On the Beneficial Action of Certain "Poisons"; and on the Influence of Poisons on Protoplasm and on Enzymes Respectively.

—By D. Fraser Harris, M. D., D. Sc., F. R. S. E., F. R. S. C., Professor of Physiology in Dalhousie University, Halifax, N. S.

(Read 22 February 1916)

It would no longer be in accordance with the teachings of physiology to regard such katabolites as CO_2, lactic acid or urea as poisonous or wholly deleterious in the animal body. Just as the heat evolved along with the motion in muscle is not waste or undesirable heat, as much of the heat in the steam-engine is, so the CO_2 evolved by tissue-katabolism is not under all circumstances a deleterious or noxious substance to be instantly eliminated. It may serve some good purpose on its way to be excreted. For, first of all, there is no doubt that it is one of the normal chemical stimuli to the activity of the respiratory centre. Normally CO_2 constitutes 5-6% of the volume of alveolar air. Any rise above normal in the CO_2 concentration in the outer air must retard the elimination of CO_2 from the alveoli; this causes CO_2 in alveolar air to increase and therefore to increase in the blood, and so produce hyperpnoea, thus:—

When CO_2 is 3% of the inspired air, there is acceleration of breathing, when it is 11% of the air, there is distinct hyperpnoea, when it amounts to 15% of the air, there are generalized convulsions, when CO_2 rises to 40% of the outer air, it acts as a direct narcotic to the central nervous system. The converse of all this is that the excessive elimination of CO_2 gives an apnoea, a chemical apnoea (Acapnia).

(2) In the second place, CO_2 causes maximal diastolic filling of the heart when it exists in the blood at 5-8%. This is

its optimum tension; for a rise to 12 to 20% causes diminished systolic output. Conversely, a considerable diminution of CO_2 produces tachycardia at the same time that it stimulates the vaso-constrictor centre. This naturally leads to an unsatisfactory condition of the circulation, for the rapid, weakening heart works badly against increased peripheral resistance. Over 8% of CO_2 in the blood stimulates the vasoconstrictor centre.

(3) It is now definitely known that excess of carbon dioxide in the blood dilates the coronary vessels. Barcroft and Dixon in 1906 wrote; "we have given reasons to show that the liberation of metabolic products from the heart, the chief of which is carbonic acid, controls the vaso-motor changes in the coronary arterioles." This is comparable with the dilator action of muscular katabolites on the arterioles of muscle demonstrated long ago by Gaskell.

(4) Both CO_2 and lactic acid hasten the rate at which arterial blood with low tensions of oxygen gives up its oxygen, that is, is reduced. When hydrogen or nitrogen is bubbled through arterial blood saturated with oxygen, the saturation falls from 100% to 80% at the end of twenty five minutes. but—

Blood containing

0.04%	lactic acid falls to about	68%	saturation	in	25 minutes
20.00%	CO_2 falls to about	60%	"	" "	"
0.09%	lactic acid falls to about	50%	"	" "	"
45.00%	CO_2 falls to about	25%	"	" "	"
0.20%	lactic acid falls to about	15%	"	" "	"
100.00%	CO_2 falls to about	7%	"	" "	"

Both CO_2 and lactic acid are, therefore, beneficial to the organism in that they accelerate the reduction of capillary blood. After vigorous exercise there may be 0.07% of lactic acid in the blood.

THE EFFECT OF CO_2 ON THE RATE OF OXIDATION AND OF THE REDUCTION OF BLOOD RESPECTIVELY.

At 37.5°C, the time taken by bubbling hydrogen or nitrogen to reduce oxygen-saturated blood to 50% saturation is twice as long as the time to oxidize blood from zero to 50% to saturation, no CO_2 being present in either case. The oxygen present in the oxidative process is 13.5% volumes, hydrogen being the rest. Barcroft has shown that the addition of CO_2 (six volumes %) to the hydrogen in the reduction process shortens the time (for reduction from full saturation to 50%) and makes it equal to the time for oxidation. The practical import of this is very clear; it means that the velocity of the uptake of oxygen by the pulmonary blood is just the same as that of its loss to the tissues. The rates of pulmonary oxidation and of tissue reduction of haemoglobin are the same, but only in presence of CO_2.

Another example of the beneficial action of a katabolite or poison is urea. Matthews says in his recent textbook: "it is found that the addition of a little urea to artificial perfusion solutions when one is perfusing the heart or other organs, is, as a rule, advantageous. This action is not so well marked in mammals as in teleostean and lasmobranch fishes."

In addition to all this, urea is itself a good diuretic, which circumstance is too obviously of advantage to the organism to merit further remark. Ammonia is a katabolite, but it is a valuable agent for warding off acid intoxication in the body. Certain amines, katabolites, are vasodilators and wash acid out of the tissues.

A *poison* may be defined in more than one way. Something that is not a food and not inert in the body, is one good description. A poison might be described as something which compromises or tends to compromise the vitality of the tissues; but unless we qualify the "something," we shall have to include such a thing as a rope tied round the neck producing

suffocation. Comprising the vitality of the tissues by *chemical* means, is inherent in the notion of a poison. Any chemical substance which interferes with the action of the heart or of the respiratory centre to such an extent as to imperil life is a poison. Unquestionably certain drugs are included under this heading. Here we are not thinking of such things as crude acid or alkali which has been swallowed and has destroyed the very tissues themselves; these indeed compromise life and are therefore poisons. Nor are we thinking of such a substance as strychnine which kills in a round-about way by asphyxia. It causes such prolonged inspiratory spasms of the diaphragm that breathing is interfered with and the due entrance of oxygen into the blood and thence to the tissues effectually prevented.

In a sense, strychnine kills as mechanically as does a rope tied round the neck; both ultimately prevent the access of oxygen to the living tissues. It is the narcotic poisons which present the typical problem to the physiological chemist.

Why and how are alcohol, chloroform, ether, morphia, cocaine, and hydrocyanic acid poisons? On what precisely do they act; and is that thing the same substance as that on which perchloride of mercury, for instance, exerts its baneful influence?

Do poisons act on protoplasm or on the products of protoplasmic activity, such as the enzymes? For it is practically certain that a poison must either compromise the proper functional activity of the biogens or living molecules, or it must interfere with the due action of some one or more of the enzymes possessed by or liberated by the protoplasm. Thus for convenience we speak of a protoplasmic poison which kills living protoplasm, and of an enzymic poison which prevents its fermentative activity.

Prof. A. D. Waller, F. R. S. in 1910 investigated the typical case of the poisonous alkaloid aconitine: he found that, whereas aconitine was distinctly poisonous for frog's muscle, it did not in the least restrain the activity of such an enzyme as ptyalin. A 0.01 Normal solution of aconitine after twelve hours' contract with ptyalin and starch was found to have exerted no restraining influence on the enzyme at all. It saccharified starch as rapidly as did an unpoisoned control. Now aconitine is a deadly poison, but to bioplasm itself, not to its product the secretion-enzyme, ptyalin. The relative toxicity of some poisons for frog's muscle is given by Waller (A), and along side it, I place a table of poisons for reductase (B).

A.		B.	
Aconitine	1000.00	Chloroform	5
Quinine	100.00	Ether, Morphine, Caffeine	3
Nicotine	33.00		
Theobromine	18.00		
Caffeine	12.00	Alcohol, Quinine	2.5
Chloroform	6.00		
Ether	0.72	Aconitine	1
Alcohol	0.06		

From these figures we see that whereas aconitine is the most deadly for protoplasm, it is actually the least injurious "poison" for the reducing, respiratory tissue-enzyme, reductase. Again, chloroform is low down as to toxicity in Waller's list; it is at the top of the poisons of reductase.

Once more, take the case of alcohol, a poison to living protoplasm. Protozoa dosed with alcohol are immobilized and die; cilia are killed in water containing alcohol. By the power it has to compromise the activity of the "vital" centres in the Medulla Oblongata, that is by interfering with the innervation of respiratory and cardiac muscle, alcohol in sufficiently high concentration is a poison; but alcohol does not interfere to the same serious extent with pepsin, a secretion enzyme.

No doubt very large draughts of alcohol do inhibit pepsin by precipitating it and so throwing it out of the sphere of chemical activity (solution); but we know quite well that both gastric and intestinal digestion can proceed in the presence of notable quantities of alcohol. Alcohol is a poison both to protoplasm and to its enzymic secretions, but it is more toxic to the former.

As Ehrlich long ago insisted, a poison can exert its influence only so long as it unites chemically with the molecules of the living protoplasm, the biogens; a substance that cannot unite, even temporarily, with the living stuff cannot be a poison; if it cannot get into relations with it, it cannot influence it.

Now it is abundantly clear that poisons do enter into union with the living stuff; chloroform continues to be eliminated by the breath for many hours after the anæsthetized person awakes. It is, of course, by its more or less firm union for the time being with the *living* heart-muscle that chloroform "acts" in high doses so profoundly as a depressant of the cardiac myoplasm which it immobilizes so that the fibres tend to die in diastolic atony.

The respiration of tissues is their chief "vital" characteristic, their taking in oxygen and giving out carbon dioxide—internal respiration— is of the essence chemically speaking of tissue-life.

In a recent research Dr. H. J. M. Creighton and I studied more particularly the inspiratory aspect of tissue respiration, namely that carried out by the reducing ferment of the tissues hitherto called "reductase."

The problem we put to ourselves was this,—Do the alkaloids and other deadly narcotic poisons, substances which kill animals in a very short time, act as inhibitants (poisons) of "reductase" to anything like the same extent? The answer in the negative was so unexpected that we tried to verify it in every possible way. Our method was as follows—the tissue juice from a cat's liver crushed in physiological (1cc) saline was mixed with a dilution of cat's blood in

water one in twenty-five. This—the control—was kept at 40°C and examined from time to time with the spectroscope to ascertain the exact moment at which the oxyhaemoglobin became reduced to haemoglobin. The other tube contained poisoned liver-juice and blood, the juice and the poison having been kept in contact for ten minutes before being added to the blood. Some of the deadliest protoplasmic poisons had very little retarding effect on hepatic reductase, in fact caffeine (the citrate), in 0.01 normal solution prolonged the time of reduction to 24.5 minutes, whereas hyoscine hydrobromide of the same strength only prolonged it to 6.5 minutes, the normal time for the control being 4.5 minutes. Some of our results are given in full in the table below;

TABLE I.

4.5 minutes required for reduction of normal mixture. Time in minutes required for the reduction of mixtures containing 1cc of liver juice, 2cc of blood solution and 1cc of an aqueous solution of the poison having a normal concentration of

Poison	0.1	0.02	0.01	0.001	0.0001	0.00001
Hyoscine hydrobromide	5.5	7.5	6.5	4.0	3.5	4.5
Cocaine hydrochloride	5.5	8.0	8.5	10.0	4.5	.....
Morphine sulphate	6.0	7.0	7.5	6.5	7.5	4.5
Atropine sulphate	8.0	6.0	6.5	....	.....	.....
Strychnine sulphate	destroys blood	8.0	6.0	6.5	6.5	.....
Quinine hydrochloride	.....	11.0	9.0	8.0	9.0	6.0
Caffeine citrate	destroys blood	25.0	24.5	12.5	10.0	0.9
Alcohol	10.0	....	10.0	....	9.0	.....
Ether	.....	5.0	....	5.0	.....	.....
Chloroform	.....	10.0	....	9.0	8.0	7.0

The general inference from these and many similar experiments was that, just as Waller had found aconitine had no influence on ptyalin, a secretion enzyme, we found that other deadly alkaloids had only a slight retarding action on the enzyme of internal respiration in the liver, hepatic reductase. An alkaloidal poison is, therefore, not deadly because it compromises tissue respiration, at least on its inspiratory side, as studied in the liver.

Realizing that the narcotic poisons act characteristically on the central nervous system, we next tried the effect of deadly narcotic poisons on brain-juice.

Six and a half minutes was the time required for cat brain-pulp to reduce the oxyhaemoglobin; the following table shows that aconitine only added 2.5 minutes, hyoscine 3.5 and morphine 6.5 minutes respectively.

TABLE II.

Time in minutes required for reduction of mixtures containing 1cc of brain juice, 2cc of blood solution and 1cc of an aqueous solution of the poison having a normal concentration of

Poison	0.1	0.05	0.001	0.00001
Hyoscine hydrobromide	9.5	....	.....	9.5
Morphine sulphate	13.0	....	.....	13.0
Aconitine	.....	....	9.0	8.5
Caffeine citrate	destroys blood	....	.....	13.0
Alcohol	.....	....	11.5	12.0
Ether	.....	....	13.5	13.5
Chloroform	.....	....	22.0	12.0

Alcohol, ether and chloroform were all much more inhibitory to the velocity of the tissue respiration of brain than were such lethal poisons as hyoscine, morphine and aconi-

tine. Our inference once again was that the deadly alkaloidal narcotics do not compromise life by interfering seriously with the inspiratory phase of tissue-respiration. But if this be admitted, then the deadly narcotics must be assumed to act on the living molecules of bioplasm, the biogens themselves. The deadly character of the narcotic alkaloids is exerted not on any substance in the outer sphere of influence of the bioplasm, not even on the endo-enzyme of tissue respiration, but on the very centre and citadel of life itself. Doubtless this is much as we should have expected *a priori*; and some critic may remark that it is not throwing much fresh light on protoplasmic poisons; but it at least tells us where the toxicity of alkaloids does *not* preeminently exert its power. This may not be much by itself, but it clears the ground for the next inquiry, namely:

What is it in the biogen for which the alkaloid has affinity, what is it precisely that is immobilized in fatal poisoning?

The poisonous action of alcohol on the lowliest forms of life is well brought out in a set of curious experiments by Professor Woodhead of the University of Cambridge. Using plate-cultures of the phosphorescent bacillus of Byerinck, he actually contrived to photograph the light produced by it in a twenty minutes' exposure. When 7 to 12 per cent. of alcohol was introduced into the culture, the light was abolished altogether; when 5% was introduced, the exposure required was 2.5 hours to obtain the same depth of result as was given by the unpoisoned bacilli in twenty minutes. Here we may say we have the toxic power exerted on the organism as a whole, for we may not in all cases of plants be able to discriminate between protoplasm and enzyme.

In certain animals which produce light, for instance the fire-fly, Photinus pyralis, the light production has actually been attributed to a ferment luciferase an oxidase carrying oxygen to a substance luciferin. It is claimed that these substances can be separated; each alone produces no light, whereas

the intraction of the two does so. It is therefore conceivable that the toxicity of alcohol against light-production might, in the case of animal bioplasm, be exerted on the enzyme and not on the biogens.

One of our general conclusions was thus stated; substances which are known to be rapidly fatal to animals and which depress the vitality of isolated tissues (muscles, nerves, etc.) that is, are deadly protoplasmic poisons, are not poisons in the same sense for reductase from liver or from brain. Another was; substances which are virulent poisons to protoplasm (e.g., aconitine, hyoscine hydrobromide) inhibit reductase no more effectively than substances very much less poisonous to protoplasm, namely, alcohol and caffeine citrate.

We were not prepared for these results; we had expected that such virulent poisons as aconitine, hyoscine and morphine would seriously interfere with tissue respiration. These alkaloids interfere with tissue respiration to a trifling extent compared with what they do as regards putting the vital machinery itself out of action. These deadly poisons compromise the activity neither of such separated substances as the secretion-enzymes, nor the non-separated endo-enzymes, but they do interfere in some way not at present understood with the inmost molecular activity of the biogens themselves. They put their enemy out of action, not by destroying his ammunition but by destroying the factories themselves. Yet the various narcotics differ *inter se* in regard to their toxicity both for neuroplasm and for neural reductase: thus chloroform is about 2.3 times as poisonous as hyoscine towards neural reductase.

Poisons of Bioplasm, of Endo-Enzymes and of Exo-Enzymes.

Certain poisons for bioplasm are poisons also for both its endo-enzymes and its exo-enzymes; such a substance is mercuric chloride. Its toxicity for the whole organism is

well known. Dr. Creighton and I found it fairly poisonous for hepatic reductase: when the normal liver (cat) needed ten minutes to reduce diluted blood, the addition of 0.01 normal $HgCl_2$ prolonged the time to 17 minutes. Waller and others have shown the high toxicity of $HgCl_2$ for ptyalin and for pepsin, both exo-enzymes. Mercuric chloride is therefore poisonous not only for the biogens, but also for their products, the enzymes which remain in the cells as well as for those which leave them.

Another poison HCN or KCN has been examined in regard to this threefold conception of the vital machinery. Its toxicity for the entire organism is notorious. Dr. Creighton and I found it very poisonous towards hepatic reductase; it added 24 minutes to the time (10 minutes) for the reduction of the blood by unpoisoned liver juice. Mr. J. B. Reed° found 1% KCN destroyed all the oxidase (for indo-phenol) in vegetable cells; he further found that the ventral nerve-cord of crawfish after treatment with KCN was quite unable to exhibit any oxidizing power. KCN also prevented any oxidase activity on the part of the leycocytes of human blood. But KCN, except in very high concentration, does not affect the activity of pepsin. KCN is therefore highly toxic towards living matter and towards both the reducing and oxidizing endo-enzymes, but *not* towards secretion-enzymes.

Another substance poisonous for protoplasm and endo-enzyme, but not for secretion-enzyme is arsenious acid (As_2O_3). It is highly toxic for naked protoplasm. Dr. Creighton and I found it added 23 minutes to the time (10 minutes) for hepatic reductase to reduce blood. It was used in 0.01 normal concentration. But against pepsin it has little power except in very high concentration. Ether for instance, does not inhibit the action of lipase.

°The role of oxidases in respiration. J. B. Reed, Jl. Biol. Chem. Aug. 1915.

The following description of quinine not written at all from our present point of view shows that it belongs to the last mentioned group of poisons[1].

"Quinine is frequently called a protoplasm poison because of its action on undifferentiated protoplasm. In small amounts it stimulates movement in Infusoria, but in larger amount paralyzes these minute organisms immediately. The alkaloid also retards the action of some unorganized ferments, especially that of the oxidases."

It appears, then, that all substances which we may call "poisons" do not act in exactly the same manner on the same constituent of the vital mechanism. One might attempt a classification of poisons somewhat as follows:—

1. Substances which act by destroying the histological integrity of the tissues altogether: strong acids, alkalies and nascent elements, as Cl in the "gassing" by Germans. The lesions may be so extensive that death results from shock or from internal haemorrhage.

2. Substances which replace O_2 in air breathed or prevent O_2 gaining access to the Haemoglobin such are CO, CO_2, and CH_4. They kill by asphyxia as truly as if the person had been suffocated or strangled.

3. Substances which cause either spasm of the muscles of respiration or of the diaphragm or paralysis of them, so that the inspirations are suspended, no O_2 reaches the tissues which are asphyxiated, e. g., strychnine and allied alkaloids; also curare which immobilizes muscles by making them inaccessible to nerve impulses.

4. Substances which immobilize the biogens of the neuroplasm of the central nervous system, so that consciousness is ultimately abolished and the respiratory centre paralyzed. All the narcotics proper fall into this group. The vegetable alkaloids are the typical members of this group inasmuch

[1] The Plant alkaloids. Henry. J. and A. Churchill, 1913, p. 171.

as they act specifically on the neuroplasm, but not nearly so directly on the enzymes of tissue-respiration.

5. Substances which both immobilize the biogens of neuroplasm and *also* retard the velocity of action of one or other of the enzymes of internal respiration, namely reductase and oxidase, but do not destroy secretion-enzymes.

Examples of these are chloroform, ether, alcohol, quinine, KCN, As_2O_3.

6. Substances which act injuriously on all the three, the biogens, the endo-enzymes, and the secretion-enzymes. Examples:—Mercuric chloride, silver nitrate, and all salts of heavy metals when in sufficient concentration. Thus Uranium salts as weak as 0.0001% retard ptyalin, and in solution no stronger than 0.008% completely inhibit it.