authors; but it became a little turbid. It is extremely viscid, and alkaline in reaction.

In the case of one dog, the action of the juice on milk exactly resembled that of rennet. The clot was rapidly produced (within a few minutes) especially at the temperature of 40° C. This contracted and squeezed out a clear whey.

Our results with the remaining dogs have been different, and we are inclined to attribute the behaviour of the juice of this one exceptional dog to the fact that it was a puppy. The remainder of the dogs were full grown.

We may now proceed to describe the results which are usually obtainable.

The first experiment we did was as follows:—a few drops of the juice were added to a few c.c. of milk in a test-tube. The tube was put into the warm bath (35°—40° C.). No change was observable in the hour we watched it; it was therefore put aside in a test-tube rack at the ordinary temperature of the laboratory (12°—14° C.), and next morning it was found set into a solid curd. It was by accident put into the warm bath once more, and to our surprise we found after a few minutes that the clot had disappeared, and the milk was apparently as liquid as before. It was taken out and cooled by placing it under the tap, and once more it solidified, and this operation could be repeated any number of times.

The same result was obtained with the juice of the other seven dogs, and in addition to the main result just mentioned we have made a number of other experiments to ascertain the explanation of what appears to be a unique phenomenon.

Other results noticed in milk acted on with pancreatic juice were the following:—

(*a*) The clot contracts very little; it has the consistency of a thick gummy jelly; after the operation of cooling and warming has been repeated a number of times, however, it does contract and squeeze out a small quantity of whey. If it is left for a long time in the warm bath, it gradually disappears, forming caseoses and peptone; this is due to the action of trypsin.

(*b*) The milk very rapidly becomes acid; the action of the fat splitting ferment of the pancreatic juice is responsible for this and the rapidity of the action of this ferment was noted by Claude Bernard. The smell of fatty acids is quite perceptible.

As a rule the effect of pancreatic juice on milk curdling is noticeable in from 15—20 minutes after it has been put into the warm bath. No change visible to the naked eye is observable; but on cooling it under the tap it sets immediately into a jelly. We, however, examined the milk under the microscope on the warm stage, after it had been left in the warm bath for twenty minutes, and found that there was an amorphous precipitate in addition to the fat globules. This indicated to us that the milk although apparently quite fluid had in reality deposited its caseinogen in the form of a fine precipitate. This was confirmed by filtering the milk through a filter surrounded with a hot water funnel. The filtrate was slightly opalescent from the presence of fat globules, but gave no precipitate on adding acetic acid; it was therefore free from caseinogen. We have always found this to be the case. A correct way of stating the action of dog's pancreatic juice on milk curdling is therefore as follows:—

When the juice is added to the milk, and the mixture kept at 35°—40° C., in about a quarter of an hour the milk though apparently unaltered, has really deposited its caseinogen in the form of a finely granular precipitate, which is removable by filtration. When this milk is cooled the granular precipitate sticks together and produces a coherent clot. The coherence of the clot is lost when the mixture is again warmed to 35°—40° C., and this operation may be frequently repeated.

The idea occurred to us that the phenomenon might be due to the action of the juice on the fats, the liberation of fatty acids precipitating the caseinogen. But this was negatived by the following experiments.

(*a*) Addition of acetic acid produces a precipitate of caseinogen; but this precipitate does not behave in the least like the precipitate produced by pancreatic juice. It comes down more rapidly in warm than in cold milk, and on cooling the precipitate produced in warm milk it undergoes no change.

(*b*) Addition of oleic acid to milk in small or large quantities produces no precipitate of caseinogen at any temperature.

(*c*) An artificial emulsion made with dilute sodium carbonate, olive oil and a little white of egg undergoes no clotting on the addition of pancreatic juice, though of course it rapidly becomes acid.

(*d*) Pancreatic juice added to a solution of caseinogen prepared by Ringer's method causes the formation of a granular precipitate of the proteid in about an hour at 40° C., but there is no formation of acid, the solution of caseinogen having been obtained free from fat. This

precipitate, however, does not become more coherent when cooled. This latter appearance is produced in milk only, that is when caseinogen has undergone no treatment of precipitation and re-solution previously.

(*e*) Another experiment which may be placed under this head was the following: it consisted in adding a small quantity of oleic acid to milk and then rennet[1] in order to see if the presence of free fatty acid modified the action of rennet. The result was that casein was precipitated in a more finely divided way than in a control specimen to which rennet alone was added, but there was no other resemblance seen to the action of pancreatic juice.

The next question we took up was whether it was possible that the existence of the proteolytic ferment with the milk curdling ferment could account for the phenomenon. This was negatived by the fact that dog's gastric juice which also contains a proteolytic ferment curdled milk in the usual way, just like rennet essence does. We further found that trypsin prepared according to Kühne's method has no curdling action on milk[2]; and that a mixture of trypsin and rennet acts just like rennet alone in producing a curd.

In another series of experiments, we found that potassium oxalate, added to milk to the extent of 0·3 per cent. (an amount which completely inhibits the activity of rennet), only hinders the curdling action of pancreatic juice on milk to a slight degree; the oxalated milk behaves just like ordinary milk when pancreatic juice is added, but the onset of the change is slower. Previously published analyses of pancreatic juice show that it contains only a small amount of calcium salts so it is difficult to suppose these are present in sufficient quantity to neutralise the action of the potassium oxalate added.

In these same experiments we noticed also that the onset of acidity was slower than in normal milk. The potassium oxalate used gave a perfectly neutral solution with water. The experiments suggest that oxalates hinder the action of other ferments than coagulating ones, but we have not at present followed up this interesting suggestion any further.

The question next arose whether pancreatic extracts are to be relied on as a criterion of the action of pancreatic juice. At the present time

[1] The preparation of rennet which we employed throughout was made by Martindale; this preparation is the one which contains least calcium salts of those we have examined.

[2] We, however, like Edkins find that Kühne's trypsin produces the 'metacasein reaction' to be referred to immediately.

when the action of internal secretions is being largely worked out by means of extracts of organs, this question is a serious one. We accordingly made several glycerine and aqueous extracts of the dog's pancreas; and we find that the extract acts on milk just like the natural juice. In the case of the young dog, where the juice acted like rennet, the glycerine extract of the pancreas acted like rennet also. In the case of three other dogs in which the juice acted in the characteristic manner described in this paper, the extracts behaved in the same way; in two cases the extracts made were aqueous ones; in the third case glycerine was employed. In the case of the glycerine extract we, however, noticed the following peculiarity; the day after the glycerine was added to the minced gland, some of the glycerine extract was filtered off and added to milk; it produced the usual action, namely, a coagulation which was not apparent to the naked eye till the mixture was cooled; on warming, the milk became fluid once more, but really contained as already explained a fine granular deposit of casein. On repeating this experiment a fortnight later, the glycerine having been in contact with the gland all the time, the addition of the extract to milk produced no coagulation at all, but a very rapid conversion of the proteid into proteolytic products. If coagulation did occur, it was so transient a phase that it was missed; the increase in proteolytic activity (due probably to more trypsin having gone into solution in the glycerine) masking the action of the curdling ferment.

We found, as many have noted before, that Benger's Liquor Pancreaticus curdles milk[1]. The phenomena described above as obtained with dog's pancreatic juice were however not observable. Mr Benger was good enough to inform us that the liquor pancreaticus is made from the pancreases of young pigs. He also drew our attention to the following fact which we have confirmed with the specimens he was good enough to supply us with: viz. that the freshly prepared liquor pancreaticus has little or no curdling action on milk, but after the deterioration that occurs in specimens that have been kept a long time, the curdling action becomes evident; the deterioration thus falls apparently chiefly on the tryptic ferment, which when in excess (as in our experiment with the glycerine extract) masks or hinders the activity of the curdling ferment.

That pancreatic extracts do act differently from rennet upon milk was originally noted by Roberts, and confirmed by Edkins. Roberts

[1] Compare Edkins, *loc. cit.* p. 202, Exp. VI.

described what he termed the 'metacasein reaction'; that is after a variable period during which boiling the milk causes no curdling, heating gives a flocculent heat coagulation. Our experiments suggest an explanation of this phenomenon; it may be that when the metacasein reaction is observable, the casein has been already deposited in a finely flocculent form by the action of the pancreatic extract and boiling simply renders this apparent by the coalescence of the minute particles into larger flocculi. As however the metacasein reaction is obtained with Kühne's trypsin, the production of metacasein appears to be a function of the tryptic rather than of the milk curdling ferment.

But apart from this, the question which we have still to answer is: What is the nature of the precipitate produced by pancreatic juice? Is it caseinogen, or casein, or something which is neither casein nor caseinogen? The experiments which we have performed in attempting the solution of this question show that 'pancreatic casein' as we may term it is something new, and its solubilities are intermediate between those of caseinogen and casein.

The experiments already recorded point in this direction; namely, the peculiar behaviour of pancreatic casein to changes of temperature, and the fact that its formation is not prevented but only slightly hindered by such an addition of potassium oxalate as completely inhibits the activity of rennet.

We further found that the action of rennet on the pancreatic casein is to convert it into true casein.

If a specimen of milk is treated with pancreatic juice at 40° for twenty minutes, pancreatic casein is formed; if then rennet is added, a clot is formed having all the solubilities of casein, and this undergoes no change when it is subsequently cooled. If oxalated milk is employed pancreatic casein is formed, but on the subsequent addition of rennet there is no alteration, unless a little calcium chloride is added as well; it is then converted into casein.

Rennet then will convert pancreatic casein into true casein. The question next arose, will pancreatic juice convert true casein into pancreatic casein? The answer to this is, no. The addition of pancreatic juice to a curd produced by rennet causes no change in its solubilities: the prolonged action of pancreatic juice, however, leads naturally to the formation of caseoses and peptone, the curd being eroded from the exterior, which is the usual action of the tryptic ferment.

The conclusion drawn from this is that pancreatic casein is some-

thing intermediate in its method of formation between caseinogen and casein; this was confirmed by examining its solubilities.

In testing this question we have been much assisted by the work of Dr Ringer[1], who worked out the principal differences between the solubilities of caseinogen and casein.

The following are our experiments :

A. Three equal quantities of milk were taken, *a*, *b*, and *c*.

To *a* acetic acid was added.

To *b* rennet was added.

To *c* pancreatic juice was added.

In all three cases a precipitate occurred; in *a* of caseinogen, in *b* of casein, in *c* of pancreatic casein.

Each was well washed with distilled water, but on account of the gummy nature of the pancreatic casein, it was found impossible to wash it so thoroughly as the other two; the same was the case throughout these experiments.

Each was then rubbed up with calcium carbonate and water added; each was centrifugalised and the fat rose to the surface and was skimmed off; the calcium carbonate fell to the bottom, and the intermediate layer was pipetted off. In the case of *a* (caseinogen) this fluid gave an abundant precipitate with acetic acid; in the cases of *b* (casein) and *c* (pancreatic casein) acetic acid caused only a faint cloud. Pancreatic casein therefore resembles casein and differs from caseinogen in its insolubility in water and calcium carbonate.

B. In this case, caseinogen, casein, and pancreatic casein were dissolved in lime water; after about 12 hours this was filtered, and the extract found to contain abundance of proteid in solution in each case.

Extract *a* (caseinogen) gave a very slight precipitate on the addition of calcium chloride (10 °/₀ solution); an equal bulk of the calcium chloride precipitate was necessary to produce a perceptible precipitate.

Extract *b* (casein) was precipitated in the cold by a very small quantity of calcium chloride solution. 3 or 4 drops of a 10 °/₀ solution to 10 c.c. of the extract was sufficient. 1 drop caused no precipitate in the cold, but on heating the mixture to 35° C., an abundant precipitate formed, which dissolved upon cooling to the temperature of the air (14° C.).

Extract *c* (pancreatic casein) was readily precipitable like extract *b* by calcium chloride. In adding one drop, there was no precipitate in the cold, but an abundant one formed on heating to 35°; and this did not dissolve upon cooling.

Pancreatic casein therefore resembles casein and caseinogen in its ready solubility in lime water; it resembles casein and

[1] This *Journal*, XI. 464. 1890 and XII. 164. 1891.

differs from caseinogen in its ready precipitability from this solution by calcium chloride; but differs from casein in the fact that the precipitate produced by a very small quantity of calcium chloride *plus* heat is not soluble on cooling.

C. The solubility in 5 % sodium chloride of the precipitate produced by the addition of calcium chloride to a lime water solution in each of the three cases was then examined. It was found that the precipitated caseinogen was readily soluble, the precipitated casein quite insoluble, and the precipitated pancreatic casein only slightly soluble.

D. The lime water extracts were also tested as follows :—

a. The solution of caseinogen was not precipitated by a drop of 0·5 % solution of phosphoric acid to 10 c.c. of the solution.

It was not precipitated by rennet.

It was precipitated (converted into casein) by a drop of 0·5 % phosphoric and *plus* rennet.

b. The solution of casein was precipitated by a drop of 0·5 % phosphoric acid to 10 c.c. of the solution. The combined action of phosphoric acid and rennet could therefore not be tested. Rennet alone gave a flocculent precipitate. This was increased by warmth and disappeared on cooling, and is in fact the effect one always gets on adding a dilute solution of a calcium salt to a lime water solution of casein. We have been unable as yet to obtain a specimen of calcium-free rennet, and the effect just described is therefore explicable on the supposition that it is due to the calcium salts in the rennet preparation, rather than to the rennet itself.

c. The solution of pancreatic casein behaved exactly like the solution of caseinogen.

E. Caseinogen, casein, and pancreatic casein were prepared as before and dissolved in 0·5 % sodium bicarbonate. They are all readily soluble.

a. Solution of caseinogen; this is precipitated in the cold by 1 drop of 10 % calcium chloride solution to 10 c.c. It is not however precipitated by 1 drop of a 2 % solution to 10 c.c., but on warming the mixture a heavy precipitate occurs at 35°—40° C., which disappears on cooling.

b. Solution of casein; this requires more than an equal bulk of 10 % calcium chloride solution to precipitate it in the cold; but a much smaller quantity, 4 or 5 drops to 10 c.c., will cause a precipitate if the mixture is warmed to 40° C.; this precipitate redissolves on cooling.

The behaviour of casein and caseinogen in a solution of sodium bicarbonate is almost the reverse to what occurs when they dissolved in lime water. (See *B.*)

c. The solution of pancreatic casein however dissolved in sodium bicarbonate behaves to calcium chloride exactly the same as the lime water solution previously described. (*B. c.*)

These facts are summarized in the following table :—

	a. Caseinogen.	*b. Casein.*	*c. Pancreatic casein.*
A. In water + $CaCO_3$	Soluble	Insoluble	Insoluble
B. In lime water	Soluble ; precipitable with difficulty by $CaCl_2$	Soluble ; precipitable with ease by $CaCl_2$; precipitate produced by trace of $CaCl_2$ at 40° soluble on cooling	Soluble ; precipitable with ease by $CaCl_2$; precipitate produced by trace of $CaCl_2$ at 40° not soluble on cooling
C. The precipitate produced by adding $CaCl_2$ to B.	Soluble in 5 % NaCl	Insoluble in 5 % NaCl	Slightly soluble in 5 % NaCl
D. Lime water solution	Converted into casein by trace of phosphoric acid and rennet.	So readily precipitable by trace of calcium salts that the action of rennet could not be properly tested	Behaves like caseinogen
E. In 0·5 sodium bicarbonate solution	Soluble ; precipitable with ease by $CaCl_2$; precipitate produced by trace of $CaCl_2$ at 40° dissolves on cooling	Soluble ; precipitable with difficulty by $CaCl_2$	Soluble ; precipitable with ease by $CaCl_2$; precipitate produced by trace of $CaCl_2$ at 40° C. not soluble on cooling

Summary.

1. The pancreatic juice obtained from temporary pancreatic fistulæ from dogs produces a change in the caseinogen of milk.

2. This action differs from the action of rennet in the following particulars.

(*a*) The precipitate of casein occurs in the warm bath (35°—40° C.) in the form of a fine granular precipitate, the milk to the naked eye undergoing no change in its fluidity. On cooling this to the temperature of the air, it sets into a coherent curd which contracts to only a small extent, and is again broken up into fine granules by warming to 35° C., the milk to the naked eye becoming again fluid. This may be repeated a great number of times.

(*b*) This phenomenon is not prevented, but only slightly hindered by such an addition of potassium oxalate as completely inhibits the activity of rennet.

3. The experiments performed with extracts of the gland lead to similar results, which may be masked if the action of the tryptic ferment is very energetic.

4. The precipitate produced may be provisionally termed '*pancreatic casein.*' By the action of rennet it can be converted into true casein. Its solubilities, as summarized in the immediately preceding table, are partly like those of caseinogen, partly like those of casein. It is probably something intermediate between the two.

June 1, 1896.

[*From the Journal of Physiology. Vol. XX. No.* 2, 1896.]

NOTES ON CREATININE. By P. C. COLLS, *late Assistant Demonstrator in Physiology, King's College, London.*

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

About two years ago, a lengthy correspondence was published in the *Lancet*[1] on the question whether normal urine contains any sugar. The principal contributors to the discussion were Dr Pavy and the late Sir George Johnson. The former maintained that normal urine contains sugar to the extent of about 0·05 per cent.[2]; he succeeded in precipitating from urine by means of lead acetate a substance which gave the reactions for sugar including the fermentation test with yeast, and the formation of a typical osazone with phenylhydrazine. Sir George Johnson maintained on the other hand that the principal reducing substance in urine is creatinine, and that many of Dr Pavy's facts were explicable on the assumption that he had neglected to recognise the reducing properties of this substance.

The fact that small quantities of carbohydrate material including dextrose are obtainable from normal urine has been recently demonstrated by Baisch[3], who used benzoyl chloride as a precipitant. The percentage of dextrose given by this observer is however much smaller (0·005) than that given by Dr Pavy.

It was therefore suggested to me by Prof. Halliburton that I should undertake some experiments in order to clear up some of the points raised, and the following is an account of what I have done under his superintendence.

The main question of the existence of sugar in the urine I have not touched, but my work has fallen under two main heads:—

[1] 1894, vol. II.

[2] See also Pavy, *Physiology of the Carbohydrates*, p. 187. Dr Pavy's words are: If the cupric oxide reduction found to occur be read off as produced entirely by sugar, the amount of this substance in normal urine may be said to be about 0·5 per 1000.

[3] *Zeitsch. f. physiol. Chem.* XIX. p. 339. 1894. This observer gives the quantity of glucose as 0·005 per cent. and of total carbohydrate in normal urine reckoned as glucose as about twice that amount.

1. Whether the method used by Dr Pavy for precipitating sugar does also precipitate creatinine? If so this would in part account for the high figure given by Dr Pavy. The answer to this question I have found to be in the affirmative.

2. Whether any creatinine exists in the blood? The answer to this question was also found to be in the affirmative. Creatinine has not been previously described in the blood, but considering the relatively large amount of creatinine in the urine, its presence in the blood would be expected.

If the sugar of the blood is estimated by the reduction method, this factor will therefore have to be allowed for in the future; some means must be taken by which the sugar obtained is not contaminated with creatinine.

The details of my experiments in these two directions are the following:—

1. *The Precipitation of Creatinine from the Urine.*

The older method of precipitating creatinine from urine by means of zinc chloride has been recently replaced by a more efficacious method introduced by G. S. Johnson[1], which is as follows:—To the urine a twentieth of its volume of a saturated solution of sodium acetate, and then one-fourth of its volume of a saturated solution of mercuric chloride are added; this produces an immediate abundant precipitate of urates, sulphates and phosphates, which is removed by filtration; the filtrate is then allowed to stand for twenty-four hours, when the precipitation of a mercury salt of creatinine $(C_4H_5HgN_3OHCl)_4\,3HgCl_2 + 2H_2O$ occurs; the compound takes the form of minute spheres, a photo-micrograph of which is reproduced in the paper just quoted. They are quite typical on microscopic examination. On account of its heaviness this compound lends itself very well to quantitative analysis. It may be collected, dried, and weighed. One-fifth or, more correctly, 20·19 per cent. of the weight found is creatinine. Creatinine may be obtained from it by suspending it in water, decomposing it with sulphuretted hydrogen and filtering. On concentrating the filtrate in a vacuum over sulphuric acid, it deposits creatinine hydrochloride, and then lead hydrate may be used to liberate creatinine from it.

Johnson has shown that this method does not precipitate sugar; and further that the urinary creatinine he obtains is different in some of its properties (such as reducing power, solubility and character of its

[1] *Proc. Roy. Soc.* XLIII. p. 493. 1887.

gold salts) from the creatinine obtained artificially from the creatine of flesh; the two creatinines are, however, isomeric.

An important point in Johnson's process is that all the operations are carried out in the cold; if heat is applied, one obtains the creatinine of former writers which has no reducing power.

I have repeated and confirmed these observations, and find that urinary creatinine reduces both picric acid, and Fehling's solution.

I then went on to ascertain whether the method used by Pavy (Brücke's method) for the separation of sugar from urine also precipitates creatinine. I select the following typical experiments from a large number which I have done.

Experiment 1. A 0·2 per cent. solution of Johnson's urinary creatinine was taken. To 10 c.c. of this, 2·5 c.c. of saturated solution of lead acetate, and 2·5 c.c. of a solution of tribasic lead acetate were added. The mixture was then filtered and 10 c.c. of liquor ammoniae fortior added, and the precipitated lead hydrate filtered off; 2·5 c.c. of the filtrate then showed with picric acid considerably less reducing power than 1 c.c. of the original solution to which this quantity would correspond, which showed that some of the creatinine had disappeared. I however subsequently found that the creatinine had entirely disappeared, and that in this experiment which was one of the earlier ones I did, the residual reducing power of the final filtrate was due to some lead which was held in solution by excess of ammonia.

Experiment 2. This experiment was performed in the same way as the last, but only 5 c.c. of the ammonia solution were added, and the filtrate was acidified and treated with sulphuretted hydrogen to remove any remaining lead. The sulphuretted hydrogen was evaporated off and the final filtrate had then no reducing power.

Experiment 3. Experiment 2 was repeated; the final filtrate as before had no reducing power; but the reducing substance was found in the precipitate mixed with the lead hydrate. This precipitate was dissolved in warm dilute acetic acid, treated with sulphuretted hydrogen to get rid of lead, the filtrate evaporated to get rid of sulphuretted hydrogen; it then gave the usual reactions for creatinine.

Experiment 4. Normal urine was tested by the picric acid test and gave a good colouration due to creatinine, for none was given by the urine after the creatinine had been removed from it by Johnson's method. This urine was treated in the same way with lead acetate, etc. as just described in the case of the aqueous solutions of urinary creatinine, and the final filtrate found to be free from reducing action on picric acid. This test is a very delicate one for creatinine, being given by a solution of 1 part of that substance in 200,000 of water.

These experiments show that urinary creatinine like sugar is precipitated from aqueous solutions, and from urine when Brücke's method is employed. It is therefore probable that Pavy's high figure for the percentage of sugar in normal urine is due to this circumstance.

2. *Creatinine in Blood.*

Johnson's method appeared to be the most promising for discovering the presence of creatinine in blood, and it was adopted with a slight modification. The great difficulty was to get rid of proteid matter.

For this purpose, I first used trichloracetic acid, but abandoned it when I found that the presence of this acid in an amount sufficient to precipitate proteids, prevents the formation of the spherical mercury salt of creatinine. This is true for urine as well as blood.

I then used saturation with ammonium sulphate to get rid of proteids. The admixture of this salt with the urine does not precipitate creatinine[1], nor does it hinder the formation of Johnson's spherical mercury salt. I employed this method for some time with defibrinated blood and serum[2], and after removal of the proteid precipitate always obtained in the filtrate the typical spherical salt of creatinine. I, however, discarded this method in the end for a simpler one, which is as follows:—

To fresh defibrinated blood, a quarter of its bulk of saturated solution of mercuric chloride was added; the mixture was shaken and filtered: to the filtrate more mercuric chloride was added until no further precipitate occurred, and the proteid was thus almost altogether removed; but no creatinine had been precipitated because for this, as Johnson has shown, sodium acetate is also necessary.

Then to the proteid-free filtrate, one-twentieth of its bulk of a saturated solution of sodium acetate was added, and the mixture set aside. As fresh blood came in, it was treated in the same way. In each case there was a typical spherical precipitate mixed with some pigment and amorphous matter probably due to traces of proteid left in solution.

The resulting precipitates from many litres of blood were mixed and washed. It was then suspended in water and treated with sulphuretted

[1] See Edmunds. This *Journal*, XVII. 452. 1894–5.

[2] My experiments have been made throughout with sheep's blood, of which it is possible to obtain large quantities with ease.

hydrogen. It was then filtered; excess of sulphuretted hydrogen was evaporated off in a vacuum. From this the creatinine was again precipitated as the spherical mercury salt, and this process repeated; on evaporating the final solution in a vacuum crystals were obtained which gave the following tests:—

1. Weyl's test. On adding sodium nitro-prusside, heating, and then adding dilute potash, a ruby red colour was produced, which changed to green on adding acetic acid.

2. Jaffe's test. On adding an equal amount of a saturated solution of picric acid, and then dilute potash, a red colour was developed intensified on boiling.

3. Mixed with mercuric chloride and sodium acetate a spherical compound separated out.

4. Mixed with mercuric chloride and caustic potash a white precipitate occurred soluble in excess of potash. After a few seconds a yellow precipitate was deposited which became dark from reduction.

5. Reduction occurred on heating with Fehling's solution.

6. On the addition of an alcoholic solution of zinc chloride, and waiting a few days, crystals resembling zinc chloride creatinine were seen.

Although I had not sufficient material to make an elementary analysis, I think I am perfectly justified in concluding that the substance I was dealing with was creatinine.

One of the experiments I made was a quantitative one; from 2000 c.c. of defibrinated sheep's blood, I obtained the spherical mercury salt, which after re-solution and re-precipitation was found on microscopic examination to be composed entirely of the characteristic spherical crystals; it was dried and weighed, and the percentage of creatinine in the blood was from this calculated to be 0·000095.

From this part of my work, I therefore draw the general conclusion that blood contains a small but ponderable amount of creatinine.

June 15, 1896.

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EXPERIMENTS ON THE HEARTS OF MAMMALIAN AND CHICK-EMBRYOS, WITH SPECIAL REFERENCE TO ACTION OF ELECTRIC CURRENTS.

BY J. W. PICKERING, D.Sc. (Lond.), *George Henry Lewes Student.*

(*In part from the Physiological Laboratory of King's College, London.*)

IN a series of papers[1] published during the last few years I have recorded the action of thermal and chemical stimuli on the hearts of chick-embryos. In this communication I record a number of observations on the hearts of mammalian embryos, the mammals used being rats, dogs and rabbits. In all cases where the mother lived during the experiment, a mixture of ether and chloroform was the anæsthetic used, while in the larger number of excision experiments the mother was anæsthetised by ether.

The object as in my previous work was to study the action of various media and stimuli on the heart prior to the development of its intrinsic nervous "mechanism", and that associated with heat-regulation; and further to ascertain whether the hearts of mammalian embryos, behaved in a similar or dissimilar manner, to the adult heart under the influence of certain stimuli, and what were the differences if any, between the physiological reactions of the embryonic and adult heart.

More than one-half of the work recorded here deals with the results produced by the action of constant and interrupted electric currents, of various intensities, on the hearts of mammalian and chick-embryos. Deductions from these observations will be found to bear on the current theories that have been advanced concerning the phenomena of cardiac inhibition.

[1] Pickering. *Proc. Roy. Soc.* LII. p. 461; This *Journal*, XIV. p. 383. 1893; *Trans. Odont. Soc.* XXII. p. 42. 1893; This *Journal*, XVII. p. 395. 1894; *Ibid.* XVIII. p. 470. 1895.

Previous work.

Prior to the researches of Bischoff[1] it was considered doubtful whether the hearts of earlier mammalian embryos pulsated. This was due, as pointed out by W. Preyer[2], to the fact, that exposure to the temperature of the surrounding atmosphere rapidly slows their cardiac rhythm to stoppage. The latter observer made six experiments on early guinea-pig embryos, at a time just before the development of their teeth, and found that their cardiac rhythm when stopped by exposure, could usually be restored by subsequent heating of the embryos. He also showed that a weak interrupted electric current accelerated their cardiac rhythm, while stronger currents produced a condition which he terms tetanus. He also states that a constant electric current has no action on the hearts of mammalian embryos, but that it causes an acceleration of the heart rhythm of chick-embryos. I am unaware of any observations on the action of chemical stimuli on mammalian hearts. The previous work on chick-embryos has been fully dealt with in my papers cited above.

My paper can be conveniently divided as under:—

PART I.

ON MAMMALIAN EMBRYOS IN GENERAL.

(1) Methods of observation.
(2) The sustaining power of various proteid solutions. (Tables 1 to 4.)
(3) The action of normal saline. (Table 5.)
(4) The action of Grimaux's synthesised proteid-like colloids. (Tables 6, 7 & 8.)
(5) The action of saline solutions. (Tables 9, 10 and 10a.)
(6) The influence of ordinary and pure distilled water. (Tables 11 and 12.)
(7) On oligodynamic action.
(8) The influence of traces of various colloids, and other organic bodies on oligodynamic action. (Table 13.)
(9) The comparative action of isotonic and other solutions. (Tables 14 and 15.)
(10) The action of various drugs comprising—caffein, veratrin, nicotin, digitalin, strophanthin, antiarin, morphine acetate, muscarin nitrate, atropin sulphate, chloroform and ether.

PART II.

ELECTRICAL OBSERVATIONS ON THE EMBRYONIC HEART.

(1) The action of interrupted currents on chick-embryos. (Tables 16 to 21.)
(2) The action of various drugs on the subsequent action of interrupted currents on chick-embryos. (Tables 22 to 32.)
(3) The action of oxygen and carbonic dioxide on the subsequent action of interrupted currents on chick-embryos. (Tables 33 to 36.)

[1] Bischoff. *Entwicklung des Kaninchen-Eies*, 1842. pp. 120—123.
[2] Preyer. *Specielle Physiologie des Embryo*, 1885. p. 30.

(4) The significance of the preceding experiments.
(5) The influence of constant currents on chick-embryos.
(6) The action of constant currents on the subsequent action of interrupted currents on the hearts of chick-embryos. (Tables 37 to 48.)
(7) The action of single and a series of induction shocks on the hearts of chick-embryos. (Tables 49 to 52.)
(8) The action of single and a series of induction shocks on the subsequent action of interrupted currents on chick-embryos.
(9) The action of single induction shocks, constant currents and interrupted electric currents on the hearts of mammalian embryos. (Table 53.)

PART III.

General conclusions.

PART I.

On Mammalian Embryos in General.

(1) *Methods of Observation.*

(*a*) *Mammalian Embryos.* At first I attempted to observe the embryos *in situ* in the uterus operating on the animals so as to expose them.

The animal, except its head, was covered by a wooden box; the space between the animal and the box was packed with silicate of "cotton". A small window, covered with glass, allowed the observations to be made. Some of the experiments performed in this manner are recorded in Section 10, Part I., others in Section 9, Part II.

Notwithstanding these precautions it was found very difficult to maintain the exposed surfaces at a constant temperature; and the embryos' rhythm consequently became irregular. Further, the anæsthetic given to the mother would probably affect the embryos by diffusion through the fœtal circulation, and thus vitiate the results. Subsequently, I entirely removed the embryos from the mother, leaving them attached to a small portion of the walls of the excised uterus. Care was taken to ligature all the larger blood vessels. The embryos were then placed in a shallow porcelain dish containing a mixture of equal volumes of defibrinated blood of the mother and a solution of ·75% sodium chloride, warmed to the required temperature. The whole was placed in an observing incubator similar to that figured and described in my previous papers; five of the sides of which were made of a water-jacket coated with silicate of cotton, while the top consisted of a glass roof. The temperature of the interior of the incubator was kept constant, or varied at pleasure, by a small gas flame controlled by a

regulator. A pair of silver electrodes were so fixed that when desired they could be brought into contact with the surface of the embryo.

When the excised mammalian embryos are first placed in the incubator, their cardiac rhythm is irregular, but after remaining in a uniform temperature for a period varying from 15 to 30 minutes, the rhythm becomes constant, and if the conditions of the experiment remain unchanged, can be maintained unvaried for several hours. In the younger mammalian embryos it has been found that the rhythm is better maintained by surrounding them with a mixture of defibrinated blood or of serum, and ·75 % sodium chloride, than by a solution of ·75 % NaCl alone. It is, however, important as will be shown in a subsequent section, that the blood of an animal of the same species be used.

(β) *Chick-Embryos.* In the experiment performed upon chick-embryos recorded in Part II, the methods adopted were those already described in my previous papers on the embryonic heart.

(γ) *Solutions Used.* Except where otherwise stated, all substances used were dissolved in ·75 % NaCl, and were administered at the temperature of the embryos. The doses are expressed in milligrams per cubic centimetre of the fluid given.

(2) *Action of Various Proteid Solutions.*

The first table records the cardiac frequency in beats per minute of twelve excised rat embryos kept at a constant temperature of 38° C. The embryos were bathed in a mixture of equal volumes of defibrinated rat's blood and ·75 % of sodium chloride. Those taken from the same mother are denoted by the same letter.

TABLE 1.

Number of the embryo.	Age in days.	Cardiac frequency.	Maximum and variation of cardiac frequency during 180 mins.
1 a	17	22	0
2 a	17	26	+ 1
3 a	17	30	− 1
4 a	17	21	0
5 b	18	20	1
6 b	18	19	+ 2
7 b	18	21	0
8 c	19	20	0
9 c	19	22	+ 1
10 c	19	24	0
11 d	21	26	− 2
12 d	21	20	0

From the above table it will be evident that although each individual embryo has, when kept under unvaried conditions, a practically constant cardiac rhythm, yet embryos of the same age taken from the same mother have different cardiac frequencies.

These results are similar to those I have recorded[1] as characteristic of the hearts of chick-embryos and of those of daphniæ. I have always observed the cardiac contraction to be in the form of peristaltic wave, and not sometimes simultaneous as stated by Preyer[2]; my observations being in accord with those of Fano and Badano[3] on embryo-chicks.

If a mammalian embryo be allowed to cool, its ventricle ceases beating before the auricle; on raising the temperature the auricle recommences to beat before the ventricle. Mechanical stimulation of the quiescent ventricle leads to a contraction commencing from the auricle.

The second series of experiments shows the results of bathing excised early rat embryos in a mixture of defibrinated dog's blood and ·75 % NaCl.

TABLE 2.

Temperature of embryos 38° C.

No. of embryo.	Cardiac frequency when bathed in mother's blood.	Average cardiac frequency in beats per min. when bathed in dog's blood.	Remarks on rhythm.	Age of embryo in days.
13e	22	29	Varies between 20 & 36, very weak and irregular.	15
14e	17	18	Much weaker than normal.	15
15e	20	26	Ditto.	15
16e	24	27	Varies between 18 & 36, fluttering and irregular.	15
17f	20	16	Has long diastole & bouts of rapid irreg. beating.	
18f	22	20	Ditto.	16
19g	17	14	Ditto.	14
20g	20	24	Varies between 18 & 28, rhythm weak and irreg., sometimes reversed.	14

An interval of 15 minutes was allowed after changing the fluid surrounding the embryos before the observations were recorded. Control experiments in which the surrounding fluid was drawn off and immediately restored, showed that the irregularities of cardiac rhythm resulting from the transfer only lasted 1 or 2 minutes. Care was

[1] *Op. cit.* and This *Journal*, XVII. p. 356. 1894.

[2] *Loc. cit.*

[3] *Archives Italiennes de Biologie*, XIII. p. 387. 1890.

taken that the new liquid administered was at the same temperature as the embryo.

Later embryos are not influenced in so marked a degree by a change of the blood used as their bathing fluid. This is probably due to the fact that they contain in their own circulation sufficient blood for their immediate metabolism.

I have, however, found that a mixture of equal volumes of egg-albumen and ·75 °/₀ NaCl has not such an inimical action on the hearts of excised rat-embryos as the blood of another species, as is shown by the following table:—

TABLE 3.

Temperature of embryos 38° C.

No. of embryo.	Age of embryo in days about.	Cardiac frequency when bathed in mother's blood.	Cardiac frequency when bathed in egg-albumen.	Remarks on rhythm.
21 g	14	22	23 to 24	Slight irregularities.
22 h	14	20	18 to 20	Ditto.
23 h	16	18	18 to 20	No change of characteristics observed.
24 h	16	21	19 to 26	Rhythm weaker.
25 h	16	20	20 to 21	Very slight irregularities.
26 i	15	18	18 to 22	Ditto.

The life of early rat-embryos will even be sustained for a considerable time by a mixture of egg-albumen and ·75 °/₀ NaCl in equal parts. The experiments recorded in the next five tables lasted three days, during which period in some cases the cardiac rhythm of the embryos was found to be well sustained, but the growth of them was apparently retarded. In each case the uterine wall to which the embryo was attached was reduced to a minimum and the embryos were washed in warm normal saline (at 38° C.) to remove as much blood as possible. The temperature during the whole period of the experiments only varied between 37·5° C. to 38·8° C. A maximum and minimum thermometer was placed inside the incubator to indicate any variations.

The record in table four summarizes the results obtained with five rat-embryos kept in the above-mentioned mixture of egg-albumen and ·75 °/₀ NaCl for three days. The observations were taken three times each day at intervals of three hours, and the maximum and minimum of cardiac frequency observed on each day is recorded.

TABLE 4.

No. of embryo.	Age at commencement of exp.	Cardiac frequency 20 mins. after immersion.	Cardiac frequency after 1 day.	Cardiac frequency after 2 days.	Cardiac frequency after 3 days.
24 h	16 days	19 to 26	17 to 20	16 to 18	14 to 18
25 h	16 ,,	20 to 21	20 to 22	18 to 20	18 to 20
26 i	15 ,,	18 to 22	16 to 22	16 to 20	14 to 16
27 i	15 ,,	16 to 18	12 to 14	12 to 14	8 to 10
28 i	15 ,,	13 to 15	13 to 15	10 to 1	12 to 14

(3) *Action of Normal Saline.*

In the next table the results obtained with five other rat-embryos of similar ages, kept under similar conditions except that the embryos were bathed in ·75 % sodium chloride without the addition of any proteid or other matter. The maximum and minimum frequency is recorded each day.

TABLE 5.

No. of embryo.	Age of embryo at commencement of exp.	Cardiac frequency 20 mins. after immersion.	Cardiac frequency 1 day after immersion.	Cardiac frequency 2 days after immersion.
28′ i	15 days	17 to 20	8 to 10	2 to 4
28″ i	15 ,,	16 to 18	13 to 14	0
29 j	14 ,,	18 to 22	7 to 9	0
30 j	14 ,,	15 to 16	8 to 10	0
31 j	14 ,,	17 to 18	0	0
32 k	15 ,,	12 to 14	3 to 4	0
33 k	15 ,,	10 to 12	0	0
34 k	15 ,,	13 to 15	0	0

It will be evident that under these conditions the embryos' hearts lose their activity, and further, it was found that, except in one case, they were on the third day irresponsive to both thermal and electrical stimuli. There is therefore a marked difference in the sustaining power of a mixture of egg-albumen and ·75 % NaCl, to that of ·75 % NaCl alone.

(4) *Action of the Synthesised Proteid-like Colloids A, B, and C (Grimaux's Colloids).*

In a series of papers recently published by Prof. Halliburton and myself[1] it has been shown that certain synthesised colloids behave in a

[1] Pickering. *Proc. Physiol. Soc.* Feb. 16, 1895; (This *Journal*, XVII.); This *Journal*, XVIII. p. 54. 1895; *Comptes Rendus*, T. CXX. p. 1348. 1895; Halliburton and Pickering, This *Journal*, XVIII. p. 285. 1895.

manner not unlike proteids. Of these substances the colloid *C*, which is formed by the action of a current of gaseous ammonia heated to 170° C. on solid aspartic anhydride, exhibits the following four striking characteristics:—(1) It gives with $CuSo_4 + KHO$ a violet colour reaction. (2) After treatment for some days at 38° C. with pepsin and ·2 °/₀ HCl it gives with the same reagents a pink reaction like a proteose or a peptone. (3) With neutral salts as precipitants, or on heating with soluble salts of Ba, Sr, or Ca, it behaves like a proteid. (4) When injected in a ·5 °/₀ to 1·5 °/₀ solution into the veins of black rabbits, dogs, rats or cats it causes extensive intravascular clotting like a nucleo-albumin.

Bearing in mind these observations it became of interest to ascertain whether solutions of this substance and of allied bodies behave like a proteid towards embryos immersed in them.

The following table shows the effect of keeping six rat embryos in a 7·5 °/₀ solution of the colloid *C* for three days under similar conditions to those described in the preceding experiments:—

TABLE 6. (With Colloid *C*.)

No. of embryo.	Age at commencement of exp.	Cardiac frequency 20 mins. after immersion.	Cardiac frequency 1 day after immersion.	Cardiac frequency 2 days after immersion.	Cardiac frequency 3 days after immersion.
35 l	14 days	16 to 18	15 to 18	12 to 14	8 to 12
36 l	14 ,,	17 to 19	12 to 14	10 to 12	7 to 10
37 l	14 ,,	20 to 24	13 to 16	8 to 14	8 to 10
38 m	15 ,,	19 to 26	16 to 19	14 to 17	8 to 12
39 m	15 ,,	18 to 28	12 to 14	8 to 10	6 to 8
40	15 ,,	16 to 18	12 to 16	7 to 9	0

A study of the preceding tables shows that the hearts of rat embryos sustain their rhythm in a 7·5 °/₀ solution of the colloid *C* almost as well as in a solution of egg-albumen. This fact is all the more striking as on incineration the colloid *C* was found not to contain salts like egg-albumen and serum, so that the result cannot be attributed to the salts in combination or solution. The colloids *A* and *B* referred to the papers previously cited, which are formed by the interaction of phosphorous pentachloride on meto-amido benzoic acid at 125° C. to 130° C., and which exhibit certain characteristics in common with proteids do not behave in a like manner to the colloid *C*. They apparently do not sustain a cardiac rhythm more than a ·75 °/₀ solution of NaCl alone.

The subjoined tables record these results. (Compare table 5.)

TABLE 7. (7·5 % Colloid *A* used.)

No. of embryo.	Age at commencement of exp.	Cardiac frequency 20 mins. after immersion.	Cardiac frequency after 1 day's immersion.	Cardiac frequency after 2 days' immersion.
41 n	14 days	18 to 20	12 to 14 (feeble)	0
42 n	14 "	19 to 20	0	0
43 n	14 "	20 to 24	8 to 10 "	0
44 o	15 "	22	0	0
45 o	15 "	24 to 26	0	0
46 o	15 "	18 to 20	6 to 8 "	0

TABLE 8. (With 7·5 % solution Colloid *B*.)

No. of embryo.	Age at commencement of exp.	Cardiac frequency 20 mins. after immersion.	Cardiac frequency after 1 day's immersion.
47 p	12 days	16 to 18	3
48 p	12 "	25 to 27	0
49 p	12 "	19 to 21	0
50 q	15 "	18	0
51 q	15 "	24 to 28	0
52 q	15 "	26 to 28	0

Reference to the papers cited on synthesised colloids will show that the colloids *A* and *B* do not approach in their chemical characteristics, so near to proteids as the colloid *C*. It is therefore interesting to note that the sustaining power of the two former bodies is far inferior to that of the colloid *C*, which is almost as marked as that of albumen.

(5) *Action of Saline Solutions.*

The next series of experiments were performed to determine the relative sustaining power of various saline solutions, when directly applied to the hearts of mammalian embryos. The first solution used was that known as "Ringer's" Circulating Fluid[1].

As in the preceding experiments excised rat-embryos were the subjects of the observations. During the two days the experiment lasted a temperature of which the extreme limits of variation were 37·8° C. and 38·6° C. was maintained. Prior to the transference from

[1] The composition of the fluid is as stated under; a saturated solution of tribasic phosphate of lime in ·6 % saline solution, with the addition of 1 c.c. of 1·5 % solution of potassium chloride to 100 c.c. of the mixture (Ringer, This *Journal*, VII. p. 291). Ten cubic centimetres of this solution were used for the immersion of each embryo.

the mother's blood the embryos were washed in the saline medium used. The other conditions were maintained constant. The results summarized in the following table are recorded as beats per minute:—

TABLE 9.

No. of embryo.	Age of embryo.	Cardiac frequency bathed in mother's blood.	Cardiac frequency after 15 mins. in Ringer's fluid.	Cardiac frequency after 1 day in Ringer's fluid.	Cardiac frequency after 2 days in Ringer's fluid.
53 r	10 days	19	18 to 20	12	0
53 r	10 ,,	24	16	8	2
54 s	14 ,,	26	14 to 15	0	0
55 s	14 ,,	30	25 to 26	7	4
56 t	15 ,,	17	12 to 13	6	3
57 t	15 ,,	28	16	4	0

A comparison with tables 5 and 6 shows that Ringer's fluid exhibits a power of sustaining the cardiac rhythm of mammalian embryos far inferior to that of the colloid *C*, but superior to that of ·75 % NaCl. No growth of the embryos was observed during immersion.

The rhythm was comparatively constant during the first four hours after immersion, but subsequently, a diminution both of force and frequency was observed, which culminated in death in diastolic stoppage usually after 50 hours' action.

A result only slightly differing in degree is obtained when the salts of blood ash are substituted for "Ringer's Fluid." They are apparently of about equal sustentive power, but far inferior to that of the colloid *C*, as is shown by the summary in the following table. The animals used were rats. The conditions of the experiments were unchanged from that of the previous series.

TABLE 10.

No. of embryo.	Age of embryo.	Cardiac frequency bathed in mother's blood.	Cardiac frequency after 15 mins. in sol. of blood ash.	Cardiac frequency after 1 day in sol. of blood ash.	Cardiac frequency after 2 days in sol. of blood ash.
58 u	10 days	20	18 to 20	9 to 11	0
59 u	10 ,,	21	19 to 21	5 to 7	0
60 u	10 ,,	19	18 to 19	—	0
61 v	14 ,,	17	15 to 17	9 to 13	6
62 v	14 ,,	18	17 to 18	13 to 15	5

A strikingly similar result is obtained when the ash of gum arabic is substituted for that of blood in the water acting as the medium

surrounding the embryos' hearts. The next table records the result at 38° C.

TABLE 10a.

No. of embryo.	Age of embryo.	Cardiac frequency bathed in mother's blood.	Cardiac frequency after 15 mins. in sol. of blood ash.	Cardiac frequency after 1 day in sol. of blood ash.	Cardiac frequency after 2 days in sol. of blood ash.
58 u	10 days	18	16 to 18	9 to 10	0
59 u	10 ,,	20	20	18 to 13	3 (feeble)
60 u	10 ,,	21	19 to 22	17 to 19	7
61 v	14 ,,	17	16	11 to 13	0
62 v	14 ,,	20	18 to 20	6 to 8	6

Two similar series of experiments were performed where the salts in solution were respectively KCl (strength 1 c.c. of 10 °/₀ solution to 200 c.c. of pure distilled water) and a mixture of the same strength of KCl plus 2 c.c. of a 10 °/₀ solution of calcium nitrate added to every 200 c.c. of the solution.

For reasons explained in a subsequent section, care was taken that only water that had been distilled into glass receivers was used. These solutions were chosen in order to make a comparison with the work of Ringer and Sainsbury[1] on Tubifex rivulorum.

The potassium chloride solution was found to have stopped the hearts of rat embryos kept at 38° C. after from 6 to 8 hours' action, while in those cases where there was an addition of calcium nitrate to the solution, the embryos' hearts maintained a fairly strong rhythm after 10, and in some cases 15 hours, and in one case a strong rhythm existed after 22 hours had elapsed.

Cardiac stoppage usually supervened after 10 or 12 hours, but in the extreme case above cited, did not take place until after 24½ hours.

There is therefore a general correspondence between these results and those cited above as obtained by Ringer and Sainsbury on Tubifex rivulorum.

(6) *Action of Distilled Water.*

Ringer[2] working in collaboration with Sainsbury and Phear[3] has shown that distilled water prepared in metal containers is fatal to Tubifex rivulorum, causing death and subsequent disintegration.

[1] This *Journal*, XVI. 1. 1894.

[2] Ringer and Sainsbury. This *Journal*, XVI. p. 1. 1894.

[3] Ringer and Phear. This *Journal*, XVII. p. 423. 1895; Ringer, *ibid.* XVIII. p. 425.

Nägeli[1] found that commercial distilled water was rapidly fatal to vegetable protoplasm; but that water distilled into glass receptacles did not exert a toxic action. More recently Locke[2] has extended these observations to animal organisms, and has found that both Tadpoles and Tubifex rivulorum will survive for a considerable time in water that has been distilled into glass receivers, while "Aqua distillata" prepared in metal containers was extremely toxic. I have, therefore, tested the action of these two kinds of distilled water on the hearts of embryos. In the following table the action of water at 38° C. (distilled into glass receivers) on 10 day rabbit embryos is recorded.

TABLE 11.

No. of embryo.	Cardiac frequency in mother's blood.	Cardiac frequency after immersion for 15 mins. in pure water.	Cardiac frequency after 4 hrs in pure water.	Cardiac frequency after 12 hrs in pure water.	Cardiac frequency after 18 hrs in pure water.
63 w	22	17	16 to 18	8 to 12	0
64 w	20	14 to 16	14 to 16	0	0
65 w	26	22	20 to 22	14 to 17	6 (weak)
66 x	18	15 to 16	10 to 12	8 to 10	0
67 x	24	24	8 to 10	4 to 6	0
68 x	14	14	7 to 9	0	0

A comparison of the foregoing table with the results recorded in table 5, will make it evident that the sustaining power of pure distilled water is considerably inferior to that of ·75 °/₀ sodium chloride. It is, however, noteworthy that in each experiment a rhythm of moderate frequency and force was maintained after 4 hours' action, and even after 12 hours, four of the hearts were beating rhythmically. The force of the beats was, however, diminished and their diastoles drawn out. My results are in accord with those of Locke[3] in as far as pure distilled water does not seem to have a toxic action on animal contractile tissues.

In the next table the results obtained with aqua distillata on the hearts of 10 day rabbit-embryos is recorded. The water had been distilled into a copper receiver and left in contact with a bright strip of copper for one day. The temperature at which the experiments were conducted was again 38° C.

[1] Nägeli. *Denkschriften der schweizerischen naturf. Gesellschaft.* XXXIII. p. 1. 1893.

[2] Locke. This *Journal*, XVIII. p. 319. 1895.

[3] *Loc. cit.*

TABLE 12.

No. of embryo.	Cardiac frequency in mother's blood.	Cardiac frequency 10 mins. after immersion.	Cardiac frequency 15 mins. after immersion.	Cardiac frequency 20 mins. after immersion.	Cardiac frequency 25 mins. after immersion.
69 y	27	16	8	0	0
70 y	20	0	0	0	0
71 y	26	12	8	4	2
72 y	18	14	12	2	0
73 z	20	10	5	0	0
74 z	16	3	0	0	0

The statistics recorded above show that distilled water (in copper receivers) exerts a markedly toxic action on the hearts of 10 day rabbit-embryos. The cardiac stoppage was always in diastole; for a short interval of time the hearts remained responsive to an interrupted electric current, but the rhythm resulting from the stimulation was in each case weaker than the normal. On shutting off the current the hearts continued to beat for a time varying from 2 to 10 minutes. After a second stoppage a repetition of the stimulation in some cases again partially restored the rhythm, but it became progressively weaker, and finally after the immersion in the water had lasted from 20 to 30 minutes, the hearts became irresponsive to both electrical and thermal stimuli. These results correspond with those usually deemed characteristic of the action of distilled water on animal tissues. The post-mortem disintegration described by Ringer and his collaborators as characteristic of its action on tubifex was also observed.

(7) *Oligodynamic Action.*

An explanation of the difference of action of the two kinds of distilled water on vegetable protoplasm has been advanced by Nägeli[1]. He suggests that the water distilled into metal receivers owes its toxic action to small quantities of metal it has taken into solution, and he terms the action of these minute quantities of the heavy metals on organized matter "oligodynamic action." To illustrate the extremely minute quantities of metal producing a toxic action, he estimates that $\frac{1}{77000000}$ of copper present in one experiment caused pronounced toxic action on the protoplasm of a filament of spyrogyra. Further the addition of small quantities of various colloidal substances such as gum, albumin, dextrin or gelatin, renders the otherwise poisonous water harmless.

[1] *Op. cit.*

(8) *The influence of traces of Colloids and other bodies on Oligodynamic action.*

The preceding experiments make it evident that water distilled into metal containers acquires a toxic action on the myoplasm of mammalian embryonic hearts, and further it is very probable that this action is due to traces of metallic substances in solution. Ringer and Phear[1] have recently shown that the addition of minute quantities of various salts, acids, and alkalis, remove the toxic and disintegrating action of water distilled into metal containers on the contractile tissue of tubifex rivulorum. For the sake of comparison I quote the following solutions used by these observers:— $\frac{1}{400000}$ of potassium ferrocyanide, $\frac{1}{20000}$ potassium citrate, $\frac{1}{1000000}$ of hydrocyanic acid, $\frac{1}{200000}$ of sulphuric acid and $\frac{1}{5000}$ of sodium hydrate. Any of which considerably retard the toxic action. The results obtained with minute quantities of the following bodies, acting on early rat embryos, are recorded in the next table. The water used had been distilled into copper receivers.

TABLE 13.

Temperature of experiments 38° C. The frequencies recorded in each case are the average number of beats per minute observed in six experiments.

Solution used as surrounding medium.	Cardiac frequency 10′ after immersion.	Cardiac frequency 20′ after immersion.	Cardiac frequency 30′ after immersion.	Cardiac frequency 1 hr after immersion.	Cardiac frequency 2 hrs after immersion.
Water distilled into metal receiver (for comparison)	9·5	1	0	0	0
Solution of $\frac{1}{10000}$ gelatin (slightly acid)	12	12	8	8	6
$\frac{1}{10000}$ colloid *A* (neutral sol.)	9	7·3	6·1	5	3
$\frac{1}{10000}$ colloid *C* (neutral sol.)	10	8·2	6·3	4·8	3·1
$\frac{1}{20000}$ gelatin	9·3	8·1	5	0	0
$\frac{1}{20000}$ colloid *A*	6	4	0	0	0
$\frac{1}{20000}$ colloid *C*	5	3	2	0	0
$\frac{1}{10000}$ caffein	8	6	3	0	0
$\frac{1}{20000}$ caffein	8	5	0	0	0
$\frac{1}{10000}$ digitalin	9	6	5	3	0
$\frac{1}{20000}$ digitalin	8	7	4	0	0
$\frac{1}{50000}$ H_2So_4	7	5	3	2	2
$\frac{1}{5000}$ NaOH	6	4	3	2	0

[1] *Loc. cit.*

It is evident that each of the substances experimented with modifies to a certain degree the toxic action of water distilled into copper retainers. It may be noted here that in order to remove the factor of the oligodynamic action of metals from these experiments all the distilled water used in preparing ·75 % NaCl, which was the medium for the administration of the various substances, was distilled into glass receivers.

(9) *The comparative action of isotonic and other solutions.*

The statement of Albanese[1] and of Ohrn[2] that a 2 % solution of gum arabic to which a trace of sodium carbonate had been added, provides an excellent nutrient fluid for the frog's heart, has led me to investigate its sustaining action on the hearts of rat-embryos. The results obtained are recorded in the next table:—

TABLE 14.

Embryos kept at 38° C.

No. of embryo.	Age at commencement of exp.	Cardiac frequency 20 mins. after immersion.	Cardiac frequency after 1 day's immersion.	Cardiac frequency after 2 days' immersion.	Cardiac frequency after 3 days' immersion.
104	14 days	16 to 18	14 to 17	8 to 10	3 (weak)
105	14 „	17 to 20	16 to 18	5 (weak)	0
106	14 „	18 to 20	18 to 20	9 to 10	2 (weak)
107	12 „	21 to 26	14 to 16	6 (weak)	responds to stimuli
108	12 „	24 to 25	18 to 19	0	0
109	10 „	27 to 28	14 to 16	5 to 7	0
110	10 „	16	12 to 13	8 to 10	3
111	9 dys 12 hrs	14 to 17	9	5	0
112	9 „ 12 „	16 to 18	7 to 9	0	0

The preceding table shows that a 2 % solution of gum arabic plus a trace of sodium carbonate "maintains" the cardiac rhythm of rat-embryos for a longer period than any of the following substances:— ·75 % sodium chloride, the synthesised proteid-like colloids *A* and *B*, "Ringers' fluid," or solutions of blood ash, of the ash of gum-arabic, but that it is far inferior in its sustaining power to either a mixture of egg-albumen and 75 % NaCl, or of a mixture of the proteid-like colloid *C* and ·75 % NaCl.

Notwithstanding numerous and exhaustive researches, it is still

[1] Albanese. *Archiv für exper. Path. u. Ther.* XXXIII. p. 297. 1893.
[2] Ohrn. *Ibid.* XXIV. p. 29. 1894.

undecided whether the heart can maintain its rhythm at the expense of its own substance, although more recent work tends to show that it is unable to so do. The recent paper by White[1] emphasises the sources of error in the various experiments on frogs' hearts. Apart from these errors it would seem to me not a fair test to work with hearts that have been thoroughly exhausted by prolonged perfusion of normal saline, for in such cases it is probable that the metabolic changes are profoundly altered, and that the organ behaves differently than under normal conditions, so that its power of contraction must become progressively decreased. The fact demonstrated by White that after complete exhaustion, and at a time when Ringer's fluid fails to induce contraction, the subsequent application of serum albumen will restore the rhythm, only proves that the heart is not dead and that when supplied with suitable nutritive material it is capable of assimilation and subsequent contraction.

It is possible that part of its contractile substance is available for use, that on its exhaustion the rhythm fails until there is a supply of fresh material.

The following table gives the comparative results of the immersion of 14 day rat-embryos in various media for a prolonged period. The embryos were excised and washed in normal saline, and maintained at 38° C. throughout the experiments.

TABLE 15.

The figures record the average number of heart-beats per minute in 12 experiments after the elapse of the various periods of time as therein stated.

	After 6 hrs	After 9 hrs	After 12 hrs	After 18 hrs	After 24 hrs	After 2 days	After 3 days
Embryos surrounded by pure distilled water	13	9	8	0	0	0	0
Surrounded by ·75 % sodium chloride	16	15	9	6	5	0	0
Surrounded by Ringer's fluid	11	8	7	6	5	2	0
Surrounded by solution of blood ash	17	15	15	14	12	0	0
Surrounded by ash of gum arabic	16	14	14	13	12	3	0
Surrounded by 2 % sol. of gum arabic	17	16	15	12	12	6	1

[1] A. H. White. This *Journal*, XIX. p. 344. 1866. Contains a *résumé* of conflicting views on this subject.

	After 6 hrs	After 9 hrs	After 12 hrs	After 18 hrs	After 24 hrs	After 2 days	After 3 days
Surrounded by 7·5 % sol. of colloid *A*	17	13	11	4	0	0	0
Surrounded by 7·5 % sol. of colloid *C*	18	18	16	15	12	10	6
Surrounded by mixture of egg albumen & ·75 % NaCl in equal parts	18	18	16	16	14	14	11
Surrounded by mixture of defibrinated rats' blood & ·75 % NaCl in equal parts	18	17	18	16	15	15	14

(10) *The Action of Various Drugs.*

All experiments except where otherwise stated were performed prior to the development of the intrinsic cardiac nervous system. The drugs were applied dissolved in normal saline; and administered direct to the embryonic heart, with the various precautions as detailed in my previous papers. Observations were taken both on embryos *in situ*, and on excised embryos. Great care was taken that the application of the drug should not vary the temperature of the heart.

(α) *Caffein.* If applied in doses of ·1 mg. dissolved in 1 c.c. of normal saline, slight tonic contraction of the cardiac myoplasm results, while in ten experiments the average increase of the cardiac frequency was four beats per minute in the case of rat-embryos, and in a similar series of experiments on rabbit-embryos, the average increase of frequency in five observations after the application of ·1 mg. of caffein was three beats per minute.

Larger doses produce pronounced tonic contraction culminating in systolic stoppage, which cannot be removed by the application of heat, or dilute acid solutions (*e.g.* $\frac{1}{20000}$ lactic acid).

The physiological action on the hearts of mammalian embryos is therefore practically identical with its action on the adult heart and on the hearts of chick-embryos, and independent of the nervous system.

(β) *Veratrin.* In a similar manner the action of this substance is practically identical with that previously described as characteristic in its action on the hearts of embryo chicks, small doses causing an acceleration of the cardiac frequency, larger doses rapidly reducing it, and culminating in diastolic stoppage. The hearts of cats and rabbits that have been stopped by the application of ·2 mg. of veratrin can usually be restored to their normal rhythm by the application of a

dilute solution of potassium chloride, or by raising their temperature 5 or 6 degrees.

(γ) *Nicotin.* The action of this substance on the hearts of embryo rats and cats is similar to its action on the chick's heart and need not consequently be described. The action of smaller doses is antagonised by the subsequent application of dilute solutions of potassium chloride.

On older mammalian embryos (cats and rabbits) after the development of the intrinsic cardiac nervous system, the application of 1 c.c. of normal saline containing ·05 mg. of nicotin prevents the inhibition normally produced by the application of an interrupted current. The subsequent application of dilute solution of potassium chloride to the hearts of older embryos, although it partially restores the cardiac rhythm, fails to restore the appearance of inhibitory phenomena when an interrupted electric current is subsequently applied.

(δ) *Digitalin.* The action of this substance on the hearts of early cat and rat embryos is practically the same as previously described as characteristic of its action on early chick-embryos.

(ϵ) *Strophanthin.* The action of ·1 mg. to ·3 mg. of this substance on the hearts of cat and rat embryos is the same as described as characteristic of its action on early chick-embryos.

Prof. Fraser of Edinburgh has informed me in a private letter that he has found that minute traces of strophanthin when applied to the frog's heart produces, after prolonged action diastolic stoppage, the result being opposite to the action of small doses of this substance acting over a comparatively short period of time. He has asked me to ascertain whether there is a similar action on the heart prior to the development of its nervous system, and in order to test this point I have tried the action of ·05 mg. of strophanthin on rat embryos, allowing the substance to act for two days on excised embryos kept under constant conditions in my observing incubator. Control experiments with rat embryos taken from the same mother, and kept in the same incubator, under precisely the same conditions, were performed so as to ensure that the results obtained were not due to any deterioration of the cardiac rhythm owing to prolonged separation from the mother. The embryos in both series of experiments were kept bathed in a solution consisting of a mixture in equal parts of normal saline and the mother's blood. In those experiments in which the strophanthin (·05 mg.) was added, the hearts of the embryos were found to be in diastolic stoppage at periods varying from 18 to 26 hours after application, while in all the controlled experiments except one the

hearts of the embryos were found beating after this time. In three cases out of the eight observations, where the diastolic stoppage had occurred after 18, 22 and 34 hours respectively, the subsequent application of heat (a rise of 5° C.) partially restored the cardiac rhythm. There is therefore a pronounced difference between the action of ·1 mg. of strophanthin over a short period of time, and of ·05 mg. of strophanthin acting over a prolonged period of time, and this action is not associated with the intrinsic cardiac nervous mechanism, but is direct on the cardiac myoplasm itself.

(ζ) *Antiarin.* Doses of ·1 to ·3 mg. act in a manner not distinguishable from the action of either digitalin or strophanthin on the hearts of early mammalian embryos, producing tonic contraction culminating in systolic stoppage. Smaller doses, *e.g.* ·05 mg. acting over from 18 to 40 hours, produced diastolic stoppage, sometimes removable by heat in a manner similar to that previously described as characteristic of the action of minute doses of strophanthin. I may here remark that minute doses of digitalin will not produce a similar result.

(η) *Acids and Alkalis.* $\frac{1}{20000}$ lactic acid and $\frac{1}{20000}$ sodium hydrate were used. The general action and reciprocal antagonism of these substances is similar to that described long ago by Dr Gaskell, as characteristic of their action on frogs' hearts.

(θ) *Morphine Acetate.* The action of this substance, as on the chick's heart, varies with the temperature of application. Doses of ·1 mg. at a temperature of 28° C. producing slowing of the rhythm culminating in diastolic stoppage, while the same dose at 40° C. causes an immediate large increase of the frequency, great irregularities, stoppages, alternating with violent bouts of beating and reversal of rhythm. Lowering the temperature will often restore the rhythm to its normal condition. Doses of ·2 mg. and upwards if applied at 40° C. act in a similar manner to doses of ·1 mg. applied at 28° C.

(ι) *Muscarin nitrate and atropin sulphate.* The action of these substances, even on the earliest embryos, is apparently identical with their action on the frog's heart. Typical muscarin depression is produced after the application of ·05 mg. of that substance, and is removed by the subsequent application of ·05 mg. of atropin sulphate. This result is the converse of that I have obtained in my experiments published on the chick's heart. There is apparently no difference in the action of muscarin nitrate and atropin sulphate on the hearts of earlier and later mammalian embryos. I have performed an exhaustive series of experiments to test this point, the animals used being rats, rabbits and cats.

(κ) *Chloroform and Ether*[1]. These substances were administered either by inhalation to the mother in the ordinary manner, and the effect watched on the embryos' hearts *in situ*, or direct to the cardiac myoplasm dissolved in normal saline, of strength ·001 of a c.c. to 1 c.c. of ·75 °/₀ NaCl, in the same manner as previously applied to the hearts of chick-embryos.

In both series of experiments it was found that chloroform acted as a marked depressant to the embryonic hearts, but even when the mother animal was killed by inhalation of the chloroform the embryos' hearts continued beating. Direct application of chloroform (·001 c.c. to 1 c.c. of ·75 °/₀ NaCl) always induced cardiac stoppage in diastole.

The direct application to the embryos' cardiac myoplasm of a mixture of chloroform and ether did not produce such toxic results. Thus embryos to which ·002 of a c.c. of chloroform mixed with ·001 of c.c. of ether was applied, sustained a fairly strong rhythm in which the average cardiac depression, in ten experiments, was only eight beats per minute. Ether applied either through inhalation of the mother, or direct to the blastoderm, in strength of ·001 of a c.c. to 1 c.c. of ·75 °/₀ NaCl, acts as a cardiac stimulant, rapidly increasing the cardiac frequency, while apparently not decreasing the force. Large doses, *e.g.* ·0015 of a c.c., still further increase the cardiac frequency, but decrease the rhythmic force of the hearts. A dose of ·002 of a c.c. stops the hearts in diastole. I have attempted by various means to antagonise the toxic action of chloroform on the hearts of mammalian embryos, and have entirely failed with the following substances, digitalin, strophanthin, antiarin, caffein, and atropin sulphate. They seem rather to increase than antagonise the depression produced.

The addition of an equal part of alcohol (·001 of a c.c. of alcohol to ·001 of a c.c. of chloroform) somewhat mitigates the depression produced by the latter substance, but if the dose of chloroform be increased to ·0015 of a c.c. the addition of alcohol does not decrease the cardiac depression.

A mixture of ·001 of a c.c. of chloroform, + ·001 of a c.c. of ether, + ·001 of a c.c. of alcohol is not so depressant as either chloroform alone, or a mixture of chloroform and ether, but here again if the strength of the chloroform be increased to ·0015 of a c.c. to each 1 c.c. of ·75 °/₀ NaCl the typical diastolic stoppage results.

The addition of ·001 of a c.c. of ammonia will sometimes partially

[1] The experiments were performed on both rabbit and dog embryos.

restore the cardiac depression produced by the application of ·0005 of a c.c. of chloroform direct to the heart, and will also more completely restore the cardiac depression produced in the embryos' hearts during inhalation by the mother of pure chloroform. Even when the mother is killed by chloroform the direct application of ammonia will considerably restore the embryos' cardiac rhythm. Thus in three experiments where the mother, a rabbit, was killed by chloroform the depression of the embryos' hearts was found to be after the direct application of ·001 of a c.c. of ammonia only four beats per minute.

PART II.

Electrical observations on the Embryonic Heart.

(1) *Action of Interrupted Electric Currents on the Hearts of Chick-embryos.*

These experiments fall under three heads:—

(1) Action on embryos aged from 50 to 100 hours.

(2) On embryos aged 100 to 200 hours.

(3) On embryos aged 200 hours and upwards.

In each of the series of experiments the current was furnished by two Daniell cells and a "Du Bois-Reymond" Inductorium. The temperature of the observing incubator was kept constant during the observations at 38° C., and the surface of the blastoderm was bathed, except where otherwise stated, in a ·75 % NaCl solution. The following experiments are selected from a number, to illustrate the results obtained:—

Table 16.

Embryo age 60 hours, with normal cardiac frequency of 42 beats per minute.

Time	Distance of primary from secondary coil.	Cardiac frequency in beats per minute.	Remarks on rhythm.
1.30	Current off	42	Normal
1.31	on : 50 cm.	46	ditto
1.31 till 1.38	ditto	46	ditto
1.39	off	44	ditto
1.40	off	42	ditto
1.40 till 1.50	off	42	ditto

Time	Distance of primary from secondary coil.	Cardiac frequency in beats per minute.	Remarks on rhythm.
1.51	on: 25 cm.	54	Stronger than normal
1.53	ditto	54	ditto
1.53 till 2.0	ditto	varies from 53 to 58	ditto
2.1	off	48	ditto
2.3	off	42	Normal
2.5	on: 15 cm.	60	Irregular and weak
2.7	ditto	—	"Herz delirium"
2.8	ditto	—	ditto
2.9	off	59	Irregular and weak
2.10	ditto	55	Weaker than normal
2.15	ditto	40	Apparently normal

The next table records the results obtained with a 100 hour embryo in an abbreviated form.

TABLE 17.

Time.	Distance of primary from secondary coil.	Cardiac frequency in beats per minute.	Remarks on rhythm.
2.50	Current off	120	Normal
2.51	on: 50 cm.	128	ditto
2.53 till 2.55	ditto	134	ditto
2.56	off	—	ditto
2.57 till 3.17	ditto	120	ditto
3.17	on: 25 cm.	134	Tonic contraction increased
3.20 till 3.24	ditto	130	ditto
3·25	on: 18 cm.	140	Apparently normal
3.26	off	120	ditto
3.27	on: 17 cm.	140	ditto
3.30	ditto	140	ditto
3.31	on: 15 cm.	146	Tonic contraction marked
3.33	on: 12 cm.	160	Delirium cordis
3.34	ditto	—	Too rapid to count and very irregular
3.34 till 3.36	ditto	—	ditto
3.37	ditto	—	Stopped in systole
3.38	off	0	ditto
3.39	ditto	twitches	Expands to diastole
3.40 till 3.50	ditto	30 to 33	Impaired action

If the current be passed through other axes of the embryo which do not go through its heart, there is no noticeable effect produced on the cardiac rhythm.

It will be noticed that the increase of strength of the current culminates in heart delirium, not in inhibition. Although most varied strengths of current have been used I have always failed to obtain a cardiac inhibition in embryos aged from 50 to 120 hours.

In the observations of which an abbreviated record will be found in the next two tables, the action of the current was tried, at all strengths corresponding to the variation of one half cm. of the distance of the coils between the extreme limits named in the tables.

In embryos varying in age from 140 to 160 hours, but rarely inhibition results. In one case inhibition was obtained in a 150 hour embryo.

The following table embodies the principal results:—

TABLE 18.

No. of embryo.	Age of embryo.	Distance of coils (ranging between)	Normal rhythm at 38° C. in beats per minute.	Inhibition if present.
I	120 hrs	50 to 8 cm.	65	No
II	126 "	50 to 8 cm.	78	No
III	150 "	23 cm.	112	Yes
IV	150 "	28 to 8 cm.	100	No
V	156 "	50 to 8 cm.	118	No
VI	162 "	22 cm.	106	Yes
VII	180 "	50 to 5 cm.	102	No
VIII	206 "	26 cm.	80	Yes
IX	250 "	20 cm.	104	Yes
X	250 "	25 cm.	106	Yes
XI	300 "	24 cm.	120	Yes

A comparison of the above with the preceding table will show that a current of sufficient strength to induce cardiac inhibition in a 250 hour embryo will, if applied to 100 hour embryo, produce tonic cardiac contraction, and if increased in strength, culminates in heart delirium. If a further comparison be made between these results and those I[1] have recently recorded on the action of muscarin nitrate and atropin sulphate on embryo-chicks, it will be found that the ages, during which these drugs exhibit no action on the cardiac rhythm, fairly correspond with the period during which interrupted electric currents fail to produce inhibition. The earliest time at which I obtained at 38° C. a reduction of rhythm due to the action of muscarin nitrate, was at 160 hrs., and in one case only have I obtained cardiac inhibition in an

[1] Pickering. This *Journal*, XVIII. p. 478. 1895.

embryo younger than this (*e.g.* aged 150 hrs.); while in another case where the embryo was aged 180 hrs. I failed to obtain this result. In embryos aged over 250 hours currents of a strength greater than that requisite to induce cardiac inhibition, cause an augmentation of the rhythm which often culminates in heart delirium. Thus in later chick-embryos weak interrupted currents produce inhibition, and stronger currents a condition of "herz delirium" possibly comparable to tetanus of a skeletal muscle.

In considering the significance of these results, it is well to bear in mind that Dew-Smith[1] found that interrupted currents when applied to the heart of a 'Salpa' did not produce inhibition, on the other hand on the heart of 'Helix pomatia' in which Lea had after histological examination failed to detect any nervous elements—that author in collaboration with Foster[1], obtained, by means of interrupted electric currents, typical cardiac inhibition.

It is however interesting to note that long ago Vulpian[2] showed that muscarin nitrate and atropin sulphate exhibited their typical antagonistic action on the snail's heart. I have however found that weak interrupted currents inhibit the hearts of Daphniæ, which in a previous paper I[3] showed are not influenced by the application of solutions of muscarin nitrate, although they are stopped by atropin sulphate.

Piotrowski[4] working with an abductor limb muscle of the crayfish, has shown that lowering of the temperature of the muscle decreases its irritability, so that stronger currents are necessary to induce contraction, while raising the temperature increases its irritability up to a certain point, beyond which a further rise of the temperature leads to a decrease of the muscular irritability which is more marked than that produced by the influence of cold.

Lowering the temperature of the chick-embryo renders it within certain limits more difficult to induce cardiac inhibitory phenomena. Thus in an embryo aged 210 hours which in order to induce cardiac inhibition at 38° C. the distance of the coils was 24 cm.; when the temperature of the embryo was reduced to 28° C., the distance of the coils necessary to induce inhibition was 11·9 cm.

The following table summarises a number of experiments on this

[1] Foster and Dew-Smith. *Proc. Roy. Soc.* XXIII. p. 318. 1875.

[2] Vulpian. *Comptes Rendus*, LXXXVIII. p. 1295. 1879.

[3] Pickering. This *Journal*, XVII. p. 356. 1894.

[4] Piotrowski. This *Journal*, XIV. p. 163. 1893.

point, and emphasises the influence of temperature on the strength of current requisite to produce cardiac inhibition.

TABLE 19.

Age of embryo.	Temp. of embryo 42° C. distance of coils to produce inhibition.	Temp. of embryo 38° distance of coils to produce inhibition.	Temp. of embryo 32° distance of coils to produce inhibition.	Temp. of embryo 28° distance of coils to produce inhibition.
220 hrs	29 cm.	26 cm.	22 cm.	18 cm.
210 „	27·5 cm.	24 cm.	16·3 cm.	11·9 cm.
250 „	28 cm.	20 cm.	20 cm.	12·6 cm.
240 „	21 cm.	18·5 cm.	17·6 cm.	16·3 cm.
240 „	22·3 cm.	19·7 cm.	18·4 cm.	10 cm.

If the temperature be increased above 42° C. it is very difficult to obtain inhibition :—" Herz delirium " culminating in systolic stoppage nearly always results. Taking the difference of the contractile tissue into account there is a general correspondence of my results with those obtained by Piotrowski[1] on the muscle of Astacus.

If an interrupted current be applied for a short time (3 to 4 minutes) to an embryo-chick's heart, and be discontinued for a minute, after which time a second interrupted current be passed through the heart, the effect produced by the application of the second current is greater than that produced by the first application of the current. In other words there is a summation of the effect produced. This result obtains either in the case of the production of heart delirium or of cardiac inhibition. The following tables embody this result:—

TABLE 20.

Embryos kept at 38° C.
Time of passage of currents 3 mins.

Age of embryo.	Distance of coils necessary to induce delirium cordis.	Distance of coils necessary to induce delirium cordis on second application.	Distance of coils to induce delirium cordis eight minutes afterwards.
75 hrs	16·4 cm.	27·3 cm.	16·7 cm.
75 „	17·3 cm.	22·4 cm.	17·4 cm.
80 „	16·8 cm.	19·7 cm.	16·3 cm.
80 „	15·9 cm.	21·4 cm.	17 cm.
110 „	18 cm.	24·8 cm.	18·3 cm.
110 „	17·5 cm.	22·4 cm.	17·3 cm.
120 „	16·3 cm.	21·3 cm.	15·9 cm.

[1] *Op. cit.*

TABLE 21.

Embryo kept at 38° C.
Time of passage of current 3 mins.

Age of embryo.	Distance of coils to induce inhibition.	Distance of coils to induce inhibition on second application.	Distance of coils to induce inhibition eight minutes after.
208 hrs	24·5 cm.	28·3 cm.	24·3 cm.
208 ,,	25 cm.	27·9 cm.	22·8 cm.
210 ,,	20·8 cm.	24·3 cm.	20·3 cm.
210 ,,	21·7 cm.	22·2 cm.	21 cm.
215 ,,	22 cm.	26 cm.	22 cm.
215 ,,	19·8 cm.	23·1 cm.	20·3 cm.
220 ,,	21 cm.	24·7 cm.	20·9 cm.
220 ,,	22 cm.	26·5 cm.	21·7 cm.

In all experiments performed, care was taken after the passage of an interrupted current that upwards of eight minutes should elapse before any further experiment was performed, so as to avoid the after effects produced by the application of the interrupted currents, from vitiating the results obtained. If an interrupted current too weak to induce either delirium cordis, on early embryos, or cardiac inhibition on later embryos, be passed through their hearts for 30 minutes, and after an interval of one minute, a second current is passed through them, it is found that a stronger current is requisite to induce both delirium cordis on earlier, and cardiac inhibition on later embryos than that requisite if the first current had not been applied. These results are therefore the converse of those obtained after the passage of an interrupted current for 3 or 4 minutes, and are strictly comparable with the effects to be subsequently described as resulting from the prolonged passage of a constant current through the cardiac myoplasm.

(2) *Action of Drugs on the Subsequent Action of Interrupted Currents.*

Biedermann[1] found that the application of dilute solutions of veratrin to an abductor limb-muscle of Astacus favoured the inhibitory phenomena produced by interrupted currents.

The following experiments were made to test the action of veratrin on the inhibitory phenomena obtained by means of interrupted currents on the embryonic heart. In these experiments the first series was kept at 38° C., the second at 32° C., the third at 26° C. In each case

[1] Biedermann. *Sitz. d. k. k. Akad. in Wien* 43; *ibid.* 45 n, 47.

the blastoderm was bathed with 1 c.c. of normal saline containing ·1 mg. of veratrin.

TABLE 22.

Embryos at 38° C.

Age of embryo.	Distance of coils required to obtain inhibition when bathed in ·75 °/$_0$ NaCl.	Distance of coils required to obtain inhibition when bathed in ·75 °/$_0$ NaCl + ·1 mg. of veratrin.	Acceleration in rhythm produced by addition of 1 mg. of veratrin.
230 hrs	22 cm.	25 cm.	4 beats per min.
226 „	20 cm.	24 cm.	6 „ „
215 „	18·7 cm.	23 cm.	3 „ „
250 „	19·8 cm.	21·8 cm.	4 „ „

TABLE 23.

Embryos at 32° C.

Age of embryo.	Distance of coils required to obtain inhibition when bathed in ·75 °/$_0$ NaCl.	Distance of coils required to obtain inhibition when bathed in ·75 °/$_0$ NaCl + ·1 mg. of veratrin.	Variation in rhythm produced by addition of ·1 mg. of veratrin.
230 hrs	21 cm.	26·3 cm.	+ 2 beats per min.
224 „	22 cm.	28 cm.	− 1 „ „
215 „	19·9 cm.	29·5 cm.	+ 1 „ „
248 „	18·75 cm.	27·9 cm.	− 2 „ „

TABLE 24.

Embryos at 26° C.

Age of embryo.	Distance of coils required to obtain inhibition when bathed in ·75 °/$_0$ NaCl.	Distance of coils required to obtain inhibition when bathed in ·75 °/$_0$ NaCl + ·1 gm. of veratrin.	Variation in rhythm produced by addition of ·1 mg. of veratrin.
228 hrs	21·6 cm.	24·3 cm.	− 4 beats per min.
226 „	20·8 cm.	26·2 cm.	− 6 „ „
218 „	22 cm.	23 cm.	− 2 „ „
249 „	19·4 cm.	22·4 cm.	+ 1 „ „

A comparison of these results with those recorded in table 19 shows that the addition of the ·1 mg. of veratrin to the bathing fluid exerts a marked influence on the cardiac inhibition produced by interrupted currents. A fall of temperature ordinarily necessitates a stronger current to produce cardiac inhibition, but when a small quantity of veratrin is present, the fall of temperature aids the appearance of inhibition. At all temperatures between 38° C. and 28° C. the addition to the bathing fluid of ·1 mg. of veratrin aids inhibition.

At temperatures above 38° C., ·1 mg. of veratrin does not apparently aid the appearance of cardiac inhibition. Larger doses, *e.g.* ·175 mg. to ·2 mg., aid inhibition, and in one case where ·2 mg. was applied to a 260 hour embryo cardiac inhibition was obtained with the coils 28 cm. apart, while the embryo was kept at 43° C. At temperatures below 20° C. the depressing action of the veratrin is so marked that it is difficult to say whether the resulting cardiac stoppage is a true inhibition, or a standstill produced by the poison.

The fact discovered some years ago by Gaskell[1] that during the stoppage produced by the action of muscarin nitrate on the frog's heart, stimulation of the vagus produced a positive electromotive variation, while that of the sympathetic produced a negative electromotive variation, has led me to investigate the influence of small doses of muscarin nitrate on the electrical inhibition obtained on older chick-embryos. Working with chick-embryos aged from 230 to 280 hours I have depressed their cardiac rhythm by the addition of ·015 mg. of muscarin nitrate dissolved in 1 c.c. of normal saline.

In the following table the results are recorded:—

TABLE 25.

Temperature 38° C.

Age of embryo.	Distance of coils to obtain inhibition when bathed in ·75 % NaCl.	Depression of rhythm produced by muscarin nitrate (·015 mg.).	Distance of coils to obtain inhibition when bathed in ·75 % NaCl + ·015 mg. of muscarin nitrate.
230 hrs	22·3 cm.	18 beats per min.	26 cm.
236 "	18·7 cm.	12 " "	22 cm.
240 "	17 cm.	16 " "	26 cm.
240 "	18 cm.	12 " "	22 cm.
275 "	17 cm.	18 " "	25·3 cm.
280 "	20·4 cm.	19 " "	26·8 cm.

It will be observed that during the muscarin depression a weaker interrupted current suffices to produce inhibition than under ordinary circumstances. In a previous paper I have shown that at lower temperatures, a much smaller dose of muscarin nitrate is required to induce cardiac stoppage than at the normal temperature of the embryo (38° C.). Similarly at lower temperatures, in the presence of muscarin nitrate, the distance of the coils to produce inhibition is greater than

[1] Gaskell. This *Journal*, VII. p. 452. 1886; *Beitrage für Physiologie* (*Ludwig's*) 1887; This *Journal*, VIII. p. 404. 1887.

the distance required at higher temperatures, that is, the current requisite is less.

If to a chick-embryo whose cardiac rhythm has been depressed by muscarin nitrate, a dilute solution of atropin sulphate be added, the force and frequency of a cardiac rhythm is restored. The current necessary to inhibit the restored rhythm is greater than the current necessary to inhibit the rhythm depressed by muscarin nitrate—that is, the coils are closer together before inhibition results.

TABLE 26.

Embryo kept at 38° C. drugs dissolved in normal saline.

Age of embryo.	Distance of coils necessary to induce inhibition after action of ·015 mg. of muscarin nitrate.	Distance of coils necessary to induce inhibition after subsequent action of ·015 mg. of atropin sulphate.	Frequency of rhythm during action of muscarin.	Frequency of rhythm after subsequent application of atropin sulphate.
240 hrs	26 cm.	20 cm.	60 per min.	85 per min.
240 „	19·8 cm.	16·2 cm.	90 „	98 „
218 „	24·3 cm.	14·2 cm.	40 „	72 „
250 „	19·8 cm.	17·3 cm.	57 „	80 „
260 „	20·2 cm.	16·2 cm.	17 „	28 „
275 „	24·5 cm.	19·5 cm.	46 „	64 „

Piotrowski[1] writing of the inhibitory phenomena obtainable with the limb muscle of Astacus states, "All circumstances which increase the tone, favour the appearance of inhibition." The next series of experiments were performed to ascertain whether this generalization applies to the inhibitory phenomena obtainable by the action of interrupted currents on the hearts of chick-embryos.

In a previous paper I have shown that caffein greatly increases the tone of the embryonic heart, I have therefore tested its action on inhibitory phenomena, and the results obtained are embodied in the next two tables:—

TABLE 27. Temperatures of embryos 38° C.

Age of embryo.	Distance of coils to produce inhibition in normal embryo.	Distance of coils to produce inhibition after application of ·15 mg. caffein.	Variation of cardiac frequency after application of caffein.
200 hrs	21 cm.	23 cm.	+ 7
220 „	18·8 cm.	19 cm.	+ 4
220 „	19 cm.	24 cm.	+ 8
224 „	21·2 cm.	26 cm.	+ 14
228 „	22 cm.	28 cm.	+ 9
250 „	21·6 cm.	21·8 cm.	+ 1 (?)

[1] *Op. cit.* p. 190.

TABLE 28.

Temperature of embryos at 28° C.

Age of embryo.	Distance of coils to produce inhibition in normal embryo.	Distance of coils to produce inhibition after application of ·15 mg. caffein.	Variation of cardiac frequency after application of caffein.
206 hrs	18·8 cm.	17·8 cm.	+ 3
210 ,,	19 cm.	19·8 cm.	+ 2
200 ,,	20·9 cm.	21 cm.	− 1
220 ,,	23 cm.	24 cm.	+ 1
250 ,,	21 cm.	20 cm.	− 1
250 ,,	18·9 cm.	19·4 cm.	+ 3
250 ,,	19·8 cm.	20·4 cm.	− 3

A comparison of the two preceding tables will show that caffein exerts an influence on the inhibition produced by interrupted currents on the hearts of embryo-chicks, but that the influence varies with the temperature at which the drug is applied. Thus at 38° C. ·1 mg. of caffein decreases the strength of the current requisite to induce inhibition, while at 28° C. the same dose sometimes even increases the strength of current that is necessary to induce inhibition. The same dose if administered at 42° C. induces pronounced tonic contraction of the cardiac myoplasm, and if even a very weak interrupted current be applied, the result is always "delirium cordis" culminating in systolic stoppage.

Similar results are obtained if digitalin is substituted for caffein, and in this case there can be no question of any action of that substance on the nervous mechanism of the heart, so that any effects produced on the inhibitory phenomena are those occurring in the contractile myoplasm alone. This point will be returned to in the discussion of the significance of these observations, and their bearing on the current theories purporting to explain inhibition.

The next two tables record the observations with digitalin:—

TABLE 29.

Temperature of embryos 38° C.

Age of embryo.	Distance of coils to produce inhibition in normal embryo.	Distance of coils to produce inhibition after application of ·015 mg. digitalin.	Variation of cardiac frequency after digitalin.
210 hrs	19·7 cm.	20·3 cm.	3 beats per min.
208 ,,	22·6 cm.	23·6 cm.	4 ,, ,,
206 ,,	21·8 cm.	21·8 cm.	0 ,, ,,
230 ,,	23·2 cm.	25·8 cm.	3 ,, ,,
240 ,,	17·5 cm.	24·7 cm.	3 ,, ,,

TABLE 30.

Temperature of embryos 28° C.

Age of embryo.	Distance of coils to produce inhibition in normal embryo.	Distance of coils to produce inhibition after application of ·015 mg. digitalin.	Variation of cardiac frequency after digitalin.
208 hrs	20 cm.	16·8 cm.	– 3 beats per min.
210 „	20 cm.	19·3 cm.	+ 1 „ „
220 „	23 cm.	20·2 cm.	– 1 „ „
250 „	21 cm.	18 cm.	– 2 „ „
250 „	17·5 cm.	18·3 cm.	+ 2 „ „
260 „	21 cm.	20·8 cm.	– 4 „ „

The two preceding tables show that the cardiac inhibition produced by interrupted currents on embryos under the influence of minute doses of digitalin (·015 mg.) is modified in a marked degree by the temperature of administration of the drug. This factor is apparently greater than in the case of the administration of caffein. Thus in the former instance four of the experiments out of five conducted at 38° C. showed under the influence of ·015 mg. of digitalin a decrease in the strength of current requisite to induce inhibition, while in the experiments conducted at 28° C. five out of six showed a marked increase of the current requisite to induce inhibition. It should be noted that the dose of digitalin is smaller than the dose of caffein.

Larger doses of digitalin (·15 mg.) induce pronounced tonic contraction of the embryonic myoplasm and even at low temperatures (28° C.), very weak interrupted currents; such as are obtained when the coils are 30 cm. apart throw the heart into pronounced delirium culminating in systolic stoppage. I have failed with the larger doses of digitalin to induce inhibition although the tone of the organ is exaggerated.

At higher temperatures (*e.g.* 42° C.) digitalin in smaller or larger doses induces a condition of pronounced tonic contraction, and on the application of a very weak interrupted current (coils 30 cm. apart), delirium cordis results.

The salient feature of the four preceding tables is that the application of small doses of either caffein or digitalin to the cardiac and myoplasm of chick-embryos at 38° C. decreases the strength of the interrupted current requisite to induce inhibition, that is, it favours the appearance of inhibitory phenomena. Bearing this fact in mind I have tried whether the converse statement is true—if substances which induce an atonic condition of the cardiac myoplasm retard the appearance of inhibitory phenomena. To this end I have selected lactic acid

of strength $\frac{1}{20000}$ dissolved in ·75 % NaCl which Gaskell[1] has shown to induce a typical atonic condition of cardiac contractile tissue.

The results obtained are summarised in the following two tables :—

TABLE 31.

Embryo kept at 38° C.

Age of embryo.	Distance of coils to produce inhibition in normal embryo.	Distance of coils to produce inhibition after application of 1 c.c. of above solution of lactic acid.	Distance of coils to produce inhibition after 1 c.c. lactic acid and 1 c.c. $\frac{1}{20000}$ NaHO solution.
250 hrs	21·2 cm.	20·8 cm.	21·6 cm.
248 ,,	20·6 cm.	18·8 cm.	21 cm.
216 ,,	19·8 cm.	15 cm.	17·9 cm.
216 ,,	17·9 cm.	17·3 cm.	20·4 cm.
227 ,,	23·2 cm.	21·6 cm.	22 cm.
230 ,,	20 cm.	16·8 cm.	24 cm.
232 ,,	21·3 cm.	21 cm.	25 cm.
240 ,,	18·75 cm.	16·2 cm.	19·2 cm.

TABLE 32.

Temperature of embryo 28° C.

Age of embryo.	Distance of coils to induce inhibition in normal embryo.	Distance of coils to induce inhibition after application of 1 c.c. $\frac{1}{20000}$ lactic acid solution.	Distance of coils to induce inhibition after 1 c.c. of lactic acid 1 c.c. of $\frac{1}{20000}$ NaOH solution.
218 hrs	19·8 cm.	19·5 cm.	18·75 cm.
260 ,,	12·8 cm.	9·8 cm.	11·7 cm.
210 ,,	16·5 cm.	15·9 cm.	15·9 cm.
214 ,,	20·4 cm.	18·3 cm.	16·9 cm.
240 ,,	18 cm.	18 cm.	18 cm.
240 ,,	11·4 cm.	8·8 cm.	8·4 cm.
260 ,,	12·3 cm.	13·1 cm.	13·1 cm.
275 ,,	15·7 cm.	14·8 cm.	13·4 cm.

It is evident from the statistics in table 31 that dilute solutions of lactic acid ($\frac{1}{20000}$) at temperature of 38° C., which may be considered the normal temperature of the embryo, retard the appearance of the inhibitory phenomena produced after the application of interrupted electric currents. In each of the experiments performed a stronger current was requisite to induce inhibition after the action of the lactic acid, and the consequent atonic condition of the embryonic heart.

[1] Gaskell. This *Journal*, III. p. 45. 1882.

A subsequent application of a $\frac{1}{20000}$ solution of sodium hydrate restored the cardiac tone and frequency, and after its action there was usually a more or less complete return of the ordinary readiness to induce inhibition by interrupted currents.

At the lower temperature of 28° C. the action of the lactic acid, although the atonic condition of the cardiac myoplasm was more pronounced, did not increase to so marked a degree the strength of current requisite to induce cardiac inhibition. The average decrease of the distance of the coils requisite to induce inhibition after application of $\frac{1}{20000}$ lactic acid to the heart was at 38° C. 1·8 cm. and at 28° C. 1·2 cm.

(3) *Action of Gases on Subsequent Action of Interrupted Currents.*

Piotrowski[1] has put forward the view that the external conditions which induce allonomic disassimilation favour the appearance of inhibitory phenomena, instancing veratrin in support of his view, and conversely that circumstances which induce autonomic assimilation favour the appearance of contraction; as the accuracy of the theory has an important bearing on the explanations put forward concerning inhibition, it is well to devise some simple series of experiments to put the matter to a test.

The presence of an excess of oxygen instead of air hastens the metabolic changes in a tissue, and as Fano[2] has pointed out increases the cardiac frequency of the embryonic heart. Embryos therefore surrounded by an atmosphere containing an excess of oxygen should have more disassimilation going on in their tissues than those surrounded by air. Conversely if an embryo be surrounded by atmosphere containing 15 °/₀ of carbon dioxide its cardiac frequency is diminished and concomitantly the metabolic activity of that organ is diminished, and theoretically there should be autonomic assimilation, with a consequent tendency on the application of a suitable stimulus towards contraction.

As in the previous series of experiments the observations were conducted at various temperatures. In the next two tables a record is made of the action of interrupted currents when the embryos are surrounded by an atmosphere of Brin's[3] oxygen.

[1] *Op. cit.* p. 192.

[2] Fano and Badano. *Archives Ital. de Biologie*, XIII. p. 387. 1890.

[3] Brin's oxygen contains 7 °/₀ of a mixture of nitrogen and argon.

TABLE 33.

Embryo kept at 34° C.

Age of embryo.	Distance of coils to induce inhibition in normal embryo.	Distance of coils to induce inhibition when embryo is surrounded by oxygen.	Increase of cardiac frequency after action of oxygen.
230 hrs	23 cm.	21·4 cm.	9 beats per min.
240 ,,	19·8 cm.	19·7 cm.	7 ,, ,,
218 ,,	21·4 cm.	20·3 cm.	8 ,, ,,
226 ,,	18·5 cm.	14·8 cm.	2 ,, ,,
248 ,,	22·4 cm.	21·2 cm.	8 ,, ,,
275 ,,	21·3 cm.	19·5 cm.	4 ,, ,,
220 ,,	22 cm.	24·2 cm.	9 ,, ,,
224 ,,	22·5 cm.	24·2 cm.	6 ,, ,,
246 ,,	16·8 cm.	16·4 cm.	4 ,, ,,
250 ,,	18·7 cm.	17·1 cm.	7 ,, ,,
250 ,,	22 cm.	23·4 cm.	6 ,, ,,

TABLE 34.

Embryo kept at 38° C.

Age of embryo.	Distance of coils to induce inhibition in normal embryo.	Distance of coils to induce inhibition when embryo is surrounded by oxygen.	Increase of cardiac frequency after action of oxygen.
217 hrs	20 cm.	21·3 cm.	10 beats per min.
220 ,,	19·8 cm.	19·75 cm.	5 ,, ,,
230 ,,	23 cm.	26 cm.	13 ,, ,,
246 ,,	21·7 cm.	18·8 cm.	2 ,, ,,
218 ,,	18·9 cm.	19·5 cm.	4 ,, ,,
250 ,,	16·4 cm.	20·4 cm.	8 ,, ,,
250 ,,	16·8 cm.	23 cm.	11 ,, ,,
260 ,,	19·6 cm.	17·75 cm.	?3 ,, ,,
260 ,,	17·6 cm.	23·1 cm.	9 ,, ,,
275 ,,	20 cm.	17·9 cm.	1 ,, ,,
275 ,,	18·2 cm.	16·75 cm.	5 ,, ,,

If an interrupted current be applied to the hearts of chick-embryos surrounded by an atmosphere containing an excess of oxygen, and kept at temperatures above 38° C., even if the current be very weak (distance of coils of inductorium 30 to 35 cm.), the result is no longer cardiac inhibition but pronounced delirium cordis.

An inspection of the two preceding tables emphasizes the important *rôle* played by temperature in modifying the action of interrupted currents on the heart in the presence of an excess of oxygen. At a temperature of 34° C., out of experiments on eleven different chick-embryos of ages varying from 217 to 275 hours, in six cases, after the

action of the oxygen, there was a decrease in the strength of current, in some cases of considerable amount, requisite to induce cardiac inhibition, but in one of these cases the decrease in the strength of the requisite current amounted to only the amount due to an increase of ·6 cm. between the distance of coils. In the remaining five experiments there was an increase in the strength of current requisite to induce inhibition, after the action of the oxygen, which in three cases amounted to that produced by a decrease of the distance of the coils of 2·9 cm., 2·01 cm., and 2·1 cm. respectively, while in the remaining two experiments the decrease of the coils was ·05 cm. and 1·55 cm. respectively. It is therefore evident that the influence of oxygen on the strength of current requisite to induce cardiac inhibition at 34° C. is very variable.

The experiments conducted at 38° C. yield more constant results, eight show an increase in the strength of current requisite to induce inhibition. The increase requisite is however very variable, in one case being only that produced by decrease of the distance of the coils of ·1 cm., in two other cases of ·2 cm., and in the most marked instance of 3·7 cm. The average decrease of the distance of the coils in the seven cases is 1·45 cm., or excluding the one extreme experiment where the decrease was 3·7 cm., the average decrease between the coils was 1·2 cm. Of the three cases where after the application of oxygen there was at 38° C. a decrease of the strength of current requisite to induce inhibition, the increase in the distance of the coils was respectively 2·2 cm., 1·7 cm. and 1·4 cm. In every experiment the oxygen produced an increase of the cardiac frequency, so that the variation of strength of current cannot be associated with variations of cardiac frequency prior to its application. I cannot see an explanation of these variations, other substances exerting, in general, at a constant temperature, a remarkably constant action on the strength of current requisite to induce cardiac inhibition. It is however possible that the oxygen exerts a variable action on the metabolism of the heart, sometimes favouring allonomic disassimilation, while at other times favouring autonomic assimilation, or possibly both processes may be proceeding *pari passu*, and accordingly as one or the other is predominant the effect is made evident in the phenomena described above, or again it is possible that Piotrowski's explanation will not adequately explain these results.

Turning to the action of an atmosphere containing 15 % of carbonic dioxide we find the results more constant, as is shown in the two following tables :—

TABLE 35.

Embryos kept at 38° C.

Age of embryo.	Distance of coils to induce inhibition in the normal embryo.	Distance of coils to induce inhibition after action of an atmosphere containing 15 % of CO_2.	Decrease of cardiac frequency in beats per min. after action of CO_2.
218 hrs	22 cm.	17·4 cm.	12 beats per min.
220 "	21·8 cm.	18·8 cm.	14 " "
218 "	20·6 cm.	19 cm.	16 " "
230 "	22 cm.	16·75 cm.	13 " "
245 "	19·75 cm.	16·8 cm.	9 " "
275 "	20 cm.	17 cm.	15 " "
275 "	18·8 cm.	14·4 cm.	22 " "

TABLE 36.

Embryos at 28° C.

Age of embryo.	Distance of coils to induce inhibition in the normal embryo.	Distance of coils to induce inhibition after action of an atmosphere containing 15 % of CO_2.	Decrease of cardiac frequency in beats per min. after action of CO_2.
220 hrs	21·4 cm.	16·4 cm.	30 beats per min.
218 "	19·8 cm.	14·8 cm.	42 " "
230 "	22·5 cm.	13·7 cm.	41 " "
240 "	20 cm.	15·4 cm.	50 " "
275 "	19·7 cm.	—	to zero.
275 "	18·5 cm.	—	to zero.

It is evident that though carbonic dioxide decreases the tone of the embryo-chick's heart, that it also decreases the tendency towards inhibition by interrupted electric currents, and although these experiments are dissimilar in results, as regards the influence of the tone on inhibition, to those of Piotrowski on the inhibition of the limb-muscles of Astacus, yet they, in some respects, support his views as regards the influence of anabolic and katabolic processes as favouring contraction and inhibition respectively.

The carbon dioxide besides depressing the frequency of the cardiac rhythm, in all probability retards the anabolic processes, which normally proceed *pari passu* with the contractions of the cardiac myoplasm, and consequently as contractions are still occurring, the amount of disassimilation exceeds the amount of autonomic assimilation, with the consequence that a stronger interrupted current is required to produce cardiac inhibition than under normal conditions. The same explanation would apply to the action of a low temperature on the amount of current requisite to induce cardiac inhibition—the retardation due to fall of

temperature on the processes of autonomic assimilation being more pronounced than the retardation of the processes of disassimilation associated with the reduced force and frequency of the cardiac rhythm.

In comparing these results with those of Piotrowski[1] it is important to bear in mind that the metabolic processes occurring in the myoplasm of the embryonic heart are much more active than those occurring in the limb-muscles of the crayfish, and that the process of autonomic assimilation probably proceeds more *pari passu* with that of allonomic disassimilation than in the case of the limb-muscles of the crayfish.

Also the crayfish being a cold-blooded animal it is probable that variations of its temperature do not affect the normal processes of autonomic assimilation to such a degree as on the warm-blooded chick-embryo, where the normal processes of assimilation are carried on within comparatively narrow limits of temperature.

If early chick-embryos (aged 60 to 150 hrs.) be surrounded by an atmosphere of Brin's oxygen, and subjected to the influence of interrupted electric currents, the increase of force and frequency which normally results from the application of weak currents (distance of the coils 30 to 50 cm.) is augmented by the presence of the oxygen, and if the current be increased in strength 'delirium cordis' results. The appearance of delirium cordis is obtained with much weaker currents in an excess of oxygen than in air. Thus at a constant temperature of 38° C. delirium cordis was obtained in an atmosphere of air in an 85 hour embryo, with the coils 18 cm. apart, and in atmosphere of oxygen when the coils were 35 cm. apart.

Turning to the influence of dilute solutions (·015 mg.) of muscarin nitrate, on the cardiac inhibition produced by interrupted currents on older chick-embryos, we may note that there is a depression of the cardiac frequency, and a pronounced atonic condition associated with a decrease of the strength of current requisite to induce cardiac inhibition, but the experiments of Gaskell, which show that during muscarin stoppage anabolic processes are probably proceeding, are strikingly in accord with these results, where the muscarin depression favours the appearance of cardiac inhibition. Further, the fact that a restoration of the force and frequency of the cardiac rhythm of the chick-embryos by subsequent application of atropin sulphate (vide table 26) is accompanied with a decrease of the tendency towards inhibition, and a

[1] *Op. cit.*

consequent increase of the strength of the current requisite to induce cardiac inhibition, which shows that although the atropin sulphate apparently "restores" the rhythm, yet it causes a greater increase of katabolic than of anabolic processes.

(4) *The significance of the preceding Experiments.*

Prominent amongst the rival explanations of the phenomena of inhibition is the view associated with the name of Claude Bernard[1], that it is due to the interference of "nerve impulse waves" in their passage along the nerve fibre. In the case of cardiac inhibition, Ranvier[2] in his careful review of this theory suggested that the interference took place in the spiral ganglion cells of the heart, the "nerve impulse wave" taking a longer time to travel through the spiral, than the direct portion. More recent writers associate inhibitory phenomena with changes in the terminations of the nerves. Others believe that these phenomena are intimately associated with metabolic changes, and in the case of cardiac inhibition, that the more important changes take place in the heart-muscle itself. The experiments I have recorded on chick-embryos prior to the development of their intrinsic cardiac nervous mechanism, show that during the absence of nervous elements from the heart the application of interrupted currents fails to produce inhibition, and that the period during which the failure to induce inhibition occurs, fairly corresponds to the time during which muscarin nitrate and atropin sulphate fail to exhibit their typical action on the embryonic heart. On older embryos at the time when muscarin nitrate and atropin sulphate exhibit their typical antagonistic action the application of a weak interrupted current produces typical inhibition, while stronger currents induce delirium cordis, in a manner similar to their action on earlier embryos. In this connection, I would recall the previously cited observation of Dew-Smith that the application of interrupted currents to the heart of "Salpa" fails to induce inhibition and that Krukenberg[3] found that the heart of this type is not affected by the application of muscarin nitrate. Again, Foster's[4] observations that snails' hearts are inhibited by interrupted currents, correspond with the fact that they are typically influenced by muscarin

[1] Bernard. Quoted in Brunton's *Pharmacology*, vol. I. p. 325. 1891.

[2] Ranvier. *Leçons d'Anatomie Générale.* 1877—8, p. 106 et seq.

[3] Krukenberg. *Vergleichend-physiologische Studien*, Dritte Abth. Heidelberg, 1880, S. 151.

[4] Foster. *Op. cit.*

nitrate and atropin sulphate. There is therefore apparently a more than coincident correspondence between the action of these drugs and of interrupted electric currents on the heart.

Further, a study of the inhibition of the hearts of older chick-embryos shows a striking similarity to the inhibitory phenomena described by Pawlow[1], Richet[2] and more accurately by Piotrowski[3], as characteristic of the action of interrupted electric currents on limb-muscles of crayfish. It will be also evident that variations of external conditions which influence the cardiac tone, or modify the metabolic changes in the cardiac myoplasm, profoundly influence the effect of interrupted currents on the embryonic heart, and consequently the resulting inhibitory phenomena. Thus the application of caffein, digitalin or veratrin, variations of the surrounding temperature, an excess of either oxygen or of carbonic dioxide markedly influence the phenomena of inhibition. These agents also modify the action of interrupted currents on embryos prior to the development of their cardiac nervous mechanism, and thus it becomes evident that the phenomena of cardiac inhibition are influenced by two series of processes, both essential, viz. those taking place in the nervous elements, and those confined to the contractile tissue. The condition of the contractile tissue plays an all important part on the effect of the stimulus produced by an interrupted electrical current, nevertheless, these experiments apparently decide that the nervous elements are not only essential, but that they play the more important part in the phenomena of inhibition.

The absence of spiral ganglion cells at a time when cardiac inhibition was easily obtainable, entirely negatives the interference theory of Bernard and Ranvier. It would also appear not improbable that the final terminations of the cardiac nerve play the chief *rôle* in inhibition, and if an analogy can be safely drawn to the inhibitory phenomena in the limb-muscles of decapods and other types, that in these types the inhibitory phenomena are also associated with changes taking place in the final terminations of the nerves. Here again the metabolic condition of the contractile tissue probably determines the effect of the stimulus.

The association of the phenomena of inhibition with anabolic processes, and those of contraction with katabolic processes, in the

[1] Pawlow. *Pflüger's Archiv*, XXVII.
[2] Richet. *Archiv de Physiologie*, 1879.
[3] Piotrowski. This *Journal*, XIV. p. 163. 1893.

contractile tissue of the heart is supported by the observations here recorded, which in some respects support the theories of Gaskell[1] and Piotrowski[1] concerning the nature of inhibition. Nevertheless it is evident that more stress should be laid on the *rôle* played by the nerve terminations than has been done by recent writers on the subject.

(5) *Action of Constant Currents on the Hearts of Chick-embryos.*

Preyer[1] states that constant currents have no action on the hearts of chick-embryos. This statement is true only as regards very weak currents, or where the contact of the electrodes is imperfect. In my experiments the ordinary non-polarisable electrodes were used, and the current conducted from china-clay terminals to heart by means of silk threads soaked in normal saline. Currents of moderate strength[2] increase the force of frequency of the rhythm of both early and later embryos. The average increase of cardiac frequency in 20 experiments on embryos of ages varying from 60 to 300 hours was 6 beats per minute. In no case did the application of a constant current produce inhibition. If a current of moderate strength[2] be applied in a direction the reverse of that of the cardiac contraction wave, to embryos aged from 40 to 60 hours, their heart rhythm becomes reversed. Stronger[3] currents applied in the direction from ventricle to auricle, will reverse the rhythm of older embryos, but it becomes more difficult to reverse the cardiac rhythm as the embryo increases in age. It should be noted that these results correspond with those of McWilliam[4] on eels' hearts and of Foster[5] and Dew-Smith on snails' hearts, and that they are obtainable both before and after the development of the cardiac nervous system.

(6) *Influence of Constant Currents on the Subsequent Action of Interrupted Currents on the Hearts of Chick-embryos.*

If a constant current of moderate strength be applied to the hearts of chick-embryos aged up to 120 hours, for two or three minutes, and immediately afterwards a weak interrupted current be passed through them, there is a pronounced increase of the force and frequency of the

[1] *Op. cit.*

[2] Currents spoken of as of moderate strength = 1 milliampère.

[3] Stronger currents = 15 milliampères. A commutator in the circuit varies the direction of the current.

[4] McWilliam. *Proc. Roy. Soc.* XXXVIII. p. 108.

[5] *Op. cit.*

cardiac rhythm. Further, the strength of the interrupted current requisite to produce delirium cordis is much less after the application of a constant current than under ordinary conditions. This effect however is only observed immediately after the action of the constant current, and progressively decreases, so that after an interval of 4 minutes from the time of the passage of the constant current, the condition of the heart becomes normal. These points are summarized in the following table:—

TABLE 37.

Embryos kept at 38° C.

Age of embryo.	Distance of coils necessary to induce delirium cordis before action of constant current.	Distance of coils necessary to induce delirium cordis immediately after action of const. current.	Distance of coils necessary to induce delirium cordis 4 mins. after action of const. current.
60 hrs	16 cm.	24 cm.	15·3 cm.
75 „	17 cm.	18 cm.	17·1 cm.
75 „	15·8 cm.	15·9 cm.	16 cm.
80 „	21 cm.	26 cm.	20·8 cm.
110 „	16·4 cm.	22 cm.	16·7 cm.
110 „	14·8 cm.	17 cm.	15 cm.
120 „	20 cm.	24·3 cm.	20·1 cm.
120 „	19·8 cm.	25·6 cm.	20·2 cm.
120 „	19·4 cm.	24·7 cm.	19·5 cm.

In embryos aged from 200 hours and upwards the application of the constant current also influences the effect of interrupted currents if they are applied immediately afterwards, as shown in the next table:—

TABLE 38.

Embryos kept at 38° C.

Age of embryo.	Distance of coils requisite to induce inhibition before action of constant current.	Distance of coils requisite to induce inhibition immediately after action of constant current.	Distance of coils requisite to induce inhibition 4 mins. after action of const. current.
206 hrs	24·3 cm.	27 cm.	23·7 cm.
208 „	26 cm.	28·5 cm.	26·1 cm.
208 „	19·8 cm.	24·6 cm.	19·9 cm.
210 „	20 cm.	23 cm.	19·9 cm.
210 „	22 cm.	24 cm.	21·6 cm.
215 „	21 cm.	21·7 cm.	21·4 cm.
215 „	22 cm.	25 cm.	22·2 cm.
250 „	18·7 cm.	22·6 cm.	18·6 cm.
250 „	21·1 cm.	23·8 cm.	20·9 cm.

It is obvious that the effect produced by the constant current, is to

decrease the strength of the interrupted current requisite immediately afterwards to induce cardiac inhibition:—that is, the constant current favours the appearance of inhibitory phenomena, although it increases both the force and frequency of the embryo's heart rhythm.

In the observations recorded in the two preceding tables, the constant current was not allowed to pass through the embryos' hearts for more than 4 minutes. If, however, the current be allowed to pass for a longer period through the hearts the result is different, although there is apparently no change in the force and frequency of the rhythm from that produced by the passage of the current for a short time.

In earlier embryos the passage of a constant current for 30 minutes decreases the tendency towards the heart delirium produced by the subsequent application of weak interrupted currents:—that is, a stronger interrupted current is necessary to induce heart delirium than under ordinary conditions. The next table embodies these results:—

TABLE 39.

Embryos kept at 38° C.

Age of embryo.	Distance of coils to induce heart delirium under normal conditions.	Distance of coils to induce heart delirium after the passage of a constant current for 30 mins.	Distance of coils to produce heart delirium after the constant current has been off for 8 mins.
75 hrs	16·7 cm.	15·1 cm.	16 cm.
75 ,,	15·8 ,,	10·8 cm.	16·3 cm.
75 ,,	15·9 ,,	12·4 cm.	14·8 cm.
80 ,,	17·4 ,,	15 cm.	16·3 cm.
82 ,,	16·3 ,,	13 cm.	14·5 cm.
82 ,,	17·1 ,,	16 cm.	15·8 cm.
96 ,,	15·2 ,,	9 cm.	15 cm.
96 ,,	15·3 ,,	11·4 cm.	14·4 cm.
110 ,,	14·8 ,,	13 cm.	13·9 cm.
110 ,,	14·9 ,,	8 cm.	13·8 cm.

In older embryos after the passage of constant current for 30 minutes, the strength of the interrupted current requisite immediately afterwards to produce inhibition is greater than that necessary to produce inhibition under normal conditions. The effect of the constant current, however, rapidly passes off, so that 8 minutes afterwards interrupted currents of the strength necessary to induce inhibition under normal circumstances will usually again induce inhibition. The next table records these results:—

TABLE 40.

Embryos kept at 38° C.

Age of embryo.	Distance of coils to induce cardiac inhibition under normal conditions.	Distance of coils to induce cardiac inhibition after the passage of a const. current for 30 mins.	Distance of coils to produce cardiac inhibition after the const. current has been off for 8 mins.
250 hrs	21·4 cm.	17·4 cm.	20·1 cm.
250 "	23·6 cm.	16·3 cm.	22·4 cm.
300 "	22·7 cm.	20 cm.	22·3 cm.
300 "	22·6 cm.	19·3 cm.	20 cm.
220 "	19·9 cm.	19·3 cm.	20 cm.
220 "	18·6 cm.	17·4 cm.	18·5 cm.
240 "	21 cm.	18·1 cm.	20·3 cm.
240 "	20·4 cm.	18·2 cm.	19·7 cm.

A comparison of these last two tables with the two preceding ones will show that the results obtained after the prolonged passage of a constant current, are the converse of those obtained after the current has only passed for a short time through the heart. A possible explanation of this result is that the prolonged passage of a constant current induces anabolic phenomena in the cardiac myoplasm, and consequently increases the tendency towards contraction and decreases the tendency towards inhibition. The subject is however a difficult one and requires further investigation.

Variations of the temperature of the embryo influence the effect produced by constant current, thus at sub-normal temperatures (*e.g.* 26 to 32° C.) a stronger constant current is necessary to increase the force and frequency of the cardiac rhythm than at 38° C., while at hyper-normal temperatures a weaker constant current will produce an increase of both force and frequency with cardiac rhythm. A probable explanation of the failure of Preyer[1] to obtain any results on embryo-chicks' hearts after the application of a constant current, is that the method he adopted cooled the embryos and consequently rendered them very resistant to its influence.

Variations of the temperature of the embryo also influence the effect produced by an interrupted current, immediately after the passage of a constant current through the heart, lower temperatures making the phenomena observed less pronounced, higher temperatures accentuating them, but not altering their nature. This result is embodied in the next eight tables, where the effects produced by the passage of a constant current for 30 and 4 minutes respectively at

[1] *Op. cit.*

temperatures of 28° C. and 42° C. are recorded both on earlier and later chick-embryos.

TABLE 41.

Embryos kept at 28° C.

Age of embryo.	Distance of coils to induce heart delirium under normal conditions.	Distance of coils to induce heart delirium after passage of a constant current for 30 mins.	Distance of coils to produce heart delirium after the constant current has been off for 8 mins.
60 hrs	17·8 cm.	16·4 cm.	17 cm.
75 ,,	16·4 cm.	16·3 cm.	16·5 cm.
75 ,,	17·4 cm.	17·1 cm.	18 cm.
80 ,,	16·3 cm.	15·8 cm.	16 cm.
80 ,,	18·7 cm.	18·6 cm.	18·6 cm.
95 ,,	15·4 cm.	15·4 cm.	16·7 cm.
95 ,,	16·8 cm.	16·7 cm.	16·7 cm.
100 ,,	15·9 cm.	15 cm.	15·5 cm.
100 ,,	17·3 cm.	14 cm.	16·9 cm.

TABLE 42.

Embryos kept at 28° C.

Age of embryo.	Distance of coils to induce cardiac inhibition under normal conditions.	Distance of coils to induce cardiac inhibition after passage of constant current for 30 mins.	Distance of coils to induce inhibition after current has been off for 8 mins.
250 hrs	21·6 cm.	19·3 cm.	21·4 cm.
250 ,,	43·4 cm.	22·7 cm.	23·4 cm.
275 ,,	22·7 cm.	21·4 cm.	22·5 cm.
275 ,,	23·8 cm.	23 cm.	22·7 cm.
300 ,,	19·4 cm.	19·7 cm. (?)	19·5 cm.
300 ,,	20·4 cm.	18·3 cm.	20·3 cm.
250 ,,	21·3 cm.	19·7 cm.	20·1 cm.
250 ,,	20·3 cm.	18·9 cm.	20·2 cm.

TABLE 43.

Embryos kept at 42° C.

Age of embryo.	Distance of coils necessary to induce delirium cordis before action of constant current.	Distance of coils necessary to induce delirium cordis after action of constant current for 4 mins.	Distance of coils necessary to induce delirium cordis 4 mins after action of constant current.
56 hrs	18·75 cm.	25·4 cm.	20·4 cm.
75 ,,	19 cm.	23·8 cm.	20·3 cm.
75 ,,	20 cm.	22·4 cm.	21 cm.
80 ,,	21 cm.	27 cm.	21·8 cm.
80 ,,	18·9 cm.	24·8 cm.	22·4 cm.
110 ,,	20 cm.	25·3 cm.	20 cm.
110 ,,	21·3 cm.	26·4 cm.	23 cm.
120 ,,	22 cm.	30 cm.	25 cm.
120 ,,	21·4 cm.	27·1 cm.	21·3 cm.

TABLE 44. Embryos kept at 42° C.

Age of embryo.	Distance of coils requisite to induce inhibition before action of constant current.	Distance of coils requisite to induce inhibition after action of constant current for 4 mins.	Distance of coils requisite to induce inhibition 4 mins. after action of constant current.
205 hrs	26·3 cm.	29·4 cm.	27·1 cm.
206 ,,	26 cm.	28·7 cm.	28 cm.
208 ,,	21·4 cm.	25·3 cm.	23 cm.
210 ,,	24·3 cm.	26·7 cm.	24·5 cm.
210 ,,	20·1 cm.	24·8 cm.	21·3 cm.
215 ,,	22·4 cm.	23·7 cm.	22·5 cm.
215 ,,	23·7 cm.	26·5 cm.	23·6 cm.
240 ,,	24·8 cm.	30·8 cm.	25·2 cm.
250 ,,	25·7 cm.	28·4 cm.	25·1 cm.
250 ,,	26·4 cm.	29·3 cm.	26·7 cm.

TABLE 45. Embryos kept at 28° C.

Age of embryo.	Distance of coils to induce heart delirium under normal conditions.	Distance of coils to induce heart delirium immediately after the passage of a constant current for 4 mins.	Distance of coils to produce heart delirium 4 mins. after passage of a consant current.
59 hrs	15·8 cm.	16·9 cm.	15·7 cm.
75 ,,	27 cm.	23 cm.	20 cm.
75 ,,	20 cm.	22 cm.	20·1 cm.
80 ,,	18·4 cm.	19·8 cm.	18·3 cm.
80 ,,	16·8 cm.	17·75 cm.	15·9 cm.
100 ,,	16·7 cm.	20·3 cm.	17·3 cm.
100 ,,	15·9 cm.	18·1 cm.	16 cm.
115 ,,	18·2 cm.	19·3 cm.	17·75 cm.
120 ,,	17·5 cm.	18·4 cm.	18·1 cm.

TABLE 46. Embryos kept at 28° C.

Age of embryo.	Distance of coils to induce inhibition before action of constant current.	Distance of coils requisite to induce inhibition immediately after action of constant current for 4 mins.	Distance of coils requisite to induce inhibition 4 mins. after passage of constant current.
205 hrs	24·2 cm.	25·3 cm.	24·1 cm.
206 ,,	25·8 cm.	25·9 cm.	23 cm.
207 ,,	23·2 cm.	24·6 cm.	22·9 cm.
208 ,,	25 cm.	28 cm.	24·8 cm.
208 ,,	25·6 cm.	27·2 cm.	25·1 cm.
212 ,,	21·4 cm.	23 cm.	20·9 cm.
212 ,,	19·8 cm.	20 cm.	19·8 cm.
215 ,,	21·3 cm.	21·6 cm.	21 cm.
240 ,,	18·75 cm.	19·4 cm.	19 cm.
256 ,,	21·4 cm.	22 cm.	20·3 cm.
250 ,,	20·8 cm.	21·3 cm.	20·4 cm.

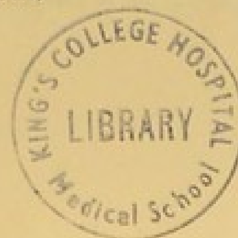

TABLE 47.

Embryos kept at 42° C.

Age of embryo.	Distance of coils to induce heart delirium under normal conditions.	Distance of coils to induce heart delirium after passage of constant current for 30 mins.	Distance of coils to produce heart delirium after the constant current has been off for 8 mins.
60 hrs	18·3 cm.	30·4 cm.	17·9 cm.
75 ,,	17·8 cm.	12·8 cm.	16·3 cm.
80 ,,	16·3 cm.	11·9 cm.	13·2 cm.
80 ,,	17·1 cm.	16·8 cm.	16·8 cm.
100 ,,	15·8 cm.	9·7 cm.	12·4 cm.
110 ,,	16·5 cm.	13·1 cm.	15 cm.
120 ,,	17·9 cm.	14·5 cm.	16·5 cm.

TABLE 48.

Embryos kept at 42° C.

Age of embryo.	Distance of coils to induce inhibition under normal conditions.	Distance of coils to induce inhibition after the passage of a constant current for 30 mins.	Distance of coils to induce inhibition after the constant current has been off for 8 mins.
250 hrs	23·4 cm.	16·4 cm.	22·4 cm.
250 ,,	23·6 cm.	16·9 cm.	23 cm.
275 ,,	22·8 cm.	18·2 cm.	21·4 cm.
275 ,,	19·6 cm.	15·8 cm.	19·8 cm.
275 ,,	20·1 cm.	16 cm.	17 cm.
240 ,,	18·7 cm.	15·7 cm.	18·4 cm.
240 ,,	21 cm.	17·1 cm.	18·7 cm.
300 ,,	20·3 cm.	17·3 cm.	19 cm.
300 ,,	19·7 cm.	16·3 cm.	19·3 cm.

It is evident from the preceding tables that the temperature of the embryo influences to a marked degree the action of a constant current, on the subsequent effects produced by an interrupted current.

(7) *Action of Single and a Series of Induction Shocks on the Hearts of Chick Embryos.*

If a single induction shock be applied during the diastolic phase to the heart of an early chick-embryo (aged 60 to 120 hours) it is found that an additional heart beat is interpolated in the rhythm. A similar result is obtained if a number of shocks be applied during the diastolic phase, providing the number does not exceed a certain amount, which apparently varies with each individual embryo. Thus if ten induction shocks be applied in a minute to an embryo, which has a cardiac rhythm

of 80 beats per minute, there is usually a resulting increase of 10 beats per minute in the cardiac frequency, as well as an augmentation of its force; but if 20 induction shocks be applied to an embryo, with a cardiac frequency of 50 beats per minute, the average number of additional beats in four experiments was 15. If 20 shocks per minute be applied to an embryo with a cardiac frequency of 120 beats per minute, there is an increase of 20 beats per minute as the result. If a single, or a series of single induction shocks be applied during the systolic phase of the embryos' cardiac rhythm, the result is more variable, the result being sometimes the interpolation of an additional beat corresponding with the stimulus, and at others an apparent slight increase in the force of the systole. The number of the series of induction shocks that will produce heart delirium is also very variable, and seems to be governed by the frequency, tone, and temperature of the heart, since all these factors cause great alteration in the number of shocks requisite to induce heart delirium. The next table summarises these various results:—

TABLE 49.

Distance of the coils throughout the experiments, 15 cm.

Age of embryo.	Frequency of heart rhythm at 38° C.	No. of induction shocks applied per minute.	Increase of cardiac frequency resulting.	Remarks on resulting rhythm.
56 hrs	60 per min.	8	8	Normal.
56 „	64 „	10	10	do.
60 „	58 „	15	15	do.
60 „	70 „	20	18	Slightly stronger than normal.
75 „	80 „	20	20	
75 „	100 „	20	16	do.
80 „	120 „	40	—	Heart delirium.
80 „	116 „	40	—	do.
100 „	108 „	30	30	Weaker than normal.
100 „	106 „	30	30	do.
120 „	116 „	40	46	do.
120 „	112 „	40	—	Heart delirium.

If a single induction shock be applied to older chick-embryos (250 to 300 hrs.) the result is similar, but a series of shocks will sometimes cause an augmentation and increase of frequency of the rhythm, and in others if the number of the stimuli be sufficiently great, either cardiac inhibition, or in other cases heart delirium results. Here again the tone, frequency, and temperature of the heart play an all-important part. The next table summarises the results obtained at 38° C.:—

TABLE 50.

Distance of the coils throughout the experiments, 15 cm.

Age of embryo in hrs.	Frequency of heart rhythm at 38° C.	No. of induction shocks applied per min.	Resulting increase of cardiac frequency.	Remarks on resulting rhythm.
250	112	12	12	Apparently normal.
250	120	20	20	Slightly stronger.
260	115	40	37	Weaker than normal.
260	96	60	—	Inhibition.
275	108	65	—	do.
275	100	65	—	do.
300	110	70	—	do.
300	115	50	—	Heart delirium.
240	116	45	43	do.
240	118	60	—	Inhibition.

If the temperature of the embryos be hypernormal, *e.g.* 42° C., the result is different, as is shown in the following table :—

TABLE 51.

Distance of the coils throughout the experiments, 15 cm.

Age of embryo in hrs.	Frequency of heart rhythm at 42° C.	No. of induction shocks applied per min.	Resulting increase of cardiac frequency.	Remarks on resulting rhythm.
240	126	45	—	Heart delirium.
240	130	60	—	do.
250	118	12	12	Stronger than normal.
250	122	20	20	do.
260	140	40	46	Heart delirium.
260	132	60	—	do.
275	116	65	—	do.
275	108	70	—	Inhibition.
300	118	50	—	do.
300	102	45	—	Partial inhibition.

At 42° C. it will be noted that only once was inhibition obtained by a series of single induction shocks, in the other experiments heart delirium resulted.

In a similar series of experiments where the embryos were kept at 28° C., the result was as follows :—

TABLE 52.

Distance of the coils throughout the experiments, 15 cm.

Age of embryos in hrs.	Frequency of rhythm at 28° C.	No. of induction shocks applied per min.	Resulting increase of cardiac frequency.	Remarks on resulting rhythm.
240	108	45	36	Characteristics unchanged.
240	102	60	—	Inhibition.
250	78	12	12	Characteristics unchanged.
250	100	20	20	do.
260	67	40	—	do.
260	82	60	—	Inhibition.
275	104	65	—	Heart delirium.
275	98	70	—	Inhibition.
300	98	50	30	Weaker than normal.
300	82	45	24	do.

Again, the strength of the current varies the effect produced, which again varies with the temperature of the embryo. Thus at the higher temperatures of 39° to 42°, a stronger series of induction shocks (distance of coils 6 to 10 cm.) tends to inhibition, while a series of equal number but of less strength (distance of coils 15 to 25 cm.) will induce heart delirium. At a sub-normal temperature the converse usually holds good, thus a series of shocks with the coils close together is more likely to produce heart delirium than the same number of shocks with the coils farther apart. Also the result produced varies with number and strength of the shocks, fewer stronger shocks applied at greater intervals of time, producing a similar effect as the larger number of weaker shocks applied over lesser intervals of time.

The following are therefore the factors which control the result produced by a series of single induction shocks on the hearts of embryo-chicks.

(*a*) Temperature of the heart.
(*b*) Frequency of the shocks.
(*c*) Strength of the shocks.
(*d*) Age of the embryo.

The result may be either :—

(α) Augmentation of force, and increase of frequency of the cardiac rhythm.

(β) Increase of frequency, accompanied by decrease of the force of rhythm.

(γ) Heart delirium (if number of shocks exceed $\frac{1}{2}$ cardiac frequency).

(δ) Inhibition (if number of shocks exceed $\frac{1}{2}$ cardiac frequency).

(8) *The action of Single, and a Series of Induction Shocks, on the subsequent action of interrupted currents on the Hearts of Chick-embryos.*

Apparently the application of a single induction shock does not influence the result produced by the application immediately afterwards of an interrupted current. A series of shocks, if of sufficient intensity and repeated sufficiently often, produce a result on the immediate subsequent action of an interrupted current like that described in section 1, part II. as characteristic of an interrupted current if applied immediately after the action of another interrupted current. The minimum number of shocks per minute to produce this effect is apparently about one-half the number of the cardiac frequency in a given embryo. The strength of the shocks should not be less than that obtained when the distance of the coils does not exceed 25 cm., but more marked results are obtained when the coil distance is from 15 to 20 cm. The effect produced on the subsequent action of an interrupted current rapidly passes off, in a manner similar to the action of one interrupted current on the subsequent action of a second interrupted current. If a series of single induction shocks not able to induce either heart delirium in earlier or cardiac inhibition in later embryos be passed for two or three minutes through the cardiac myoplasm, the distance of the coils necessary to induce either heart delirium or cardiac inhibition on the subsequent application of an interrupted current is more than under ordinary circumstances (*i.e.* the strength of current is less). If the series of induction shocks be passed through the cardiac myoplasm for a comparatively long time (*e.g.* 30 minutes), the distance of coils of subsequent interrupted currents, requisite to induce either heart delirium or cardiac frequency is less (*i.e.* the strength of requisite current is greater). These results are parallel to those obtained with interrupted and constant currents (see tables 37 to 48).

(9) *The action of Single, and a Series of Induction Shocks, constant and interrupted currents, on the Hearts of Mammalian embryos.*

The embryos used were those of rats, rabbits, and dogs. In the experiments to determine the time of inhibition, the observations were made both *in situ* in the mother, and on excised embryos in the observing incubator. All other observations were made on excised

embryos in the observing incubator, and except where otherwise stated the embryos were bathed in normal saline.

(*a*) *Single induction shocks.* The application of a single induction shock during the diastolic phase to the heart of a mammalian embryo interpolates a beat corresponding with the application of the shock. If applied during the systolic phase the result is variable, an additional beat sometimes resulting, at others a mere strengthening of the systole.

(*b*) *A series of induction shocks.* The results with the embryos of dogs, rabbits and rats closely correspond with the results recorded in section 8 (tables 48 to 51) as characteristic of the action of a series of shocks on chick embryos. Allowance should be made for the fact that the cardiac frequency of mammalian embryos is lower than that of chick embryos, and consequently a smaller number of shocks produce either delirium cordis or cardiac inhibition, according to the age of the embryo. The general remarks (with this observation), as regards the effect of temperature variations, on the effect produced by induction shocks on the hearts of chick-embryos apply to mammalian embryos.

In the next table is the record of a series of induction shocks of dog embryos kept at 38° C.

TABLE 53.

Distance of coils throughout experiments, 15 cm.

Age of embryos in days.	Frequency of heart rhythm at 38° C.	No. of shocks applied per min.	Increase of cardiac frequency resulting.	Remarks on resulting rhythm.
8	20 beats per min.	8	8	Stronger than normal.
8	22 ,, ,,	10	10	do.
10	18 ,, ,,	15	—	Delirium cordis.
10	21 ,, ,,	20	—	do.
14	24 ,, ,,	12	12	Stronger than normal.
14	21 ,, ,,	21	—	Delirium cordis.
15	20 ,, ,,	24	—	Inhibition.
16	17 ,, ,,	12	12	Weaker than normal, beats fluttering.
16	18 ,, ,,	15	—	Inhibition.
16	24 ,, ,,	20	—	do.
17	20 ,, ,,	16	—	do.

As on the chick embryo, the following factors control the result produced by a series of induction shocks on the hearts of dog, rat and rabbit embryos. (1) The temperature of the heart. (2) The frequency of the shocks. (3) The strength of the shocks. (4) The age of the embryo. The result of the shocks may be manifested as (1) An

augmentation of the force, and an increase of the frequency of the rhythm. (2) An increase of the frequency, accompanied by a decrease of the force of the rhythm. (3) Heart delirium, if the number of shocks exceeds half the number of heart beats per minute. (4) Cardiac inhibition on older embryos, when the number of shocks exceeds half the number of heart beats per minute.

It is unnecessary to burden this already lengthy paper with the statistical records on which these generalisations are based, as they are similar to those recorded in the case of chick-embryos.

(*c*) *Constant Currents.* Here again the description given of the action of constant currents on chick-embryos in section 5 applies to mammalian embryos. The influence of constant currents on the subsequent action of interrupted currents is also the same on the mammalian as on the embryo chick's heart.

(*d*) *Interrupted Currents.* These produce either delirium cordis or inhibition, according to the age of the embryo. On earlier embryos, interrupted currents (of strength when the distance of the coils is from 15 to 20 cm.) produce delirium cordis, on later embryos (16 to 18 days on the dog or cat) cardiac inhibition. It is very difficult to pronounce the exact age at which an interrupted current will produce cardiac inhibition, and although I have made a considerable number of experiments to determine the point I am not in a position to make a definite statement. It is however clear that on early embryos (up to eight days) interrupted currents fail to produce cardiac inhibition. All the same factors which influence the appearance of either delirium cordis, or cardiac inhibition, on chick-embryos also influence the appearance on mammalian embryos.

The action of caffein, digitalin, veratrin, muscarin nitrate and atropin sulphate, dilute acids and alkalis, oxygen and carbon dioxide on the effects produced by interrupted currents is the same as recorded on the chick's heart.

PART III.

General Conclusions.

The principal points to which this paper calls attention may be summarised as follows:

1. The hearts of mammalian embryos (rat, dog, rabbit and cat) will, if kept under proper conditions, beat with a rhythm constant for each

individual embryo, even when they are excised from the mother; and if supplied with suitable nutrient material (*e.g.* a mixture of the mother's blood and ·75 % NaCl) they will maintain a fairly constant rhythm for three or four days. They are very susceptible to variations of temperature. Special precautions were taken to maintain them at the desired temperatures.

2. If mammalian embryos are surrounded with a nutrient fluid consisting of equal parts of the blood of another species and ·75 % NaCl, irregularities of their cardiac rhythm are observed. If the surrounding medium is a mixture in equal parts of egg albumen and ·75 % NaCl, the irregularities of the cardiac rhythm are not so marked as when the surrounding medium is the blood of another species.

3. Embryos surrounded by this mixture of egg albumen and ·75 % NaCl, maintain a fairly strong rhythm even after three days' immersion.

4. Embryos surrounded by ·75 % NaCl alone, will sometimes continue beating for one day after exposure to this substance.

5. Embryos surrounded by 7·5 % NaCl solution of the proteid-like colloid *C*, will maintain a fairly strong rhythm for three days.

6. Embryos surrounded by a 7·5 % solution either of the colloids *A* or *B* in normal saline are usually dead after an exposure from 18 to 20 hours. In some cases when surrounded by the colloid *A* dissolved in normal saline feeble heart beats were found after 24 hours had elapsed.

7. "Ringer's fluid" will maintain the heart beats for about a day. In a few cases a very slow and feeble rhythm was maintained after two days' immersion in this solution.

8. The sustaining power of the ash of gum arabic and of blood ash is very similar to that of "Ringer's fluid."

9. A 2 % solution of gum arabic to which a trace of sodium carbonate has been added will sustain the rhythm from 2 to 3 days. It is however far inferior in sustaining power to the proteid-like colloid *C*, as the rhythm on the third day, when it exists, is always very feeble.

10. Attention is drawn to Table 15, which summarises the relative sustaining powers of various substances.

11. Water that has been distilled into metal receivers is very toxic to the cardiac myoplasm of chick embryos. Pure distilled water that has been received into glass containers is apparently innocuous to the embryonic heart. It has however no power of sustaining the heart rhythm, and thus forms a valuable control experiment in comparing the relative sustaining power of various other substances. My experiments

lend support to the theory of the oligodynamic action of minute quantities of metals in solution, put forward by Nägeli and Locke.

12. The oligodynamic action of copper can often be partially removed by the addition of traces of certain colloids and other substances, including gelatin, and the synthesised proteid-like colloids *A*, *B* and *C*.

13. An isotonic solution has apparently a better sustaining power on the cardiac myoplasm than one that has a different osmotic tension from the fluid constituents of the embryo.

14. The general action of doses of caffein, antiarin, digitalin, strophanthin, nicotin, veratrin and morphine acetate, is the same on the hearts of mammalian embryos as that described in my previous papers on those of chick-embryos.

15. The action of minute doses of strophanthin acting over a comparatively long period of time is the converse to the action of larger doses of strophanthin acting over a short period of time; inducing diastolic stoppage. The results are similar to those described by Fraser as characteristic of the action of minute doses of strophanthin on the frog's heart, and take place prior to the development of the intrinsic cardiac mechanism of the embryo.

16. The application of small doses of nicotin to later mammalian embryos, after the development of an intrinsic cardiac mechanism, prevents the appearance of cardiac inhibition, after the application of interrupted electric currents.

17. Muscarin nitrate and atropin sulphate exhibit their typical antagonistic action on both earlier and later mammalian embryos. In this respect there is marked difference from the action on earlier chick-embryos.

18. Chloroform acts as a powerful depressant to the hearts of mammalian embryos, either if applied direct to the cardiac myoplasm, or if administered through the mother's circulation, the embryo remaining *in situ* in the uterus. Ether, except in very large doses applied direct to the cardiac myoplasm, acts as a stimulant to mammalian embryos' hearts. The addition of a small quantity of alcohol to the chloroform mitigates its toxic action on the heart. A mixture of chloroform, ether, and alcohol was found not to be so depressant as either chloroform alone, or a mixture of chloroform and ether.

The subsequent application of either digitalin, strophanthin, antiarin, caffein, or of atropin sulphate, fails to restore the rhythm of embryos' hearts that have been stopped by chloroform. The direct application

of a dilute solution of ammonia will however sometimes partially restore their rhythm.

Electrical Experiments.

19. Very weak interrupted electric currents have apparently no action on early embryos (aged up to 160 hours). Stronger currents produce an augmentation of the force and an increase of the frequency of their rhythm, while if the strength of the current is further increased, its action culminates in heart delirium and systolic stoppage.

20. The earliest embryo in which cardiac inhibition was obtained after the application of an interrupted current to its heart, was aged 150 hours. The application of an interrupted current to the hearts of embryos of this age usually results in delirium cordis. Embryos aged 160 to 180 hours may or may not have their hearts inhibited on the application of an interrupted current. Embryos aged 200 hours and upwards can always have their hearts inhibited by an interrupted current. In older embryos a current will induce inhibition, when weaker than that requisite to produce this result in younger embryos.

21. In embryos aged over 250 hours a strength of current greater than that requisite to induce cardiac inhibition causes an augmentation and increase of frequency of the rhythm, which often culminates in heart delirium. Thus in older chick-embryos weaker currents produce inhibition, and stronger currents heart delirium.

22. Lowering the temperature of the embryo renders it more difficult to induce inhibitory phenomena. An increase of the temperature of the embryo, up to a certain point, renders it easier to induce cardiac inhibition. If however the temperature be increased above 42° C. it is very difficult to obtain cardiac inhibition. On the application of an interrupted current at temperatures about 42° C. heart delirium culminating in systolic stoppage nearly always results.

23. If an interrupted current of moderate strength be applied for three or four minutes to an embryo-chick's heart, and then is discontinued for a minute, after which time a second interrupted current be passed through the heart, the effect produced by the second application of the current is greater than that produced by the first application of the current. In other words there is a summation of the effect produced. This result obtains either in the case of the production of heart delirium, or of cardiac inhibition.

24. If an interrupted current of moderate strength be passed through a chick-embryo's heart for thirty minutes, and be discontinued, and if

after an interval of one minute, a second interrupted current be passed through the heart, it is found that the result is the converse of the above; that is, a stronger current is requisite immediately after the passage of the first current to produce either delirium cordis on earlier, or cardiac inhibition on later embryos.

25. Small doses of veratrin (·1 mg.) administered at either 26° C., 32° C. or 38° C. decrease the strength of current requisite to induce cardiac inhibition on chick-embryos. At temperatures above 38° C. ·1 mg. of veratrin apparently does not aid the appearance of cardiac inhibition, but larger doses, *e.g.* ·175 mg. to ·2 mg. will do so.

26. When ·015 mg. of muscarin nitrate is applied to an embryo-chick's heart, the strength of the current requisite to produce cardiac inhibition is less than under ordinary circumstances. If the rhythm that has been depressed by muscarin nitrate be partially restored by the subsequent application of ·015 mg. of atropin sulphate, the strength of the current requisite to induce cardiac inhibition, after the subsequent application of the atropin sulphate, is greater than during the muscarin depression; although there has been an increase in the force and frequency of the heart rhythm due to the antagonistic action of atropin sulphate.

27. The influence of caffein on the cardiac inhibition produced by interrupted currents varies with the temperature of administration. At 38° C. the administration of ·1 mg. of caffein decreases the strength of current requisite to induce cardiac inhibition, at 28° C. the same dose sometimes increases the strength of current requisite to induce cardiac inhibition, while at 42° C. even weak interrupted currents induce delirium cordis culminating in systolic stoppage.

28. Similar results are produced by the administration of ·015 mg. of digitalin where also the temperature of the administration plays an all-important part on the result produced by the subsequent application of interrupted currents.

29. It is evident that the tone and temperature of the heart play an all-important *rôle* in the phenomena of inhibition.

30. Small doses of lactic acid, if applied to the cardiac myoplasm, retard the appearance of the inhibitory phenomena after the application of interrupted currents. A subsequent application of dilute sodium hydrate restores the tone and frequency of the heart as well as the ordinary readiness to induce inhibition on the application of an interrupted current.

31. The influence of oxygen on the strength of current requisite to

induce cardiac inhibition is very variable. Variations of the strength of current requisite to induce cardiac inhibition cannot be associated with the variations of the cardiac frequency. It is possible that the presence of an excess of oxygen exerts a variable action on the cardiac metabolism, sometimes favouring allonomic disassimilation and at others autonomic assimilation, and according as one or other of these processes is predominant, the tendency towards the cardiac inhibition is increased or decreased.

32. Excess of carbon dioxide decreases the tone of the heart and the tendency towards inhibition. This result, though apparently contradictory to those of Piotrowski on the abductor limb muscles of the crayfish, can be explained by the probability that the excess of carbon dioxide probably retards the processes of autonomic assimilation, and as the cardiac contractions are proceeding the amount of dissimilation exceeds the amount of autonomic assimilation, so that there is a tendency towards contraction, and consequently a stronger current required to induce inhibition.

33. The older theory of the association of the phenomena of cardiac inhibition with the interference of the nerve impulse in the ganglion cells of the heart, is disproved by the observations where inhibitory phenomena were obtained before the development of such cells.

34. The phenomena of cardiac inhibition are influenced by two series of processes, both essential, viz. those taking place in the nervous elements and those confined to the contractile tissue... My experiments lend support to the theories of Gaskell and Piotrowski concerning the nature of the processes of inhibition, Nevertheless it is evident that more stress must be laid on the *rôle* played by the nerve terminations, than has been done by many recent writers.

35. Constant currents of strength of one milliampère increase the force and frequency of the cardiac rhythm of chick-embryos. If a constant current of sufficient strength be applied in a direction the reverse of the heart beat, the heart rhythm is reversed. These results occur both before and after the development of the intrinsic cardiac nervous mechanism.

36. The passage of a constant current through embryos' hearts kept at 38° C. for 2 or 3 minutes favours the appearance of heart delirium, or cardiac inhibition, on the subsequent immediate application of an interrupted current.

37. The prolonged passage of a constant current through the cardiac myoplasm kept at 38° C. produces a converse result.

38. Variations of temperature play an important part on the action of a constant current, on the subsequent action of an interrupted current.

39. A single induction shock applied during diastole causes the interpolation of an additional heart beat. If applied during systole the result is variable.

40. The application of a series of induction shocks produces various results, which depend on the temperature of the heart, the frequency and strength of the shocks, and the age of the embryo. The result may be either an augmentation of the force and frequency of the heart, an increase of cardiac frequency accompanied by a decrease of the rhythmic force; heart delirium, or cardiac inhibition.

41. A series of induction shocks influence the subsequent action of an interrupted current on the embryonic heart.

42. In general, the result produced by a constant current, a single induction shock, or a series of induction shocks, or an interrupted current on the hearts of mammalian embryos, is similar to that produced on embryo-chicks' hearts. The age of the embryo at which an interrupted current will induce cardiac inhibition is not so defined in the case of mammalian, as in that of chick-embryos.

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THE COAGULABILITY OF THE BLOOD OF ALBINOS.

By J. W. PICKERING, D.Sc. (Lond.), *George Henry Lewes Student*.

The recent researches of Prof. Halliburton and Dr T. G. Brodie[1] have demonstrated that the intravenous injection of an active solution of nucleo-proteid into albino rabbits, fails to induce intravascular coagulation of their blood, although similar doses of the same substance, when injected into the vascular system of either black or brown rabbits, produces extensive intravascular coagulation.

Working alone, and in collaboration with Prof. Halliburton I[2] have shown that the proteid-like synthesised colloids *A*, *B*, and *C* (colloïdes amidobenzoique and aspartique) produce typical intravascular coagulation when introduced into the circulation of pigmented rabbits, dogs, cats, and guinea-pigs, but fail to produce this result when injected into the vascular system of albino rabbits.

These results being anomalous, I have performed a series of experiments on the Norway or Arctic Hare (Lepus variabilis), certain specimens of which, during the winter season are albinos, but during the summer months change to a pigmented condition, in order to ascertain if there is any connection between the pigmentation of the animal, and tendency of its blood towards intravascular coagulation, after the injection of either a nucleo-proteid or of the synthesised proteid-like colloid *C* (colloïde aspartique of Grimaux[3]). I have now the pleasure of recording my best thanks to Capt. White for kindly supplying me with the animals for my experiments.

The striking contrast of the effect produced by the intravenous injection of either a nucleo-proteid (prepared from thymus), or of the proteid-like colloid *C*, during the albino condition of the animals, to

[1] Halliburton and Brodie. This *Journal*, XVII. p. 135. 1894.

[2] Halliburton and Pickering. This *Journal*, XVIII. p. 285. 1895. See also Pickering, *ibid.* XVII. p. 54. *Comptes Rendus*, CXX. p. 1348. 1895.

[3] Grimaux. *Revue Scientifique*, 18 April, 1885. *Comptes Rendus*, XCIII. p. 771; XCVIII. pp. 231, 1336 and 1578.

that obtained when the animals have become pigmented, is shown by the following précis of experiments, all of which were performed under the anæsthetic influence of a mixture of chloroform and ether. Control experiments were performed to ensure that both the nucleo-proteid, and proteid-like colloid *C*, employed were in the active condition.

Exp. 1. Animal in albino condition. Active solution of nucleo-proteid used. Sample of carotid blood coagulated six minutes after withdrawal. A second sample of carotid blood, taken immediately after the injection of 20 c.c. of nucleo-proteid, coagulated in five minutes after withdrawal. 20 c.c. more of the nucleo-proteid were then injected and the carotid blood coagulated in three minutes after withdrawal. A further injection of 15 c.c. of the nucleo-proteid killed the animal; death being apparently due to respiratory failure, with pronounced exophthalmos, and dilatation of the pupils, as symptoms. On post-mortem examination, made immediately afterwards, the blood throughout the vessels was found to be in the fluid condition.

Exps. 2, 3, and 4 were also performed on animals during their albino condition, and the results obtained were entirely in accord with those recorded in Exp. 1. In each case the injection of the solution of nucleo-proteid hastened the coagulation of the blood after withdrawal from the carotid, but entirely failed to induce intravascular coagulation. In Exp. 3 the animal was killed by asphyxia, in Exps. 2 and 4 the injection itself proved fatal. In Exp. 3 a dilute solution of a $CaCl_2$ was injected after the nucleo-proteid.

Exps. 6 *and* 7. The animals were in the pigmented condition. In the first of these experiments the injection of 25 c.c. of a nucleo-proteid solution produced death with typical symptoms, and on post-mortem examination, made immediately afterwards, extensive clots were found in the portal system, vena cava inferior, and jugular vein. In the second experiment the injection of 35 c.c. of nucleo-proteid produced a like result. The coagulability of the blood withdrawn from the carotids was also hastened in each case.

Exps. 8 *and* 9. The animals were in the albino condition and a 1·5 % solution of the proteid-like colloid *C* was used. The injection of the synthesised colloid also hastened the coagulation of the blood after withdrawal from the carotid, but entirely failed to induce intravascular coagulation. Death was caused in each case by the injection of the colloid, and the typical dilatation of the pupils and exophthalmos was observed.

Exp. 10. The animal was in the pigmented condition. 25 c.c.

of the proteid-like colloid *C* were injected. Death with typical symptoms resulted. Immediate post-mortem examination revealed pronounced clots in the right auricle, right and left ventricle, pulmonary artery, jugular, inferior vena cava and portal veins.

If the animals are in a condition in which the pigmentation is partial, that is either in the transition state between the albino and pigmented condition, or as is commonly the case, in a state when the fur has become white only in parts, then after injection of a nucleo-proteid, there may result either a partial coagulation of the intra-vascular blood, or there may be an entire failure to induce intravascular clotting. This result is shown by the following experiments:

Exp. 11. Animal in transition state (partially pigmented). 45 c.c. injected with fatal result, and typical symptoms. Small loose clot found in portal vein. Remainder of blood fluid, but coagulates very rapidly after withdrawal from the blood vessels.

Exp. 12. Animal partially pigmented. 60 c.c. of nucleo-proteid injected and animal killed by asphyxia. Blood throughout vascular system fluid.

Exp. 13. Animal nearly in albino condition. 65 c.c. of nucleo-proteid proved fatal. Post-mortem examination revealed a small loose clot in portal vein and another in the inferior vena cava.

Exp. 14. Animal in same condition as above. 65 c.c. of nucleo-proteid injected and animal killed by asphyxia. Blood throughout the vascular system was in fluid condition.

The observations recorded in this paper seem to point to the view that the intravascular coagulation of the blood, produced by the injection of either a nucleo-proteid or of the proteid-like colloid *C*, is due to a chemical interaction between a molecular group or groups in the above named substances, and another molecular group or groups existing in the proteids of the living plasma; and not to the heavy nature of the colloidal substance injected.

A slight alteration in the chemical composition of the blood of the animal is sufficient to prevent substances which normally induce intravascular coagulation from so doing. It is well to recall in this connection the influence of the season of the year (probably due to the temperature variations) on the physiological reactions produced by certain substances on the heart, as these physiological reactions are probably associated with, if not dependent on chemical changes in the heart's contractile tissue. The appearance of the albino condition of the animal being confined to winter, and the pigmented state to the

warmer seasons and the presence or absence of pigmentation being evidence of a varying metabolism it is probable that the two series of phenomena are comparable.

Experiments in Extravascular Plasma.

In a paper published last year I[1] pointed out that the synthesised proteid-like colloids *A*, *B* and *C* failed to induce coagulation in sodium sulphate plasma. It had previously been shown by Prof. Halliburton[2] and Dr Brodie that nucleo-proteids failed to produce coagulation in salted plasma. Pekelharing[3] however explained the result of Halliburton's experiments, where magnesium sulphate was the diluent used, by suggesting that the magnesium sulphate prevented the union of calcium and nucleo-proteid, essential in his opinion to the reaction of coagulation; Halliburton[4] has accepted Pekelharing's explanation, and has further demonstrated that dog's blood mixed in certain proportions with a 1 °/₀ solution of sodium carbonate has its coagulation accelerated by the addition of a nucleo-proteid, and from this and other experiments, he concludes that Schmidt's fibrin ferment is a nucleo-proteid.

Since my experiments, cited above, are also open to Pekelharing's objection that salted plasma is not a fair test plasma, I have repeated them, following the method adopted by Halliburton. I have found that the addition of either of the colloids *A* and *B* (prepared by the interaction of PCl_5 and meta-amidobenzoic acid) does not apparently accelerate the coagulation of dog's blood mixed with 1 °/₀ sodium carbonate, neither does it accelerate that of either pigmented or albino rabbits. The addition of the colloid *C* (colloïde aspartique) however causes a slight acceleration of the coagulation of the sodium carbonate plasma as is shown by the tables on p. 314.

It is evident that the colloid *C* hastens the appearance of a clot in the extravascular plasma (dissolved in 1 °/₀ sodium carbonate) of both albino and pigmented rabbits, but that its action is not so marked as that of a nucleo-proteid. This property of the colloid *C* is lost if it be kept in solution for two or three days, and specimens even if kept dry lose their power after they have been prepared three or four months.

[1] Pickering. This *Journal*, XVIII. p. 54. 1895.
[2] Halliburton and Brodie. This *Journal*, XVII. p. 146. 1894.
[3] Pekelharing. *Centralblatt für Physiologie*, IX. p. 102. 1895.
[4] Halliburton. This *Journal*, XVIII. p. 306. 1895.

(Brown rabbit's blood used.)

	Proportion of blood to solution	Sol. of 1 % sodium carbonate (control experiments)	2 % sol. of colloid *C* in 1 % sol. of sodium carbonate[1]
i.	Equal parts	No clot in 3 hours	No clot in 3 hours.
ii.	Blood + $\frac{1}{3}$ of its vol. of solution	ditto	ditto
iii.	Blood + $\frac{1}{4}$ of its vol. of solution	Clot in 23 mins.	Clot in 16 mins.
iv.	Blood + $\frac{1}{8}$ of its vol. of solution	Clot in 18 mins.	Clot in 12 mins.
v.	Blood + 6 drops of sol.	Clot in 20 mins.	Clot in 5 mins.
vi.	ditto	Clot in 16 mins.	Clot in 4 mins.

(Albino rabbit's blood used.)

	Proportion of blood to solution	Sol. of 1 % sodium carbonate (control experiments)	2 % sol. of colloid *C* in 1 % sol. of sodium carbonate
i.	Equal parts	No clot in 3 hours	No clot in 3 hours
ii.	Blood + $\frac{1}{3}$ of its vol. of solution	Faint clot in 2 hrs. 40 mins.	ditto.
iii.	Blood + $\frac{1}{4}$ of its vol. of solution	Clot in 18 mins.	Clot in 17 mins.
iv.	Blood + $\frac{1}{8}$ of its vol. of solution	Clot in 21 mins.	Clot in 7 mins.
v.	Blood + 6 drops of sol.	Clot in 15 mins.	Clot in 4 mins.
vi.	ditto		

The colloids *A* and *B* even when they have been previously warmed with a solution of calcium chloride, fail to induce coagulation in extra-vascular plasma prepared by this method.

I may here incidentally remark that I have confirmed the experiments recently made by Mr A. Edmunds[2] that the addition of the colloid *C* does not hasten the coagulability of milk, neither will the colloids *A* and *B* produce this result.

Summary.

In conclusion, I would emphasise the following points:—

The condition of the intravascular blood varies in animals that are sometimes in an albino, and at others in a pigmented condition.

During the albino condition the injection into the circulation of either a nucleo-proteid, or of a dilute solution of the synthesised proteid

[1] All the sodium carbonate used was prepared by myself from water distilled into glass receivers, to avoid the possibility of oligodynamic action; *vide* author's paper, This *Journ.* xx. p. 177. 1896.

[2] Edmunds. This *Journal*, XIX. p. 466. 1896.

like colloid *C* (colloïde aspartique), fails to produce intravascular coagulation of the blood. A subsequent intravenous injection of $CaCl_2$ also fails to induce intravascular coagulation.

During the pigmented condition of the animal, the intravenous injection of either of these substances produces extensive intravascular coagulation, which is usually well marked in the portal vein, inferior vena cava, and jugular vein.

The coagulation of the blood after withdrawal from the carotid is hastened by the injection of either of these substances into the circulation of the animal, both during its albino and pigmented condition.

During the partially pigmented condition of the animal, the intravenous injection of a nucleo-proteid may either produce, or fail to produce intravascular coagulation of the blood. Its introduction into the circulation, however, always hastens the coagulation of blood withdrawn from the carotids.

The colloids *A* and *B* (colloïdes amidobenzoique of Grimaux) fail to induce coagulation in the extravascular plasma prepared by an admixture of dog's or rabbit's blood (in various proportions) with a 1°/₀ solution of sodium carbonate. Warming these substances with $CaCl_2$ fails to give them the power to induce coagulation in extravascular plasma.

The colloid *C* hastens the appearance of a clot in the extravascular plasma (prepared by this method) of dogs, and of both pigmented and albino rabbits. The acceleration of the coagulation produced is not however so great as can be produced by the addition of a similar quantity of a nucleo-proteid to 1°/₀ sodium carbonate plasma.

The remarkable correspondence between the action of nucleo-proteids and of the synthesised proteid-like colloid *C* both on albinos' intravascular blood, and on extravascular 1°/₀ sodium carbonate plasma favours the view tentatively put forward by Prof. Halliburton and myself[1] that the colloid *C* is probably the nearest substance at present known to proteids, and may possibly even be termed an elementary proteid. The absence of intravascular coagulation after injection of large doses of the colloid *C* into the circulation of albinos, negatives the view that the action of the colloids is due solely to their heavy molecular weight.

[1] *Op. cit.*

August 10, 1896.

PROTEOSES IN SEROUS EFFUSIONS.

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Some years ago one of us[1] examined a large number of various dropsical effusions, and arrived at the conclusion that these fluids contained no proteoses (albumoses) or peptone. To this rule cerebro-spinal fluid was the only exception. We have been led to take up this subject again for two reasons. The first of these is, that since the papers referred to were written, improved methods have been introduced for the detection of the substances in question; and the second is, the appearance of a paper by Dr. A. Lockhart Gillespie[2] on the same subject. Dr. Gillespie examined twenty-two cases of various effusions (pleural, ascitic, œdema, amniotic), and found proteoses in all cases, and true peptone in seventeen out of the twenty-two. In some cases traces only were found, but in the majority sufficient was present to enable him to estimate the quantity, and to separate the proteoses into their varieties. He further states that while he does not know their significance, the presence of these substances must be due to the action of some of the digestive ferments which are known to be present normally in the blood. The only proteolytic ferment which has been found with certainty in the blood is pepsin; and as this requires an acid medium for its action, it is difficult to understand this explanation. However, before seeking an explanation we considered it best to make sure of the facts, and, as the sequel will show, our conclusion is that proteoses and peptones are always absent from serous effusions, and that Dr. Gillespie's work is vitiated by the use of faulty methods.

We will therefore first consider the question of methods, and pass subsequently to our results. The principal methods for the detection of proteoses and peptone in fluids which contain also albumins and globulins, are four in number—

[1] W. D. Halliburton, *Brit. Med. Journ.*, July 26, 1890; *Journ. Physiol.*, London, vol. x. p. 232. "Text-Book of Chemical Physiology and Pathology," chap. xviii.

[2] *Rep. Lab. Roy. Coll. Phys.*, Edin. 1894, vol. v. p. 51.

1. Heat coagulation method.
2. Devoto's modification of the ammonium sulphate method.
3. The alcohol method.
4. The trichloracetic acid method.

These we will take one by one.

1. *Heat coagulation method.*—This method consists in heating the mixture, acidifying slightly with acetic acid, filtering off the coagulated albumins and globulins, and examining the filtrate for proteoses and peptone. This was the method used by Gillespie. He says: "The albumoses were obtained by saturation of the specimen with ammonium sulphate after removal of all the proteids coagulable by heat, and separated into their three forms by means of sodium chloride and dialysis." This method is now practically entirely abandoned by those who wish to obtain trustworthy results. It has been found that heating such an acid mixture leads to the actual formation of proteoses and peptone from the native proteids; it is therefore perfectly easy to understand that he succeeded in finding these products of hydration in the effusions he examined.

The use of this method has led to many similar mistakes in the past: it led Hofmeister to describe proteoses and peptone as a constituent of the blood; it led Struve, Schmidt-Mulheim, and others to the conclusion that a peptone-like substance exists in milk; and more recently Chabrié,[1] by the use of the same method, has described a new constituent of blood serum, which has the properties of a proteose, and to which he has given the name albumone. Chabrié's mistake has been amply demonstrated by Robert Brunner.[2]

Neumeister, Sidney Martin, Starling, Halliburton, and others, in numerous papers, have also protested against the use of this method.

2. *Devoto's method.*—This method consists in saturating the proteid mixture with ammonium sulphate, boiling and filtering. The filtrate will contain the peptone, if any is present. The precipitate on the filter is then extracted with boiling water. The extract will contain the proteoses, if any are present. This method is a decided improvement on the one first described, as it avoids the use of acid; we have, however, found it is not wholly free from the same objection as was urged against the first method, for the boiling alone will cause the formation of a small amount of primary proteose; thus a fluid which, by absolutely trustworthy tests, is shown to contain no proteose or peptone, will often give a slight indication of proteose by Devoto's method.

M. Matthes[3] has arrived at the same conclusion. His special object was to search for proteoses and peptone in the blood of leucæmic patients; his preliminary experiments led him to abandon Devoto's method in favour of the alcohol method, to be immediately described.

[1] *Compt. rend. Acad. d. Sc.*, Paris, vol. cxiii. p. 557.
[2] "Inaug. Diss.," Berne, 1894.
[3] *Berl. klin. Wchnschr.*, bd. xxxi. s. 351.

Wright[1] and Pekelharing[2] have considered that the non-coagulability of dog's blood, often noted after injection of nucleo-albumin, may be due to the splitting of the material injected into a nuclein moiety and a peptone moiety, and that the latter is responsible for the non-coagulability of the blood. This was supported by Pekelharing's finding peptone in such blood. C. J. Martin[3] and Halliburton and Brodie[4] have been unable to accept this explanation, chiefly because they have not been able to confirm Pekelharing's observation. For the detection of peptone (the term as here used includes the proteoses) Pekelharing used Devoto's method, which, as we have just seen, is untrustworthy.

3. *The alcohol method.*—This method consists in coagulating albumins and globulins by the prolonged action of large quantities of alcohol. After some months, water dissolves out from the precipitate so produced, proteoses and peptone, the albumin, globulin, and other native proteids being entirely insoluble. This is the method which Sidney Martin[5] has employed with such fruitful results in his investigations into the proteoses of disease (diphtheria, tetanus, etc.) which accumulate in the spleen and elsewhere. It is the method by which Gourlay[6] investigated normal spleens, and so corrected v. Jaksch's[7] statement that normal spleens contain "peptone." It is a method which is especially adapted to the investigation of the proteids obtainable from solid organs; but it also works perfectly well with liquids—indeed the statement originally made by one of us, already referred to, that serous fluids are free from proteoses and peptone, and that cerebro-spinal fluid contains such substances, was the result of experiments principally carried out by the use of this method. It is absolutely trustworthy; the only disadvantage it possesses is the length of time it takes.

4. *The trichloracetic acid method.*—This method is also perfectly trustworthy, and possesses the additional advantage of being rapidly applied. It has been employed by Obermayer,[8] Starling,[9] C. J. Martin,[10] and Halliburton and Brodie.[11]

The method consists in adding to the suspected liquid an equal volume of a 10 per cent. solution of trichloracetic acid. An abundant precipitate thus produced is filtered off, and the filtrate tested for proteoses and peptone. C. J. Martin, however, pointed out that in the cold the proteoses would be partly in the precipitate. He therefore

[1] *Proc. Roy. Irish Acad.*, 3rd series, ii. No. 2, 1892, p. 117.

[2] *Verhandl. de k. Akad. v. Wetensch., te Amsterdam*, Tweede Sectie, Deel i. No. 3.

[3] *Journ. Physiol.*, Cambridge, 1893, vol. xv. p. 375.

[4] *Ibid.*,1894, vol. xvii. p. 158.

[5] Goulstonian Lectures, *Brit. Med. Journ.*, London, March, 1892.

[6] *Journ. Physiol.*, London, 1894, vol. xvi. p. 32.

[7] *Ztschr. f. physiol. Chem.*, Strasburg, 1892, bd. xvi. s. 243.

[8] *Wien. med. Jahrb.*, 1888, ss. 375–381.

[9] *Journ. Physiol.*, Cambridge, vol. xiv. p. 131.

[10] *Ibid.*, vol. xv. p. 375.

[11] *Ibid.*, vol. xvii. p. 169.

recommends that after the acid is added the mixture should be rapidly boiled and filtered while hot. The filtrate, on cooling, deposits some of the previously dissolved proteoses, if any are present.

Starling advises that heat should not be applied for fear of the formation of primary proteoses. But C. J. Martin found, and we have confirmed his observation, that with rapid boiling and filtration this does not occur. The strong acid appears to have such a coagulating effect on the native proteids that the hydrating tendency of the hot acid has no effect on them.

This is the method, and it is an extremely delicate one, upon which we have relied in investigating the question whether proteoses or peptone are present in serous effusions.

More in detail, the actual *modus operandi* was as follows:—The serous fluid under investigation was divided into several portions, which were lettered *a*, *b*, *c*, etc.

a. This was saturated with ammonium sulphate in the cold, and filtered. The filtrate was tested for peptone by the biuret reaction.

b. This was saturated with ammonium sulphate, boiled and filtered. The filtrate was tested for peptone.

c. The precipitate on the filter in *b* was then extracted with boiling water, and filtered. The extract was tested for proteoses by the nitric acid test and the biuret reaction.

d. To this portion an equal volume of 10 per cent. solution of trichloracetic acid was added, and filtration then performed. The filtrate was tested for proteoses and peptone.

e. This portion was treated in the same way as *d*, except that it was boiled before filtration.

f. This portion was treated with dilute acetic acid, boiled and filtered. The filtrate was tested for proteose and peptone.

The fluids investigated were the following:—Hydrocele fluid, six specimens; ascitic fluid, six specimens; pleural fluid, five specimens; fluid from cystic ovary, one specimen. For these we are chiefly indebted to Mr. Cyril Wace, House Physician, King's College Hospital; Dr. Willoughby Lyle, St. Peter's Hospital, supplied us with four of the specimens of hydrocele fluid, and Mr. P. T. Beale, with one. To all these gentlemen we beg to offer our sincere thanks for the assistance they have thus given to us.

The result of our investigations may be given under the same heads, *a*, *b*, *c*, etc., as the experiments just enumerated.

a. Peptone absent.

b. Peptone absent.

c. Traces of proteose occasionally found, but, as already explained, this method (Devoto's) is not free from error.

d. Proteose and peptone absent.

e. Proteose and peptone absent.

f. This experiment was not always performed, as we knew so well from previous work the fallacies of the method. In only one case did we fail to obtain evidence of proteose. It is no doubt possible to so adjust the amount

of acid, and the length of time during which the liquid is boiled, as to avoid the formation of proteose, but the chances are all the other way.

We may sum up our conclusions very briefly as follows :—

1. In searching for proteose or peptone in such fluids as blood, milk, or serous effusions, it is important to use such methods as will not in themselves lead to the formation of these hydration products from the native proteids present.

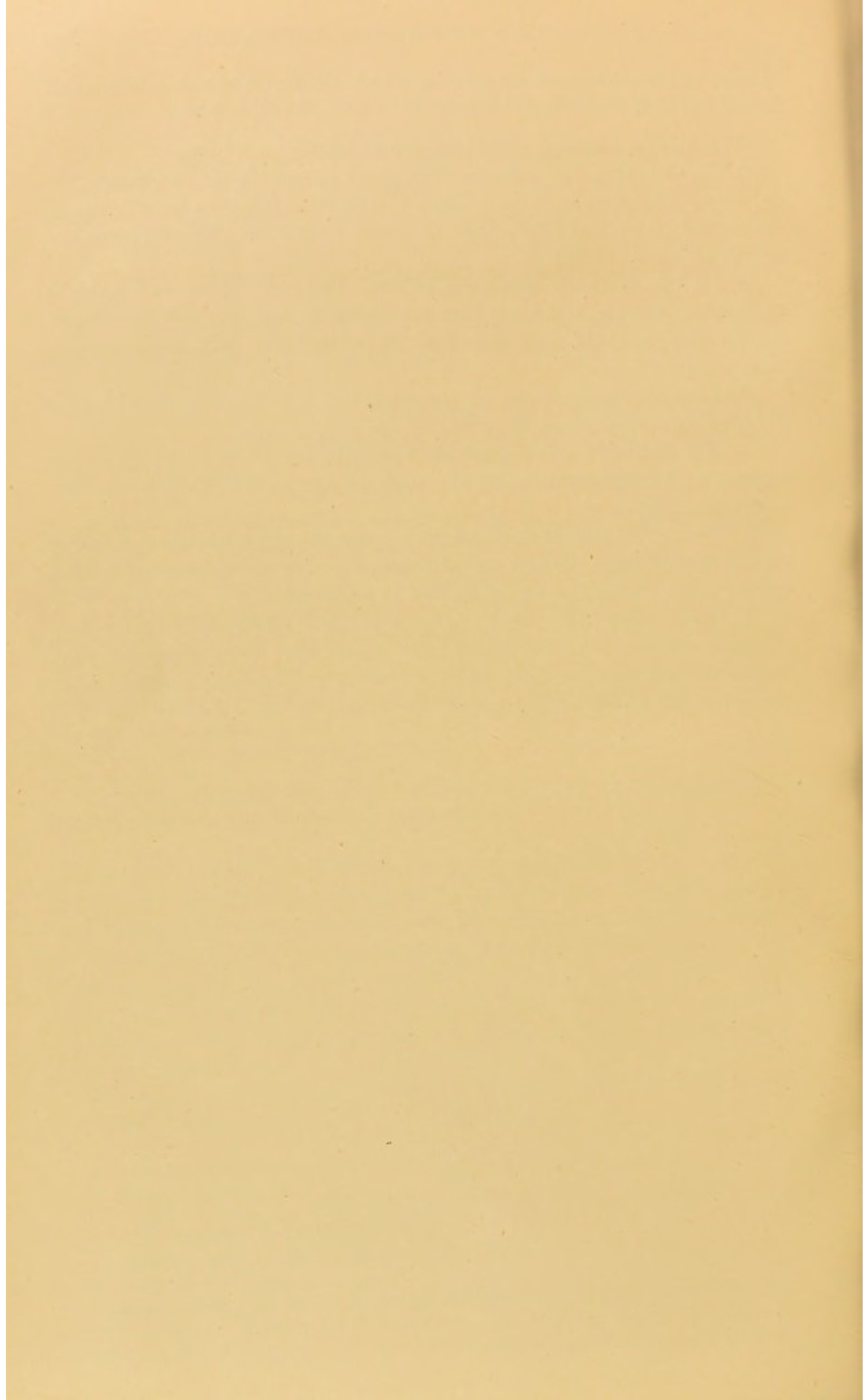
2. Of the methods described, boiling after acidulation, to coagulate the native proteids, is the one best calculated to lead to the formation of proteolytic products, and therefore the one least calculated to give trustworthy results.

3. Devoto's modification of the ammonium sulphate method is also not free from this source of error.

4. The methods which give good results are those in which either alcohol or trichloracetic acid are used as agents for coagulating the native proteids.

5. The trichloracetic acid method possesses the advantage of being rapidly performed; though in the investigation of solid organs like spleen, kidney, etc., the use of alcohol is preferable.

6. Our present experiments support previous conclusions, that serous effusions are free (like the blood from which they originate) from proteoses and peptone; and we consider that Dr. Gillespie's contrary conclusion is due to his having employed untrustworthy methods.



[*From the Proceedings of the Physiological Society*, Feb. 13, 1897.]

The Precipitation of Carbohydrates by Neutral Salts. By R. A. YOUNG.

The researches of Nasse, Pohl, and Halliburton have shown that certain colloid carbohydrates are precipitated by saturation of their solutions with neutral salts.

In the present research this method has been applied to all the common carbohydrates including the crystalline sugars, and also to the study of the digestion cleavage products of starch.

Method. In nearly all cases, the saturation has been carried out at the ordinary room temperature in neutral solutions, the salt being added till no more would dissolve.

In many cases excess of the salt has been added, and the solution allowed to stand over it for some hours or even days.

In a few cases the solutions have been saturated at higher temperatures. Sodium sulphate has its maximum solubility at about 33° C., and at this temperature is a very powerful precipitating agent.

If precipitation occurs, the precipitate is collected, well washed with a saturated solution of the salt used for precipitation, and then, if necessary, the salt dialysed away and the reactions of the substance investigated.

The principal salts used have been magnesium sulphate, sodium chloride, sodium sulphate, ammonium sulphate, and sodio-magnesic sulphate.

Results. The general results obtained may be briefly summarised as follows:

i. **Crystalline carbohydrates.** Dextrose, lævulose, cane sugar, maltose, lactose were all investigated, but with uniformly negative results.

ii. **Glycogen** is completely precipitated from its solutions by magnesium sulphate, sodio-magnesic sulphate and ammonium sulphate at ordinary temperatures, or by sodium sulphate at 33° C. It is not precipitated by sodium chloride, by half saturation with ammonium sulphate, nor by saturation with sodium sulphate at ordinary temperatures or at 100° C.

Saturation with ammonium sulphate or magnesium sulphate affords a ready means of obtaining glycogen from its solutions, and of distinguishing it from erythrodextrin.

iii. **Starch paste and soluble starch** are precipitated by ammonium sulphate, magnesium sulphate, and by sodium sulphate at 33° C.

It is incompletely precipitated by sodium sulphate in the cold, and by half saturation with ammonium sulphate, not at all by sodium chloride or ammonium chloride.

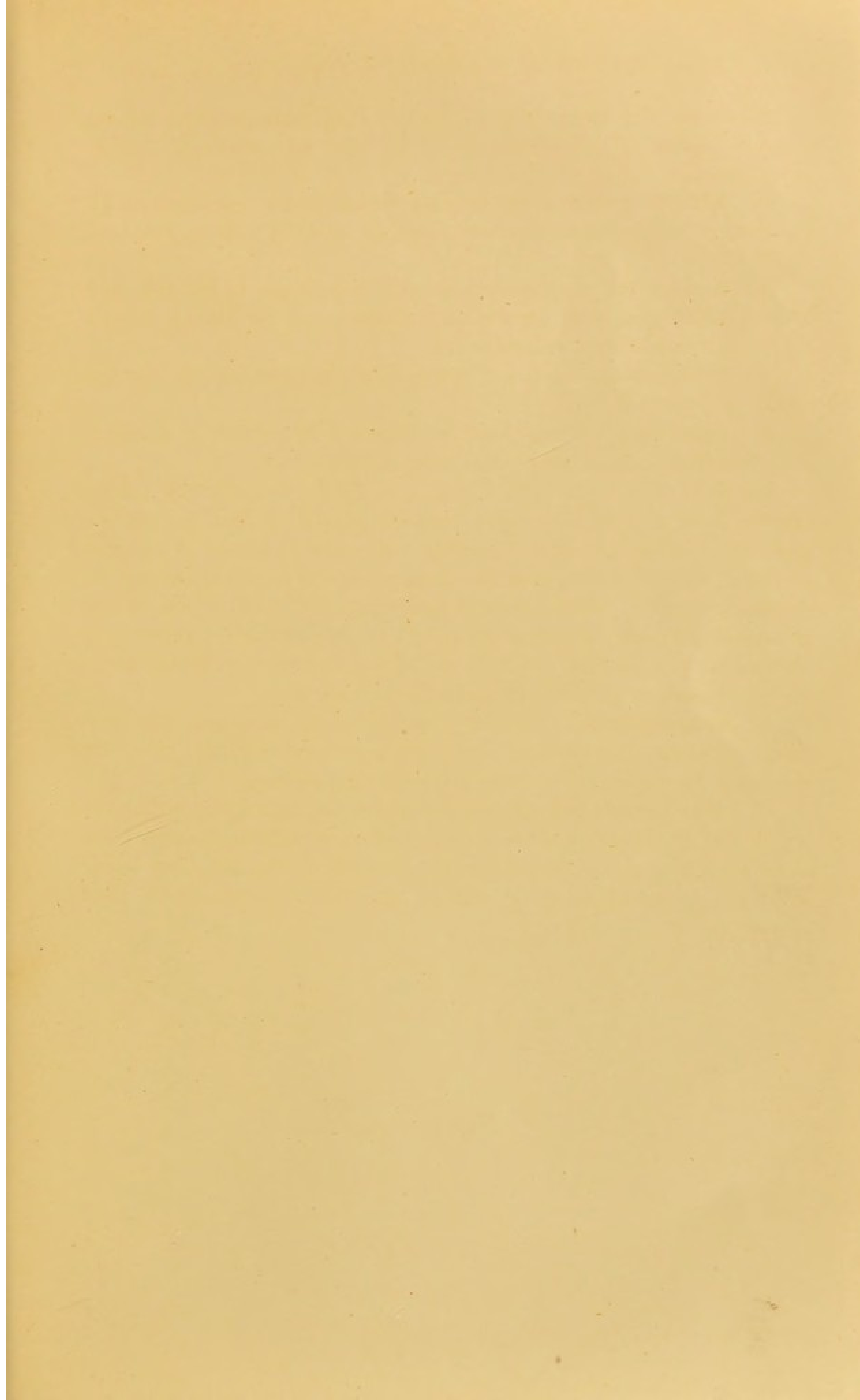
iv. **Erythrodextrin** is not precipitated by salts, though the red iodide of erythrodextrin is.

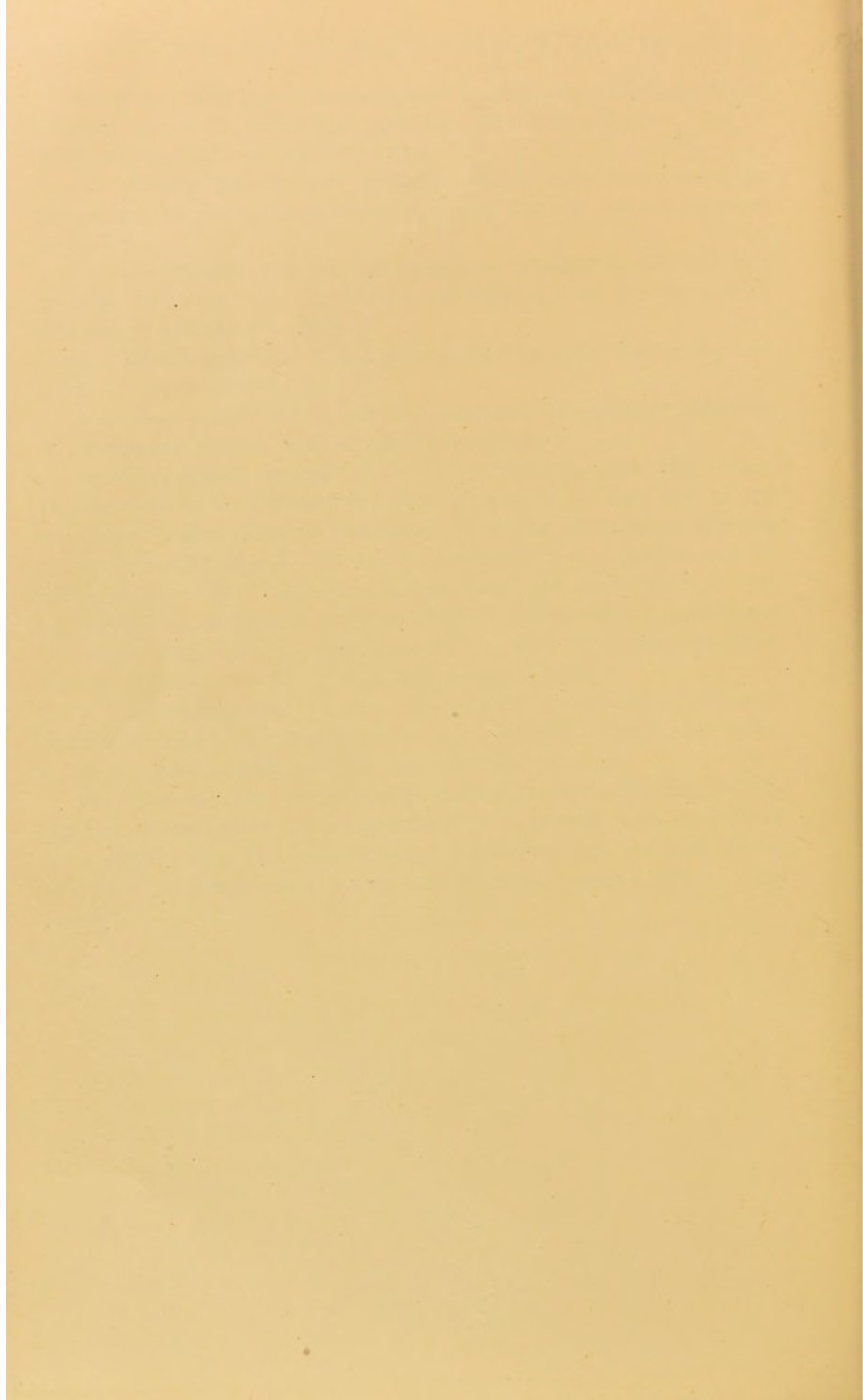
A copious precipitate is however obtained by saturating solutions of commercial dextrin with ammonium sulphate.

Solutions of this precipitate give a peculiar coloration with iodine, varying from a dark blue-violet through purple to dark red as the quantity of iodine added is increased. It seems to consist at any rate partly of soluble starch, but small quantities of a substance can be obtained from it, which gives a mahogany red with iodine, is not precipitated by magnesium sulphate but is precipitated by subsequent saturation with sodium sulphate at 33° C., and is apparently intermediate between soluble starch and erythrodextrin.

v. **Achroodextrin** is partly precipitated by saturation with ammonium sulphate, and **inulin** partly by magnesium sulphate, but so far it has not been possible to effect complete precipitation.

vi. **The soluble iodine compounds** of the carbohydrates, including that of erythrodextrin, are precipitated on saturation, in many cases more readily than the carbohydrates themselves, e.g. the blue iodide of starch is precipitated by sodium chloride whereas starch itself is not.





[*From the Proceedings of the Physiological Society*, Feb. 13, 1897.]

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.

The experiments have been conducted as follows: the animals used were dogs, anæsthetised with ether. The right external jugular vein and the left carotid artery were exposed and a cannula was introduced into each vessel. The artery was connected with a mercurial manometer in the usual way for taking a blood-pressure tracing. A simultaneous tracing of the respiratory movements was taken by the tambour method.

The fluids were injected into the vein and the results were, with certain exceptions to be afterwards mentioned in all cases similar, viz. no marked effect upon respiration; but a marked temporary fall in the blood-pressure which begins about 10 seconds after the commencement of the injection.

The fluids we used were

(1) Normal cerebro-spinal fluid taken from a case of dripping from the nose during life. For this we are indebted to Dr St Clair Thomson. This produced no effect; one of us observed a similar negative result when the cerebro-spinal fluid taken from an animal during life has been introduced into the vein of another animal of the same species.

(2) Cerebro-spinal fluid obtained *post mortem* from a considerable number of cases of general paralysis of the insane, from one case of stuporose melancholia, and from one case of cerebral hæmorrhage owing to the giving way of a cortical cerebral vessel.

To avoid fallacy of decomposition from microbic growth, it may be stated that the bodies were placed in a cold chamber (0° C. or below

that) within half an hour of death, and cultures were in all cases made from the cerebro-spinal fluid and blood of the frontal sinus, and in nearly all instances without result. This is necessary, because many of these people die with bladder affection or ulcerative colitis, and microbic toxins might arise.

As a rule 10 c.c. of the fluid were injected and although the effect varied somewhat in degree, yet in only one instance did no fall in the blood-pressure occur. That instance was the cerebro-spinal fluid from the case of cortical hæmorrhage, an acute case with no naked-eye wasting of the brain substance.

(3) The cerebro-spinal fluid was boiled and filtered, and the filtrate gave the same result. It could not therefore be due to proteid.

(4) The cerebro-spinal fluid was mixed with several times its volume of alcohol, by which all proteids and proteoses would be precipitated. It was filtered and the filtrate dried at a temperature of about 40° C. and the residue dissolved in saline solution. This when injected gave a similar fall in the blood-pressure.

(5) Solution of neurine hydrochloride 0·125 per cent. solution. 2·5 c.c. gave a similar fall: but in most instances this was followed by a return to or even above the original pressure and then a second fall which persisted to some extent, a condition we never observed with the cerebro-spinal fluid. This result is similar to that previously obtained by Prof. Schäfer and Dr Oliver[1]. Stronger doses produce slowing of the heart, and slowing and deepening of the respiration. The fatal dose is less than a decigramme, respiration ceasing before the heart.

(6) Solution of choline hydrochloride 0·2 per cent. solution. 5 c.c. gave a result identical as far as we could observe with that obtained by the pathological cerebro-spinal fluids. With stronger doses there is slowing of the heart.

(7) The blood obtained from two patients suffering with pseudo-apoplectiform convulsions of general paralysis obtained by venesection was mixed with several times its volume of absolute alcohol, filtered, and the filtrate evaporated to dryness at about 40° C. The residue was dissolved in saline solution and a quantity was injected corresponding to 50 c.c. of the original blood in each case. The result obtained corresponded entirely with that obtained with the pathological cerebro-spinal fluids and with *solution of choline*. Normal blood similarly treated gave a negative result.

[1] This *Journ.* vol. XVIII. p. 266.

It may be added that section of the vagi has no influence on the fall of blood-pressure produced by the injection.

The substance in the pathological cerebro-spinal fluid which produces the effect is precipitable by phosphotungstic acid; it is therefore probably alkaloidal in nature. Normal cerebro-spinal fluid after removal of the proteid gives no precipitate with phosphotungstic acid. The pathological cerebro-spinal fluids we have examined are rich in coagulable proteid, contain no proteose or peptone, and are usually free from reducing substance. The reducing substance of the normal fluid was considered by one of us to be allied to, or identical with pyrocatechin. In small doses pyrocatechin produces no effect on blood-pressure; in large doses it causes a very slight fall.

The effects produced upon the blood-pressure by the introduction of these various fluids into the venous circulation were experimentally demonstrated at the meeting of the society. A number of lantern slides were shown to illustrate the disintegration of the nerve-cells of the brain in some of the cases.

Since the meeting we have taken tracings of blood-pressure simultaneously with plethysmographic tracings of the limbs, and of the kidney, an air oncometer being used in connection with the latter organ. There is no peripheral dilatation of the blood vessels. That the fall of blood-pressure is cardiac in origin was confirmed by experiments on the frog's heart. These and other experiments are now in progress. This conclusion fits in very well with what is found in general paralysis of the insane; cardiac weakness and enfeebled circulation are commonly observed; and fatty degeneration of the heart is very frequently discovered *post-mortem*.

[*From the* PROCEEDINGS OF THE ROYAL SOCIETY, Vol. 60.]

"The Chemical and Physiological Reactions of certain Synthesised Proteid-like Substances. Preliminary Communication." By JOHN W. PICKERING, D.Sc. (Lond.). Communicated by Professor HALLIBURTON, F.R.S. Received November 10,—Read December 10, 1896.

The experiments of Professor Grimaux,* made more than ten years ago, have until recently attracted but little attention amongst English physiologists, although that investigator has synthesised a series of colloidal substances which, in their chemical characteristics, show striking similarities to proteids.

Working alone, and in collaboration with Professor Halliburton, I† have shown that three of the substances synthesised, viz., the "Colloids amidobenzoic A and B," formed by the interaction of phosphorus pentachloride and meta-amido-benzoic acid at 125° C., according to the details described in Grimaux's papers, and the "colloïde aspartique" formed by the passage of a current of dry gaseous ammonia over solid aspartic anhydride heated to 125° C., not only give the leading chemical reactions of proteids, but when intravenously injected into dogs, cats, or pigmented rabbits, cause extensive intravascular coagulation of the blood, in a manner indistinguishable from the physiological action of nucleo-proteids. When injected into the veins of albino rabbits or into the vascular system

* Grimaux, 'Comptes Rendus,' vol. 93, p. 771, 1881; *ibid.*, vol, 98, p. 105, 1884; *ibid.*, vol. 98, p. 1434 and p. 1578.

† Pickering, 'Journ. Physiol.,' vol. 14, p. 341, 1893; 'Comptes Rendus,' vol. 120, p. 1348, 1895; 'Physiol. Soc. Proc.,' Feb. 16, 1895 ('Journ. Physiol.,' vol. 17); 'Journ. Physiol.,' vol. 18, p. 54, 1895; *ibid.*, vol. 20, p. 171, 1896; *ibid.*, vol. 20, p. 310; Halliburton and Pickering, 'Journ. Physiol.,' vol. 18, p. 285, 1895.

b

of the Norway hare (*Lepus variabilis*), during its albino condition, these substances fail to induce intravascular coagulation of the blood, although they hasten the coagulation of the blood when drawn from the carotids, in a precisely similar manner to nucleo-proteids.

Taking these facts as the basis of my investigations, I have endeavoured to synthesise substances which will approach more nearly in their chemical and physiological reactions to proteids than those briefly described above; and to further investigate the properties of Grimaux's colloids.

I. *General Description of Experiments.*

I have up to the present synthesised seven different colloidal substances, by the interaction of either phosphorus pentachloride or pentoxide on certain well-known derivatives of proteids, and the details of their preparation, physical properties, chemical and physiological reactions are described below.

Colloid α.—Prepared by the interaction of equal parts of meta-amido-benzoic acid, biuret, and three times its weight of phosphorus pentoxide at 125° C. in a sealed tube. The best results are obtained by continuing the heating for about six hours, although a similar substance is obtained by heating for half an hour at 130° C. The product of the reaction is a pinkish-grey friable powder, which is insoluble in cold water, and almost insoluble in boiling water. This substance should be repeatedly washed until all traces of phosphoric acid are removed. When heated with Millon's reagent it fails to give the reaction characteristic of tyrosine and proteids; it also does not give the well-known colour reactions with the salts of copper, nickel, cobalt, and caustic potash. It gives the typical blue reaction associated with the name of Fröhde* when heated with sulphuric and molybdic acids, as well as the xanthoproteic reaction.

If the amount of biuret exceeds the amount of meta-amido-benzoic acid, then the excess of biuret left over gives its typical colour reaction with copper sulphate and potash.

The pinkish-grey powder, obtained by the reaction described above, should be dissolved in ammonium hydrate, and the resulting solution evaporated down at the temperature of the atmosphere *in vacuo*, when the resulting product appears as a number of translucent yellowish plates, which are tasteless and inodorous, and closely resemble in appearance both Grimaux's "colloïdes amido-benzoique and aspartique" and dried serum-albumen. These plates are with difficulty soluble in cold water, but readily pass into solution on warming. The solution obtained does not coagulate on heating, but

* Fröhde, 'Annalen der Chemie,' vol. 145, p. 376.

if a trace of a soluble salt of either barium, strontium, calcium, magnesium, or sodium be added, a pronounced coagulum is obtained on heating. This point will be returned to you in a subsequent section, but the similarity to dialysed serum-albumen may be pointed out, as that substance is stated not to coagulate when heated.*

The solution does not coagulate spontaneously on standing, neither will the addition of "fibrin ferment (*i.e.*, a nucleoproteid†) induce coagulation. It gives a typical xanthoproteic reaction, a violet with copper sulphate and potash, a dark heliotrope-purple with cobalt sulphate and potash, and a faint yellow with nickel sulphate and potash. It also gives Fröhde's sulpho-molybdic reaction; I may, however, remark that I found that several substances chemically allied to proteids yield this reaction, which is therefore not diagnostic of proteids alone. An alcoholic solution of alloxan gives with the solid plates a brilliant red coloration (Krasser's‡ reaction) similar to that produced with plates of serum-albumen. Negative results were obtained with the reactions associated with the names of Liebermann,§ Adamkiewicz,|| and Millon.¶

The solution is neutral and lævorotatory ($\alpha_D = -52$), and if treated with pepsin and a 0·2 per cent. hydrochloric acid, or by an alkaline solution of trypsin, for several days at 38° C. it does not peptonise.

Qualitative analysis shows that this substance does not contain phosphorus in its molecule.

It is precipitated from solution by mercuric chloride, silver nitrate, and lead acetate. These precipitates yield the same colour reactions as the original substance.

The precipitate formed by the addition of lead acetate, like that obtained by the addition of this substance to a proteid solution, redissolves on the passage of a current of sulphuretted hydrogen through the solution in which it is suspended, and judging by chemical tests alone, the nature of the substance is unchanged by the processes of precipitation and redissolving. Its physiological action is, however, markedly changed, as will be shown later on.

The original solution is readily precipitated by trichloracetic, phosphotungstic, phosphomolybdic acids, and by acetic acid and potassium ferrocyanide, as well as by salicylsulphonic acid; the precipitate formed by this last substance is coagulated by heating in a manner similar to the coagulation produced by heating the precipitate resulting from the addition of this substance to a proteid

* Schmidt and Aronstein, 'Pflüger's Archiv,' vol. 8, p. 75, 1874.

† *Vide* Halliburton, 'Journ. Physiol.,' vol. 18, p. 306, 1895.

‡ Krasser, 'Monat. für Chem.,' vol. 7, p. 673; 'Maly's Jahresb.,' vol. 16, p. 1.

§ Liebermann, 'Maly's Jahres.,' vol. 18, p. 8.

|| Adamkiewicz, 'Ber. d. deut. Chem. Gesell.,' vol. 8, p. 761.

¶ Millon, 'Comptes Rendus,' vol. 28, p. 40.

solution. I may here mention that salicylsulphonic acid does not precipitate disintegration products of proteids like leucine, tyrosine, xanthine, or hypoxanthine.

All the precipitates cited above give the colour reactions characteristic of the original substance.

If the original solution is saturated with either magnesium sulphate, ammonium sulphate, or sodium chloride, the whole of the colloid rises to the surface of the liquid, and may be skimmed off. On placing this scum in an excess of distilled water, it rapidly redissolves, forming a pale yellow opalescent solution, which gives all the chemical reactions characteristic of the original substance. If the amount of neutral salt be insufficient to produce precipitation, the passage through the liquid of a current of carbon dioxide or of sulphur dioxide will effect the same result. Neither of these gases will, however, cause precipitation in the entire absence of salts.

The following experiments illustrate the results produced by the intravenous injection of this substance into dogs, rabbits, and cats. The procedure adopted was identical with that described in the previous papers published by Professor Halliburton and myself,* on the intravascular injection of Grimaux's colloids. In all cases the animal was anæsthetised by a mixture of chloroform and ether, an excess of the latter substance being used when the subjects were dogs.

Experiment 1.—Fox terrier (weight 27 lbs. 10 oz.); 25 c.c. of a 0·75 per cent. solution of the colloid α was injected, and proved fatal. Pronounced exophthalmos and dilatation of the pupils, and typical stretching movements were observed.

Post-mortem examination made immediately after death revealed pronounced clots in the jugular vein, inferior vena cava, and portal vein, and a slight clot in the left ventricle and in the pulmonary artery.

Experiment 2.—Large black cat (weight 9 lbs. 6 oz.); 40 c.c. of the colloid proved fatal, with similar symptoms as above. Immediate *post-mortem* examination showed pronounced clots in the left ventricle, right auricle, inferior vena cava, portal, and jugular veins. The remainder of the blood was fluid, but coagulated very rapidly after withdrawal.

Experiment 3.—Black rabbit; 38 c.c. of the same substance produced a similar result.

Experiment 4.—Albino rabbit; 42 c.c. proved fatal. Death was accompanied by pronounced exophthalmos and dilatation of the pupils and stretching movements of the limbs. *Post-mortem* examination showed the blood throughout the vessels to be fluid. It, however, rapidly coagulated after withdrawal from the vessels, and the coagulability of samples of the blood taken from the carotids during

* *Op. cit.*

the injection of the colloid was also hastened; thus after 20 c.c. of the colloid had been injected, the time of complete coagulation of blood withdrawn from the carotids was hastened by 2 minutes, after 30 c.c. by $3\frac{1}{2}$ minutes, and after 35 c.c. by 4 minutes.

It will be evident that the results recorded above are similar to, if not indistinguishable from, those produced by the intravenous injection of a nucleoproteid.

When slowly introduced into the circulation of dogs, and to a much lesser degree of rabbits, in minute quantities, the effect produced on the coagulability of the blood is the converse of that resulting from the introduction of larger quantities. This effect is more pronounced than that obtained by the intravenous injection of Grimaux's colloids, and more resembles Wooldridge's* "negative phase," which is characteristic of a nucleoproteid, but is not so pronounced as the result obtained with that substance.

This result is illustrated by the following experiment:—

Experiment 5.—Large black mongrel. Anæsthetic, ether and morphia (weight, 60 lbs.); 1 c.c. of a 0·025 per cent. solution colloid α was injected very slowly, the injection being distributed over half an hour, at the end of which time the retardation of the time of coagulation of blood withdrawn from the animal's carotid was found to be 8 minutes 30 seconds. A second dose of 1 c.c. of the same solution injected and distributed over 20 minutes caused a further retardation in the time of coagulation of the carotid blood of 2 minutes; but a third injection distributed over a similar period of time hastened the coagulability of the blood that had been previously retarded, so that the retardation, as compared with the time of coagulation before the injection of the colloid, was only 1 minute 30 seconds. After a still further injection of the colloid, the blood coagulated more rapidly than in the normal condition, and finally, when the dose was pushed, intravascular coagulation of the animal's blood occurred, and death resulted.

If the colloid is separated from the solution by saturation with magnesium sulphate, sodium chloride, or ammonium sulphate, as before described, and the scum redissolved in distilled water, the opalescent solution obtained will, when intravenously injected into pigmented rabbits, produce typical intravascular coagulation. Repetition of the process of precipitation and redissolving however, destroys the physiological activity in a manner similar to the result produced with both nucleo-proteids and Grimaux's synthesised colloids.

If the solution formed by the passage of a stream of sulphuretted hydrogen over the precipitate formed by the addition of lead acetate to the colloid is injected intravenously into pigmented rabbits or

* Wooldridge, 'Du Bois-Reymond's Archiv,' 1886, p. 397; 'Proc. Roy. Soc.,' vol. 40, p. 134, 1886.

dogs, it is found not to induce intravascular coagulation, although its chemical and physical characteristics are apparently unchanged. This result shows that the chemical reactions used for "testing" proteids are not sufficiently delicate to indicate the chemical changes which are demonstrable by physiological methods. The following experiment illustrates this result:—

Experiment 6.—Black rabbit (weight 7 lbs. 9 ozs.); anæsthetic, chloroform and ether; 120 c.c. of redissolved solution injected produced dyspnœa, exophthalmos, dilatation of pupils. A further injection of 10 c.c. of this substance was immediately fatal. *Post-mortem* examination failed to reveal any clots in the animal's vessels. Blood withdrawn from the carotids during the injection showed only one minute's decrease in the time taken to complete coagulation.

Experiment 7.—In another experiment, where minute quantities of this substance were very slowly injected, there was no retardation of the time of coagulation, like that produced by the original substance or by a nucleo-proteid.

Colloid β.—This substance is formed by heating together tyrosine, biuret, and phosphorus pentachloride in the ratio of equal weights of the two former substances, with twice the weight of the latter, for six hours at 125° to 130° C. in sealed tubes. The product of this reaction is a grey powder insoluble in cold water, and very sparingly soluble on heating. This substance gives the xantho-proteic and Fröhde's reaction, but fails to give typical colour reactions with the other reagents commonly used in testing proteids. It should be repeatedly washed until all traces of the contaminating phosphoric acid are removed, and then dried *in vacuo* at about 30° C. It readily dissolves in concentrated ammonium hydrate, and the solution is opalescent and lævorotatory ($\alpha_D = -48$), and in appearance indistinguishable from that of the other colloids produced. It gives the following distinctive reactions as classified in the annexed table, but does not digest when subjected to the action of either pepsin and 0·2 per cent. hydrochloric acid for three days at 38° C., or of an alkaline solution of trypsin, kept at the same temperature for a similar time. It yields the following distinctive reactions:—

Colloid β.

$CuSO_4$ KHO.	$CoSO_4$ KHO.	$NiSO_4$ KHO.	H_2SO_4 and molybdic acid.	Millon's reagent.	HNO_3 and NH_4OH (heating).	Salicyl sulphonic acid.
Violet-coloured solution.	Heliotrope purple-coloured solution.	Faint yellow-coloured solution.	Dark blue precipitate.	Dark red precipitate.	Orange precipitate.	Precipitate which coagulates on heating.

It gives negative results with the reactions of Liebermann and Adamkiewicz, but gives the typical red coloration when the solid plates are heated with an alcoholic solution of alloxan (Krasser's reaction). It is separated from solution by neutral salts in a manner similar to the colloid α and Grimaux's colloids. The scum also redissolves in distilled water giving an opalescent straw-coloured solution. It is precipitated by silver nitrate, lead acetate, and mercuric chloride, as well as by phosphotungstic, phospho-molybdic, and trichloracetic acids, and by acetic acid and potassium ferrocyanide.

In the entire absence of salts it is not coagulated on boiling, but, on the addition of a trace of a soluble salt of either sodium, magnesium, barium, strontium, or calcium, a coagulum is obtained on heating to 74° C.

The fractional heat coagulation of this substance will be dealt with in a subsequent section.

The effect produced by the intravascular injection of various quantities of this body is illustrated by the following experiment:—

Experiment 8.—Brown mongrel (weight 27 lbs. 7 oz.); anæsthetised with ether and morphia. The jugular vein on the one side, and the carotid artery on the other, were exposed, and cannulæ inserted into them. The colloid β was injected into the jugular vein, and samples of blood withdrawn from the artery. The following table shows the rate of clotting of the various samples:—

(1) Before injection of the colloid, the blood clotted in 10 minutes 30 seconds.
(2) 5 c.c. of 0·75 per cent. solution of colloid dissolved in 0·75 per cent. saline injected. A firm clot formed in 17 minutes 8 seconds.
(3) 10 c.c. more injected. Loose clot in 22 minutes.
(4) 10 c.c. more injected. Firm clot in 31 minutes.
(5) 10 c.c. more injected. Firm clot in 13 minutes.
(6) After interval of 5 minutes a second sample of carotid blood formed a firm clot in 7 minutes 30 seconds.
(7) 7 c.c. more injected. Firm clot in 7 minutes 30 seconds.
(8) 10 c.c. more injected. Firm clot in 6 minutes.
(9) 15 c.c. more injected. Firm clot in 3 minutes.
(10) 10 c.c. more injected and proved fatal.

Immediate *post-mortem* examination revealed loose clots in vena cava inferior, and jugular vein, and pronounced clots in portal vein, and right ventricle.

This experiment shows the "negative phase" after injection of small quantities of the colloid β, and the typical hastening of the coagulability of the blood withdrawn from the carotid after the intravenous injection of

a larger dose, and finally the coagulation of the intravascular blood when the dose is again increased.

Colloid γ.—The colloid γ is formed by heating together at 130° C. in sealed tubes, for three hours equal weights of alloxan and metamido-benzoic acid, with twice their weight of phosphorus pentoxide. The product of the reaction is a white powder, very slightly soluble in cold water, and sparing soluble in warm water. It should be washed in ice-cold water till the excess of phosphoric acid is removed, and the remaining substance dissolved in concentrated ammonia. The resulting solution is opalescent and straw-coloured, and should be evaporated down at the temperature of the laboratory *in vacuo*, when a number of translucent, yellowish plates, closely resembling the previously described colloids are formed. These plates are soluble in warm water, and the solution is pale straw-coloured, opalescent, and lævorotatory ($\alpha_D = -41$) and shows the following reactions:—

Colloid γ.

HNO_3 NH_4OH. (heating).	Millon's reagent.	Fröhde's reaction.	$CuSO_4$ and KHO.	$NiSO_4$ and KHO.	$CoSO_4$ and KHO.	Salicyl-sulphonic acid.
Yellow solution.	Dirty brown ppt.	Blue precipitate.	Violet solution.	Very faint yellow solution.	Dark brown solution.	No precipitate.

It is separated from solution by saturation with either magnesium sulphate, sodium sulphate, sodium chloride, or ammonium sulphate, the colloid rising to the surface of the liquid as a white scum, which redissolves, forming an opalescent solution when thrown into distilled water. It is precipitated by silver nitrate, lead acetate, and mercuric chloride. If the precipitate formed by the addition of lead acetate is suspended in distilled water, and a current of sulphuretted hydrogen is passed through the liquid, the precipitated colloid again passes into solution.

When heated in the presence of a trace of a neutral salt, fractional heat-coagulation is obtained, which will be detailed in a subsequent section.

If the colloid γ is injected into the circulation of dogs or pigmented rabbits, even in large quantities, it does not produce intravascular coagulation, although it somewhat hastens the coagulability of blood withdrawn from the carotid.

The colloid γ, although yielding many of the chemical reactions that have been used as distinctive tests for proteids, and also behaving in a very similar manner to the previously described proteid-like colloids, does

not, like them, produce intravascular coagulation when intravenously injected into pigmented rabbits. Neither will the colloid γ when introduced into the circulation of dogs, very slowly and in minute quantities, produce a retardation of the coagulation of blood withdrawn from the carotids.

Colloid δ.—The colloid δ is formed by heating at 125° C. in sealed tubes for three hours, equal weights of para-amidobenzoic acid and phosphorus pentachloride. The resulting product, a grey friable powder, insoluble in cold water, was, after washing to remove the contaminating phosphoric acid, dissolved in concentrated ammonia, and evaporated down at a low temperature *in vacuo*. The resulting substance appears as a number of translucent yellowish plates, apparently similar to those previously described. They are soluble in warm water, forming an opalescent straw-coloured solution, which is lævorotatory ($\alpha_D = -42$). This solution gives the xantho-proteic and Fröhde's reaction, but fails to give the typical colour reactions of proteid-like substances with salts of copper, cobalt, or nickel and caustic potash; neither does it give the reactions of Millon, Liebermann, or Adamkiewicz. It is not precipitated by salicylsulphonic acid, but it is precipitated by salts of the heavy metals. Neutral salts separate it from solution like the preceding substances. When freed from salts, it does not coagulate on heating, but if a trace of sodium chloride or of another neutral salt be present, it coagulates on heating to 75° C. When intravenously injected into pigmented rabbits, it fails to produce intravascular coagulation, neither does it hasten the coagulability of blood withdrawn from the carotids. It fails to induce a "negative phase" in the coagulation of dogs' blood. This series of results lends additional support to the view that the coagulation of the blood resulting from intravenous injection of the colloid, is due to the interaction of the colloid with the constituents of the plasma, and not to the heavy nature of colloid molecule.

Colloid ϵ.—The colloid ϵ is prepared by heating together equal weights of tyrosine and xanthine with twice their weight of phosphorus pentachloride at 125° C. for three hours. The product of the reaction is a yellowish powder slightly soluble in warm water. After repeated washing in cold water, it is dissolved in concentrated ammonia, and the resulting solution evaporated down *in vacuo* at a low temperature. The resulting substance consists of a number of translucent yellowish plates like those previously described. It is readily soluble in warm water, forming a yellowish opalescent solution, which is lævorotatory ($\alpha_D = -38$).

This solution gives a typical red when heated with Millon's reagent, which is not due to an excess of tyrosine, since the intermediate product in the preparation of the substance fails to give this reaction. It does not give any other of the distinctive proteid colour reactions,

but is precipitated by salicylsulphonic acid, and the precipitate coagulates on heating. It behaves with neutral salts and salts of the heavy metals similarly to the previously described substances. It does not cause intravascular coagulation of the blood when intravenously injected into dogs or pigmented rabbits, neither will the very slow injection of minute quantities into the circulation of dogs induce a "negative phase." It does not induce coagulation when added to 1 per cent. sodium carbonate plasma.

Colloid ζ is prepared in a similar manner to the colloid ϵ, hypoxanthine being substituted for xanthine. It has a similar appearance to the colloid ϵ, is lævorotatory ($\alpha_D = -40$), gives Millon's reaction, and negative results with the other tests characteristic of proteids.

It also behaves with neutral salts and salts of the heavy metals in a similar manner to the previously described substances. When intravenously injected into the circulation of dogs or pigmented rabbits, it fails to induce intravascular coagulation, neither will it cause coagulation when added to extravascular 1 per cent. sodium carbonate plasma.

Colloid η.—The colloid η is prepared by the interaction of tyrosine and phosphorus pentoxide for three hours at 130° C. in sealed tubes. The product of this reaction is a pinkish friable powder, sparingly soluble in cold water and soluble on boiling. This substance does not yield Millon's reaction. After washing in cold water to remove the contaminating phosphoric acid, the powder is dissolved in concentrated ammonia, and a straw-coloured opalescent solution is obtained. This is evaporated down *in vacuo*, and the resulting substance appears as a number of plates, similar in appearance to those of the previously described colloids, and which are soluble in warm water, giving an opalescent solution. This solution is precipitated by salicylsulphonic acid and the precipitate coagulates on heating. It is also precipitated by salts of the heavy metals, and separated from solution by neutral salts. It does not yield any of the distinctive colour reactions of proteids, and fails to produce intravascular coagulation when intravenously injected into rabbits.

II. *The Fractional Heat Coagulation of Synthesised Colloids.*

The method of differentiating the members of a mixture of proteids by fractional heat coagulation was introduced by Halliburton,* and employed by him more especially in the examination of the proteids of serum. This method was subsequently used by Corin and Bérard† in separating the albumins of the white of egg, and by Chittenden

* Halliburton, 'Journ. Physiol.,' vol. 5, p. 159.

† Corin and Bérard, 'Bul. de l'Acad. Roy. de Belgique,' vol. 15, 4, 1888.

and Osborne* in studying the proteids of maize. The method was rendered more accurate by Hewlett,† who substituted a bath of cod-liver oil for the water bath usually employed as the heating medium, and exhaustively dealt with the adverse criticisms made by Haycraft and Duggan.‡

I have applied this method, using an oil bath, in the examination of the proteid like colloids synthesised by Professor Grimaux and myself. As pointed out in a previous section, in the entire absence of salts these substances do not coagulate, even when boiled. For the sake of comparison the following experiments were performed, so as to satisfy the following conditions:—(*a*) A 2 per cent. solution of the substance under examination was always used. (*b*) The diluent fluid always consisted of a 0·75 per cent. solution of sodium chloride. (*c*) In each experiment 10 c.c of the fluid under examination was used, and the test-tubes were of uniform internal diameter. By this means the mass to be heated remained constant. (*d*) The thermometer was placed in the middle of the test-tube containing the fluid under examination.

The colloid A ("colloïde amidobenzoique" of Grimaux) shows a coagulation temperature of 70° to 71° C.

The colloid B (of Grimaux) which is prepared from the same reagents as the colloid A, but the temperature at which the reaction of synthesis is conducted is allowed to rise to 130° C., shows on heating one faint appearance of flocculi at 56° to 58° C., and a second more pronounced coagulum at 70° to 72° C.

The colloid C ("colloïde aspartique" of Grimaux) on fractional heating shows three distinct sets of flocculi, appearing respectively at 58°, 67°, and 73·1° to 76·4° C.

The colloid α, if care has been taken to keep the temperature of preparation constant at 125° C., shows, on heating, only one coagulum at 70·6°; if, however, in the preparation of this colloid the temperature of synthesis is allowed to rise, a second colloid coagulating at 42° C. is often but not always formed.

The colloid β, even when the temperature of the synthesis has been kept constant at 130° C., shows, on heating, three constituents coagulating at 47° C., 56° C., and 74° C.

The colloid γ apparently only has one temperature of heat coagulation, viz., 75° C.

The colloid δ coagulates at 75° C.

The colloid ϵ coagulates only at 47° C.

The colloid ζ coagulates at 48° and 59° C.

* Chittenden and Osborne, 'Amer. Chem. Journ.' vol. 13, 7 and 8; vol. 14, 1.

† Hewlett, 'Journ. Physiol.,' vol. 13, p. 493, 1892.

‡ Haycraft and Duggan, 'Brit. Med. Journ.,' 1890, vol. 1, p. 167; 'Edin. Roy. Soc. Proc.,' vol. 16, p. 361, 1888–9.

The colloid η coagulates only at 52° C.

Adopting the conclusion of Halliburton that the precipitates obtained by the fractional heat coagulation of a proteid substance, correspond with various constituents of that substance, we may possibly conclude that those synthesised colloids which yield fractional heat-coagula are mixtures of different colloidal substances.

Thus the colloid B would consist of two substances which might be designated B_1 and B_2, and the colloid β of three substances, designated colloids β_1, β_2, and β_3 respectively, and the colloid δ of two substances, δ_1 and δ_2. I have endeavoured to ascertain in the cases of the colloids B_1 and B_2 and of the colloids β_1, β_2, and β_3 whether each of these substances will equally induce intravascular coagulation of the blood, when intravenously injected into pigmented rabbits and dogs.

The method of procedure adopted was briefly as follows:—The activity of a solution of the colloid was tested by a control experiment. One of the constituents was removed by fractional heat-coagulation and the effect, if any, produced by the intravascular injection of the remaining colloid in solution was tested.* The following is the record of some of the results obtained:—

Colloid B_2 after a removal of colloid B_1 will, if intravenously injected, induce intravascular coagulation in pigmented rabbits, and if slowly injected in minute doses a "negative phase" in dogs.

Colloids β_2 and β_3 will still, after the removal of colloid β_1, induce intravascular coagulation in pigmented rabbits, although a much larger dose is required after the removal of β_1 and β_2 than if the mixture of the three substances is injected, if only β_1 is removed the activity of the mixture is not impaired. From this I conclude that β_2 and β_3 are the active constituents of the colloid mixture I have designated as the colloid β. There is apparently no difference in the tendency to induce a "negative phase" in dog's blood after the removal of β_1 and β_2 from the solution.

III. *Other Properties of the Synthesised Colloids.*

The influence of these substances on red and white blood corpuscles, and on extravascular 1 per cent. sodium carbonate plasma will be described in a subsequent paper.

IV. *Concluding Remarks.*

It is evident from the observations recorded in the preceding pages, that if certain derivatives of proteids, and other substances of

* The solution after removal of one of its constituents by fractional heat-coagulation, was evaporated down *in vacuo* until it had the same specific gravity as the original solution.

allied chemical constitution are heated together in sealed tubes with an excess of either phosphorus pentachloride or pentoxide, a series of colloidal substances are formed which, when freed from the contaminating phosphoric acid, and dissolved in concentrated ammonia, give opalescent solutions that, on evaporating down *in vacuo*, yield substances closely resembling in physical, chemical, and physiological properties certain proteids.

These colloidal substances, although they differ from one another in minor details, are usually distinguished by the following characteristics :—

1. They are soluble in warm water, forming opalescent lævorotatory solutions.

2. The resulting solutions yield the principal colour reactions hitherto deemed diagnostic of proteids.

3. In the absence of salts, solutions of these colloids do not coagulate on heating. In the presence of a trace of a neutral salt they coagulate on heating at temperatures very similar to proteid solutions.

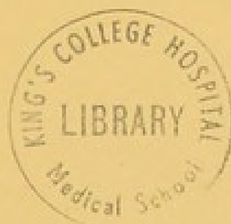
4. Fractional heat-coagulation shows the colloidal solutions are a mixture of different substances.

5. The different constituents of the colloidal solution exhibit different physiological action.

6. In the presence of an excess of neutral salts, or of salts of the heavy metals, the colloidal solutions behave in a manner similar to proteid solutions.

7. When introduced into the circulation of pigmented rabbits, dogs, and cats, certain of these substances (viz., the colloids designated A, B, C, α and β) produce intravascular coagulation of the blood in a manner similar to a nucleo-proteid. They also hasten the coagulability of the blood withdrawn from the carotid, and will, when slowly injected intravenously in minute quantities into dogs, produce a retardation of the coagulability of the intravascular blood, *e.g.*, a "negative phase."

8. Apparently these colloidal substances are, owing to both their physical and chemical properties and their physiological behaviour, the nearest synthesised bodies at present known to proteids.



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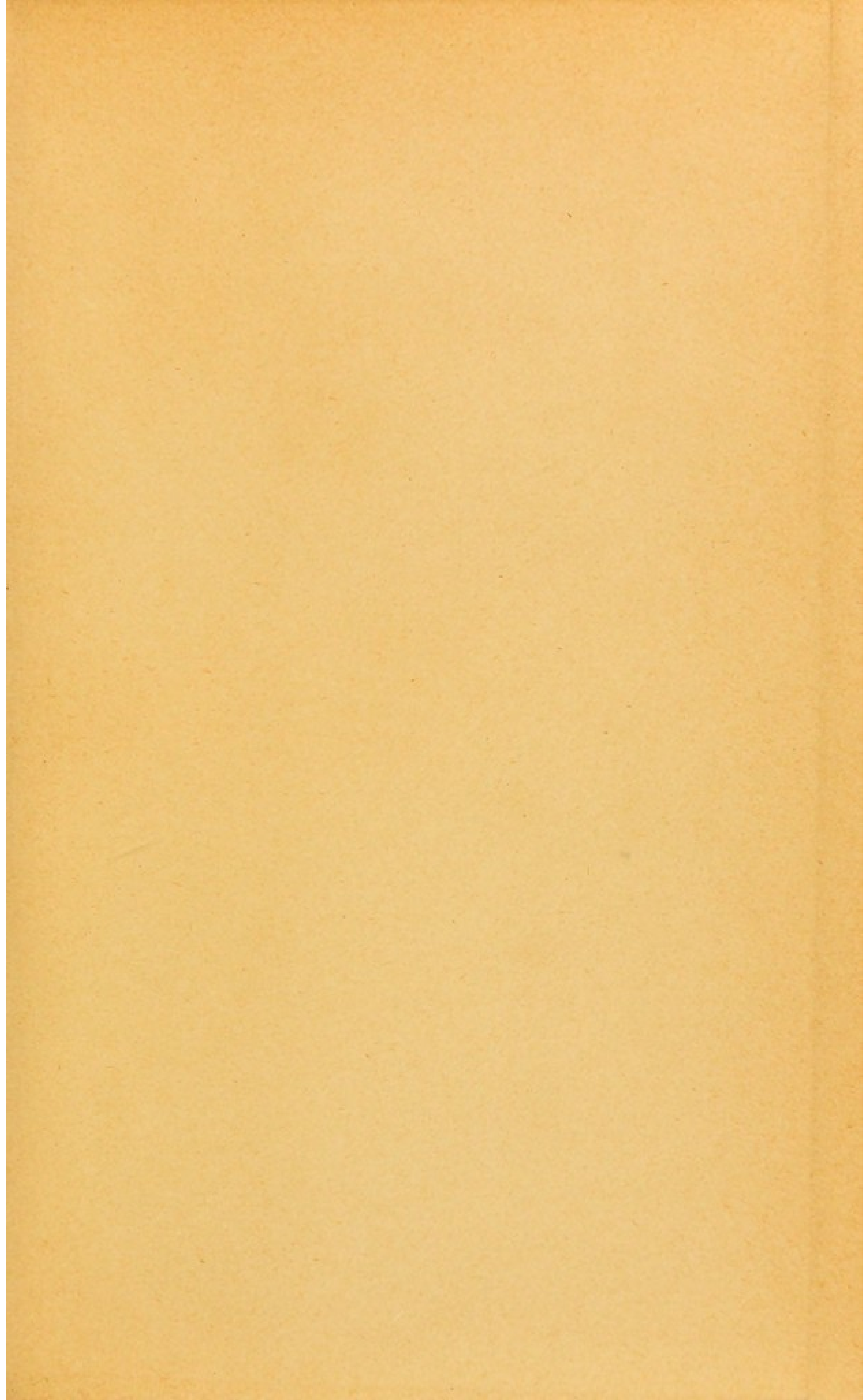

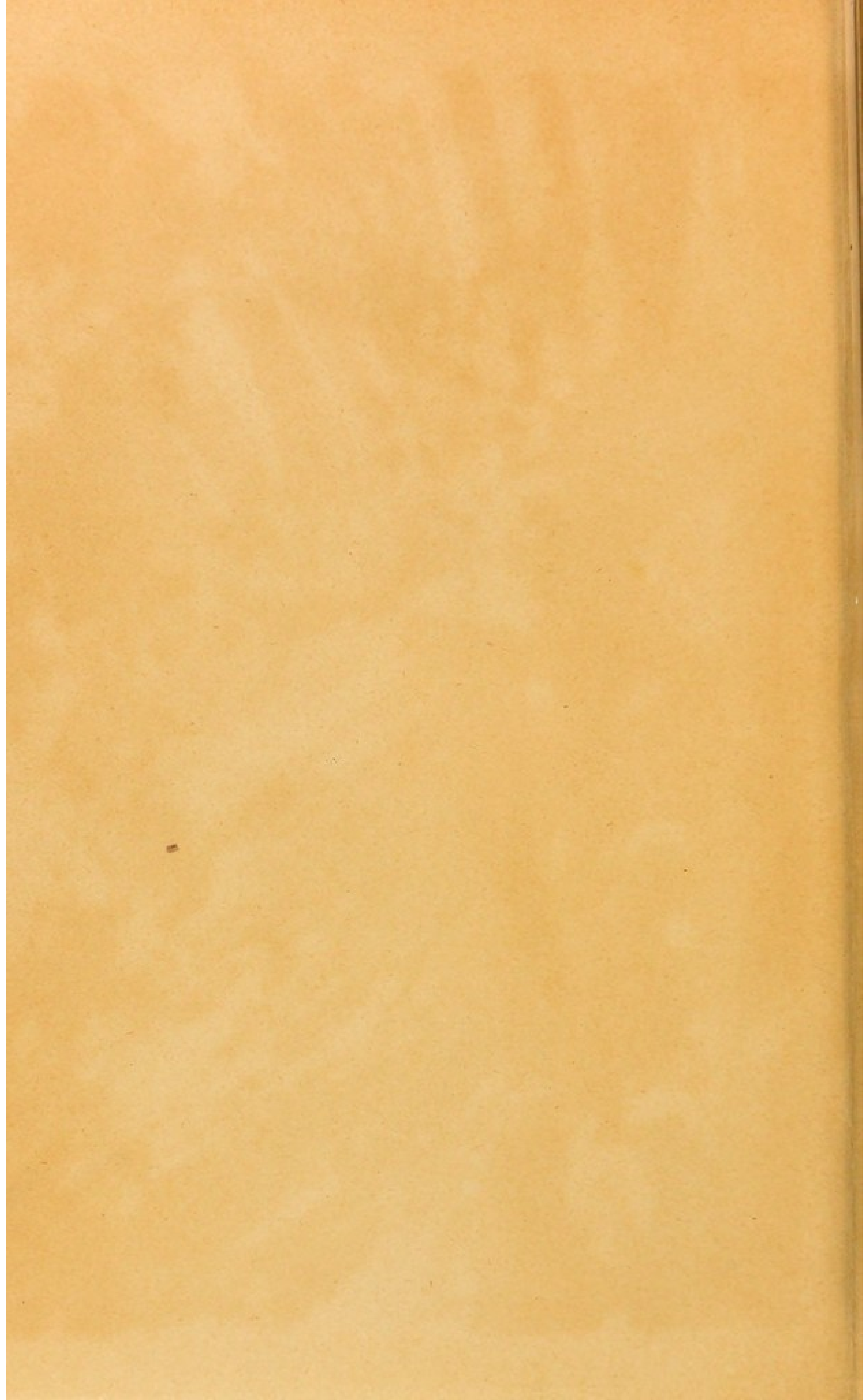

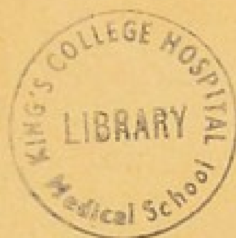
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