

ON THE INTIMATE ASSOCIATIONS OF INORGANIC IONS WITH  
NATIVE AND DERIVED PROTEINS.—BY DAVID FRASER  
HARRIS, M. D., D. SC., F. R. S. E., *Professor of Physiology  
and Histology, Dalhousie University, Halifax.\**

Read 8th April, 1912.

We must assume that unless united to the living molecules (biogens) no food would be assimilated, no drug benefit us, and no poison harm us. We must have some sort of union, incorporation or molecular linking, and that cannot be outside the sphere of atomic affinities. Protoplasm must be chemically viewed as an unstable, molecular, protein complex to which, probably as side-chains, adhere carbohydrate molecules and fat molecules and many inorganic ions both anions and cations. The unmasking of this fat is called in pathology "fatty degeneration," the unloosening of this sugar is called tissue-diabetes. We have fat necrosis after certain poisonings; for instance, phosphorus and alcohol can unmask fat in many tissues of a persons the very opposite of obese, while after chloroform or an excessive percentage of carbon dioxide in the blood we have glycohaemia and the consequent glycosuria which means that the poison has displaced the sugar and sent it into the bloodstream. But further, a salt-free (ash-free) protoplasm, that is, salt-free living protein is only a conception of the chemists; protein is ash-free only in the laboratory. No doubt these ionic side-chains constitute mere traces, but as inorganic substances they play an exceedingly important part in the activities and existence of living matter. A salt-free diet will not support life. Dogs fed on ash-free fats, carbohydrates and proteins were moribund in twenty-six to thirty-six days.

---

\* Contributions from the Science Laboratories of Dalhousie University —  
[Physiology].

But, further, the salts of the diet must be present in it in their natural unions and not merely in a solution added to the organic food. Whereas mice thrive on a diet of dried cow's milk, they were moribund in twenty to thirty days on the sugar, fat and casein of milk to which a solution of the extracted salts of milk had been added. We know that a diminution in the amount of potassium absorbed will lead to scurvy.

It used to be said that as the salts contribute no energy, they are not incorporated into the living matter; this is quite a mistake, for although they do not yield energy, they are incorporated as truly as is the fat or carbohydrate or oxygen. It would appear that all the following must be present in the tissues and fluids, not necessarily all in all: sodium, potassium, calcium, magnesium, iron, phosphorus, chlorine, iodine, fluorine and arsenic. Without these, the living matter is not functionally intact: there is a metabolism of the inorganic as truly as there is of the organic.

Take the case of the beating heart; if perfused with distilled water, even containing oxygen and dextrose, it will shortly stop beating, and a loss of salts from it can be proved to have occurred. Now give it a perfusion-fluid with sodium chloride whose osmotic pressure is equal to that of the sodium chloride in the heart, and still it stops. This is found to be because we have left out the potassium and the calcium; the addition of these, the potassium chloride as dilute as 1 in 10,000 is enough, will cause the heart to beat rhythmically. Apparently the cardiac myoplasm establishes an equilibrium between certain organic ions within itself and others in the lymph of its spaces, the point of equilibrium being dependent upon the osmotic pressure of these substances in the surrounding fluids and on the affinities of the protoplasm for these substances. If any one ion predominates somewhat over the others, that is, is present in higher concentration than exists in normal lymph, effects which have been called "toxic" will supervene; thus if potassium is too abundant we have the heart stopping in potassium diastole,

if calcium be too abundant we have the heart stopping in the systole of calcium rigor.

Now the affinities of certain kinds of protoplasm for certain ions are quite different from those of other kinds of protoplasm for them. Thus the red corpuscles fix potassium and iron, the brain, phosphorus; the muscles, potassium; the bones and teeth, calcium and fluorine; the thyroid gland, iodine; and the fluids of the body chiefly sodium. The thyroid gland can, moreover, fix more iodine per unit of tissue than can any other tissue. Chemically speaking, therefore, protoplasm in different situations is chemically different; the protoplasm of the brain has not the same atomic affinities as that of muscle or bone or thyroid gland. The tissues are, however, supplied by lymph of practically uniform composition, so that these chemical differences have been said to be due to "selective affinity." Now these differences must be very slight. Dr. Jermain Creighton<sup>1</sup> has shown that egg-albumin, a native protein and a very direct product of living matter, can distinguish in its selective affinity between iron in the trivalent and iron in the divalent state. Dr. Creighton has found that egg-albumin apparently forms a union with the ferri-ion whether that be as in ferric chloride or in soluble Prussian Blue, (potassium ferri-ferro-cyanide), both of which have trivalent iron as a cation; or in potassium ferricyanide in which trivalent iron is part of a complex anion. Some late work has shown the iron in haemoglobin to be the ferri-ion: so that it would appear that the point is not whether iron is cation or anion but whether it is tri- or di-valent. The difference is physico-chemically very slight, and yet the albumin takes cognizance of it. In accordance with these views some pharmacologists assert that simple anaemia is cured only by ferric salts. Dr. Creighton has further shown that even gelatine exhibits analogous selective affinities. Here, I think, we are in presence of some very important facts as indicating

---

1. Creighton, H. J. M. : Trans. N. S. Inst. Science xiii, (2), 61-75, (1911-1912).

the delicate nature of these unions of colloids with metallic ions, unions, which in some cases have been labelled "adsorptions." Now if this sort of thing can go on in non-living albumin what may not be chemically possible in the living bioplasm itself? Dr. Creighton speaks guardedly of "a complex" between the protein and the iron; but we may at least hold a salt-like union is effected and that the tri-valent iron is chemically bound. In accordance with this we have to remember Professor Macallum's test<sup>1</sup> for inorganic versus bound iron: a dilute (0.5%) solution of pure haemotoxylin gives with inorganic iron a blue black coloration, but with bound iron no reaction. Under the latter heading come haemoglobin and both the potassium ferri-cyanide and the potassium ferrocyanide. In these the iron atom is bound in some fashion so as not to affect the haemotoxylin in the manner in which it can do when in the unbound condition of inorganic salts presumably ionised.

It used to be said that inorganic salts given as drugs have a tendency to be deposited in the liver; in more modern terminology it would be said that the hepatic protein has the power of binding the inorganic ions—mercury, arsenic, manganese, etc.—and therefore retaining them in the liver and so preventing them reaching the circulation in anything like the concentration in which they were absorbed. This capacity of the liver is but one expression of its *detoxicating* power in virtue of which it fixes many poisons, pathogenic toxins and others, and so prevents their entrance into the circulating blood.

There must therefore be constant interchanges between the living matter and the inorganic constituents of the lymph, for inorganic salts are being constantly absorbed and constantly excreted and so, on the whole, the percentage of inorganic constituents in the tissues does not vary. Now the amount of any one constituent—iron, calcium, sodium,

---

1. Macallum, A. B: J. Physiol., 22, 92, (1897-1898).

potassium, etc.—depends on its ionic pressure in the lymph as well as on the affinity for it possessed by the particular tissue in question. Certain forms of malnutrition may depend not so much on the malabsorption of an inorganic ion as on the diminished affinity for that substance as the result of some intoxication or devitalisation of the living tissues.

But even when an ion is present in the lymph in a concentration greatly above its concentration in the cell, that substance is not absorbed in anything like the degree which one would expect of it, if one had regard only to its concentration over iso-tonicity. The living tissues have a “power of refusal.”

This explains what is so well recognized, that it is impossible to oversaturate the tissues with any of the mineral substances—iron, arsenic, calcium, or even oxygen. This fixedness of limit for saturation of protoplasm by chemical substances explains the impossibility of indefinite increase in bulk of tissues by overfeeding with nitrogenous food, of increasing the intensity of tissue-changes to any notable extent by the breathing of pure oxygen by healthy persons or of increasing, for instance, the iron or phosphorus content of the healthy red marrow or brain. After being satisfied, the tissues have a power of refusal—one of the expressions of “functional inertia.”<sup>1</sup>

The same line of reasoning applies to the gases concerned in metabolism. Thus oxygen must be under a certain pressure in order to enter properly into union with the living matter. Whereas oxygen at the partial pressure of one-fifth of an atmosphere suffices for the perfusion fluid for a frog-heart, it must be under the pressure of one atmosphere in the fluid<sup>2</sup> necessary for the mammalian heart. In the actual blood, which could not take up anything like this quantity of oxygen in solution, this high pressure is functionally represented by the loose

1. Harris, D. Fraser: *The functional inertia of living matter.* London, Churchill 1908.

2. Ringer-Locke solution consists of—

NaCl, 0.9%	NaH CO <sub>3</sub> 0.01 to 0.03%
Ca Cl <sub>2</sub> , 0.024%	Dextrose, 0.1%
K Cl, 0.042%	

Solution fed to the heart under the pressure of one atmosphere of oxygen.

chemical union of oxygen with the haemoglobin which dissociates in the neighbourhood of the living cells owing to the partial pressure of oxygen in them being always zero. In the lung-alveoli, oxygen is not present to more than 15 to 16% of an atmosphere (that is 104 mm of mercury), and this pressure of itself would be inadequate to drive the oxygen into solution in blood-plasma to an amount sufficient for the respiratory needs of the tissues, hence the blood possesses in its red corpuscles a substance capable of uniting with the inorganic oxygen in such a way that it can carry far more oxygen to the tissues than could ever possibly reach them in solution in a colloidal protein substance like the plasma. It is of advantage to the body that there be formed, therefore, complexes between proteins or protein-derivatives and certain inorganic ions; and Dr. Creighton,<sup>1</sup> by having studied some of these in detail, has thrown a good deal of light on their probable nature.

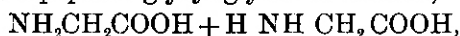
The whole of modern medicine is permeated by the notion that bioplasm is affectable, that is, is capable of responding to stimuli, a large number of which are chemical. Thus the formation of an *anti*-body is only possible because there is a reaction on the part of the affectable living matter to the chemical stimulus of the foreign substance: if toxin be the chemical stimulus, then antitoxin is the chemical response. But the toxin must first come into chemical union with the protoplasm else no antitoxin can result, just as the food molecule must come into chemical relationship with the protoplasm else no food could be absorbed.

The power of the proteins of blood-serum to absorb or take up either acids or alkalies is of fairly high importance to the bodily health. Thus, confining ourselves to the absorption of acids only, if we add normal acid to blood-serum and use methyl orange as an indicator, we shall have to add 0.18 c. c. of normal  $\left(\frac{N}{1}\right)$  hydrochloric acid to turn the indicator pink. If now we titrate, similarly, the saline dialysate from the serum, the acid-

1. Creighton, H. J. M. : loc. cit.

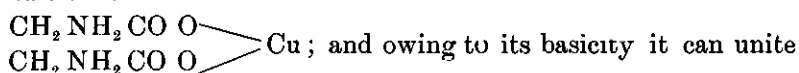
holding power is now only 0.04, so that (0.18 — 0.04) 0.14% is the figure representing the acid fixed by the proteins alone. This represents 0.51% of hydrochloric acid itself. Now this is rather a considerable amount; and its physiological significance is that, within pretty wide chemical limits, no free acid can reach the living tissues, for the circulating proteins can combine with them and so constitute a protective mechanism against acidosis or an excessively acid condition of blood. These native or serum-proteins, therefore, behave in an amphoteric fashion, for they can fix alkalis like acids and acids like alkalis. This explains how serum is acid to phenolphthalein, and alkaline to methyl-orange, while it is physico-chemically neutral. This double power proteins possess is now believed to be due to their polypeptide composition. This means that after any number of amino-acids have united together in chain fashion, there will be left an amidogen group at one end and a carboxyl group at the other, thus conferring a chemical polarity or what is otherwise called "residual affinity."

Thus the dipeptide glycyl-glycin is formed,

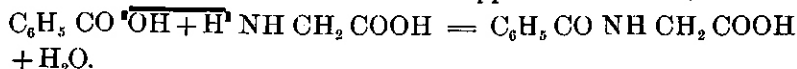


which gives us  $\text{NH}_2\text{CH}_2\text{CO-NH-CH}_2\text{-COOH} + \text{H}_2\text{O}$ , a compound is basic on account of  $\text{NH}_2$  and acidic on account of the  $\text{COOH}$ .

Hence owing to its acidity, glycyl can form the copper salt thus



with an acid like benzoic and form hippuric acid thus;



The union of oxygen with haemoglobin is, however, not merely an adsorption due to residual affinities, for it is strictly mono-molecular, and the reduced form of the pigment is different from the oxidised in colour and therefore in spectrum.

But not merely are acids and inorganic substances united

to the native proteins of blood, for 1% of fat is probably held in a quite invisible form in blood-plasma. This is exceeded by the liver which can hold as much as 5% of fat in a perfectly transparent and invisible form; the fat, for the time being, is chemically united to the tissue-proteins. Some physiologists hold that during the time that carbohydrate is in the liver, it is present in a protein-complex and they say that glycogen can be demonstrated chemically in liver cells before it can be histologically.

One of the latest views as regards the early fatigue of muscle is that potassium salts are detached and sent into the circulation depressing the motor nerve-endings. What unloosens the potassium is not yet obvious, but it appears that potassium is set free. Lactic acid is similarly free in the circulation in the later stages of muscular fatigue.

That the union is ionic as regards certain inorganic substances is interestingly shown in the part played by calcium salts in the clotting of the milk. It is known that when the rennin has transformed the caseinogen into soluble procainein there is no precipitation of the latter until it has formed a union with calcium: a drop or two of calcium chloride now causes an abundant precipitation of casein. In 1895 I showed<sup>1</sup> that barium chloride and strontium chloride were equally efficacious. Here the action must be due to the divalent ions and to the different ions indifferently, for certainly the anion chlorine is not the causal substance. Now while this is so as regards the clotting of milk, barium cannot supplant calcium medicinally. In particular, barium chloride cannot replace calcium chloride as regards efficiency in maintaining the heart's rhythm. Barium is absorbed very slowly from the intestine, and when so absorbed is found to be a direct stimulant of muscle-fibre as distinct from nerve-fibre. Just as barium can replace calcium in the clotting of milk so it can replace it in the clotting of blood. Magnesium sulphate injected into rabbits gives rise to

---

<sup>1</sup> Harris, D. Fraser: Some points in the physiological chemistry and coagulations of milk. *Pro. Roy. Soc. Edin.*, Session 1895-1896.



paralysis and anaesthesia and a low blood-pressure; it can be rapidly antagonised by either the chloride or the acetate of calcium, which revives the respiration in a surprisingly short time, but *not* by barium. It would seem as though in a non-vital union, barium and calcium were interchangeable, but not so in vital chemical complexes. Thus, the influence of magnesium is the same as that of calcium in inhibiting the spontaneous twitching of muscles immersed in solutions of sodium or lithium and in antagonising the contraction of skeletal muscle brought about by potassium salts; but in regard to its action on the heart, magnesium stands quite apart from calcium, barium and strontium, and is totally unable to replace these in the cardio-inhibitory mechanism or at the skeletal neuro-muscular junction.

And this is to a large extent comprehensible, for chemically, such substances as caseinogen, blood-germent, albumin, etc., and *not* to be taken as the equivalents of living matter, complicated as they are. The metabolism of calcium is full of lessons for us; one result of its presence in blood is to confer a certain degree of viscosity on that fluid. If there is too little viscosity, there is a tendency for the blood-plasma to exude too freely through the capillary wall so that an oedema or urticaria may be produced which is rapidly removed by the administration of a soluble salt of calcium—the chloride or lactate. It is possible that the tissues of haemophiles may suffer from a congenital inability to absorb or incorporate calcium. But indeed the whole doctrine of ionisation has been of great service in biology: for this may be taken to be the converse of the chemical condition of union of ions or atoms with the protein or living matter. Thus in the simple case of action of acids on living tissues, it is found that e. g. HCl is far more destructive to enzymes (except pepsin) than is acetic, the only satisfactory explanation of this being that HCl is far more perfectly ionised than acetic, not more than 3% of which is ionised. Since in regard to the effects of different acids, it is highly

unlikely that all the various anions ( $\text{Cl}$ ,  $\text{NO}_3$ ,  $\text{PO}_4$ ,  $\text{SO}_4$ ,  $\text{CH}_3\text{COO}$ ) are the active substances, it is customary to attribute the physiological activity of the acids to the ionised H. Similarly the alkalies, ( $\text{KOH}$ ,  $\text{NaOH}$ ,  $\text{NH}_4\text{OH}$ ) have only the OH ion in common, so that their common influence in activating enzymes is to be attributed to the anion hydroxyl. Hence, too, the "free" alkalies are physiologically more active than the carbonates, because they are more perfectly ionized. In some recent work of mine<sup>1</sup> on the presumed endo-enzyme, tissue reduction, any inhibitory action I found as the result of the presence of protoplasmic poisons was to be attributed rather to their acidity than to their so called toxicity; this is but one more verification of the statement that acids—H ions—destroy enzymes. Of course in all these problems we are dealing with very small quantities: the maximum concentration for the activating effect of alkalies is not greater than  $\frac{1}{10}$ <sup>th</sup> molecular. May the activity of certain dilutions not explain some of the results obtained in homoeopathy?

So much, then, for the sign of the ionic charge; we have still to reckon with the valency of the ion or the potential of the charge or the ionic potential.

Now the physiological activity of inorganic ions increases with their valency thus— $\text{Na}^I$ ,  $\text{Ca}^{II}$ ,  $\text{Fe}^{III}$ ; sodium being more bland than calcium and calcium than iron or conversely, iron is more active (toxic) than calcium, and calcium than sodium.

Much interesting work on the physiological activity varying with the valency has been done by my friend Mr. Mines, Fellow of Sydney Sussex College, Cambridge. Speaking of the H ion Mr. Mines writes:

"Concentration of H ions from .005 normal upwards, cause strong tonic contraction in skeletal muscle and a primary rise in electrical irritability, while the trivalent cations produce neither of these effects. On the other hand, the H ion shows striking resemblances in its action to that of the K ion. The

1. Harris, D. Fraser: *Bio-Chem. Journ.*, Vol. VI, 200 (1911).

relative concentrations of H and K needed to produce similar effects on frog's skeletal muscle are in the ratio of 1 to 5, i. e. inversely as their ionic velocities."

And again he writes:

"So far from it being possible to ascribe the physiological action of various ions to some one factor such as solution tension, valency or ionic velocity, it must be recognized that one and the same ion may exert its influence on different tissues by virtue of different characters or groups of characters. Further, two ions, which from the point of view of one tissue exhibit constellations of properties which are much alike, may present wholly dissimilar aspects towards another tissue."

Mr. Mines adopts the view that tissues are to be regarded as "emulsoid (hydrophile) colloids."

These and similar researches are of the utmost value in bringing us towards the biologist's great desideratum—greater definiteness of conception regarding the living matter itself.

Our present point of view is that not alone in terms of pure organic chemistry are conceptions of the constitution of protoplasm to be framed. We are finding we must include in these the non-organic, the non-vital substances whose presence does not indeed constitute life, but in whose absence life cannot be constituted. As we have had in the past full demonstration of the importance of the structurally "infinitely little," so at the present time we are having, each day, fresh demonstration of the importance of the chemically infinitely little.

Dalhousie University, Halifax, Nova Scotia.

April 6th, 1912.