LXXVIII. ADSORPTION AND MECHANISM OF POISONING.

PART I. IRRITANT POISONS.

By TRILOK NATH SETH.

Chemical Laboratories, Benares Hindu University, India.

(Received May 8th, 1923.)

IN order that we may evaluate the effect produced upon life functions by the substances artificially ingested into the organism it is necessary, according to our present ideas, to postulate that the substances enter into some sort of a combination with the constituents of the cells, which in turn transmit the effect to the whole organism. It is evident that such an interaction depends not only upon the structure of the ingested reagent, but quite as much upon the structure of the cell-substances. From the chemical standpoint great difficulties arise in an attempt to study the effect of poisons directly upon higher organisms, because of the numerous disturbing influences, such as physiological activities and localisation of poisons in particular regions of the animal system, etc. Therefore, an attempt has been made in the present work to simplify matters as far as possible by choosing comparatively simple substances for purposes of experimentation. Of course the results thus obtained are by no means absolute, yet they throw some light on this difficult problem and they may possibly indicate the probable trend of reactions in higher organisms, under a similar set of conditions.

Moore and Roaf [1904, 1906] have shown that chloroform and other anaesthetics combine with protoplasm to form unstable compounds and they presume that the formation of such compounds is the cause of intoxication. The close relationship between intoxication and poisoning can justify our holding the above view with regard to the mechanism of poisoning also. Again, Loew [1893] assumes a direct chemical union of the protoplasm with the so-called "substituting poisons," supposing the labile amino and aldehyde groups of the protoplasm to be active in effecting such a union. Such an explanation seems to be, at least in some instances, quite plausible. For example, hydroxylamine and the hydrazines, which are well-known aldehyde-reagents, are powerful poisons, while the ketoximes in which the reaction group is bound, are not so poisonous. Among others, the work of P. Karrer [1918] on the action of toxins, may be specially mentioned, as supporting Loew's theory of chemical reaction between poisons and the affected tissues. However, in spite of the support lent to this theory by numerous workers, it has failed to explain

satisfactorily the mechanism of poisoning in a very large number of cases in which it has been established on fairly firm grounds that no chemical action takes place, and that the effects produced are due to surface action only. Ehrlich, for example, maintains that during the course of his numerous experiments in this direction, he was never able to establish that there had been a chemical union between the poison and the affected part of the body. In fact, he found that the various poisons could be again extracted from the tissues by means of neutral chemically inactive solvents. However, Ehrlich [1909] himself mentions a number of toxins and poisons, *e.g.* cocaine, stovaine, and compounds of trivalent arsenic, etc., as being exceptions to his generalisations.

Thus it appears that at the present stage of our knowledge we cannot accept either Loew's or Ehrlich's theory to the entire exclusion of the other. On the other hand, we must accept both, for it appears that there are cases of simple chemical union, simple surface action, and cases involving both surface action and chemical action.

Howsoever this conflict of opinions regarding the mechanism of actions may be decided, at least it is certain that the production of an effect at any desired place in the organism necessitates such a constitution of the ingested substance as to make possible its local fixation, whether it be of a chemical or of a physical nature. In other words, "sorption" [McBain, 1919] of a poison by the tissues is necessary for the production of any physiological effects. The old notion that a poison may kill by its action through the nervous system without sorption, cannot be retained in the light of our present knowledge, and therefore a study of the sorption of poisons by the principal constituents of the body, is bound to be of some interest in the elucidation of the mechanism of poisoning.

Poisons belonging to the irritant type have been dealt with in the present work, and investigations on the other groups are in progress. By far the most important of the irritant poisons are salts of arsenic, lead, copper, antimony, and chromium, and these substances are characterised by their irritant effects upon the tissues, accompanied by well-marked nervous influences.

Apart from the chemical nature of the inorganic poisons the physical factors concerned in their solution, etc., are of great importance. The part played by such factors can be easily realised when it is considered that the specific effects of such poisons depend chiefly, if not exclusively, upon their constituting ions [Blake, 1839, 1881]. Quite in agreement with the assumption of ionic effect in these physiological studies, is the great importance of the degree of dissociation, which is very decidedly pointed out by the investigations of Dreser [1893] and Paul and Krönig [1896] upon the bactericidal action of mercury salts. A further support of this theory is provided by the comparative inactivity of cacodyl compounds as poisons (Crum Brown), in spite of their arsenic content and marked solubility. The inertness of such compounds is easily explained by the fact that the arsenic in them is bound up to an organic radical and is not present in the ionic state even in water.

Results obtained by Kahlenberg [1900] and Loeb [1897, 1898], during their investigations on the action of salts on bacteria, are also in general agreement with the ionic theory of poisoning.

On the basis of the view that sorption precedes poisoning and that poisoning, at least in the case of most inorganic salts, is ionic in nature, the present work was carried out with the object of determining the sorption of the various poisons in the different regions of the animal system with a view to elucidate the mechanism of poisoning.

EXPERIMENTAL.

Caseinogen, representing the class of protein substances which form the chief constituents of the animal body, was chosen as the adsorbent in the course of the present work, and a number of experiments were also performed with saliva, bile, and blood-serum. The poisons tried were lead acetate, arsenious oxide, and copper sulphate.

The general procedure followed in all the experiments is outlined below.

Aqueous solutions of each poison in six different concentrations were prepared. Equal volumes (50 cc.) of each of these solutions were pipetted out into dry stoppered bottles and then a weighed amount (1.0 g.) of caseinogen (the size of the particles being between 16–20 mesh) was introduced into each bottle. After thorough mixing the bottles were kept in a thermostat at 37° (body temperature) for a given time, after which the contents of each bottle were filtered separately through filter papers which were previously soaked with the particular poison in question, washed and dried. The amount of the poison left after sorption, was determined analytically in the filtrate and knowing the initial concentration of the poison used, the sorption of each poison per gram of the adsorbent (X/M) was calculated. The concentration of the poison in 1 cc. of each solution after sorption (volume concentration) was also determined and these results were plotted against the corresponding X/M . The results obtained with each poison are given below.

Lead Acetate.

Aqueous solutions of pure lead acetate of concentrations approximating to $N/10$, $N/15$, $N/20$, $N/30$, $N/50$ and $N/100$ were prepared and stocked after filtration. 50 cc. of the poison of each concentration was used for each experiment and the extent of sorption was determined after one, three and six hours. Lead was estimated gravimetrically as lead chromate and the results, calculated in terms of $\text{Pb}(\text{CH}_3\text{COO})_2$, are given in Table I.

The curves drawn from the data are shown in Fig. 1. The shape of these curves indicates clearly that they are typical "Adsorption Isotherms." Further support is lent to this view by the fact that logarithms of X/M and volume concentration when plotted, produce a straight line which is so characteristic of adsorption reactions.

Table I.

Concentration of original solution (in terms of $\text{Pb}(\text{CH}_3\text{COO})_2$ per litre)	After sorption for one hour		After sorption for three hours		After sorption for six hours	
	X/M	Volume concentration	X/M	Volume concentration	X/M	Volume concentration
15.916 g.	0.1105	0.01370	0.1464	0.01299	0.1482	0.01295
10.486	0.0824	0.00884	0.1196	0.00809	0.1263	0.00796
7.834	0.0711	0.00641	0.1121	0.00559	0.1225	0.00538
5.354	0.0694	0.00396	0.0966	0.00342	0.1121	0.00311
3.124	0.0448	0.00223	0.0596	0.00193	0.0655	0.00181
1.443	0.0242	0.00106	0.0376	0.00079	0.0351	0.00084

Fig. 1.

Arsenious Oxide.

Aqueous solutions of pure arsenious oxide of concentrations approximating to $N/15$, $N/20$, $N/30$, $N/50$ and $N/100$ were employed and the amount of arsenic in them was determined before and after adsorption, by titration against a standard iodine solution, in the presence of excess of sodium bicarbonate, using starch as an indicator. All other conditions of temperature, time, and procedure were the same as described before (p. 615). The results calculated, in terms of As_2O_3 are given in Table II.

Table II.

Concentration of original solution (in terms of As_2O_3 per litre)	After sorption for one hour		After sorption for three hours		After sorption for six hours	
	Volume		Volume		Volume	
	X/M	concentration	X/M	concentration	X/M	concentration
3.30 g.	0.00190	0.00326	0.00220	0.00325	0.00128	0.00327
2.477	0.00107	0.00245	0.00107	0.00245	0.00092	0.00246
1.65	0.00072	0.00163	0.00072	0.00163	0.00048	0.00164
0.9895	0.00027	0.00098	0.00027	0.00098	0.00002	0.00099
0.4949	0.00022	0.00049	0.00022	0.00049	0.00012	0.00049

The nature of the equilibrium after one and three hours is distinctly different from the usual adsorption equilibrium, and what is more striking, the equilibrium conditions in the last four concentrations appear to be the same for the first three hours, after which there is a marked change, whereby the nett results of sorption after six hours, are sufficiently influenced to be thrown into the form of an "Adsorption Isotherm."

Relation between Adsorption and the Degree of Dissociation of a Poison.

It has been clearly brought out by the investigations of Dreser, and of Paul and Krönig, that substances which are dissociated to a greater extent are more powerful disinfectants and antiseptics than others which are dissociated to a lesser degree. This and other similar observations led the author to investigate the relation between adsorption and the degree of dissociation of a poison in solution. With this end in view the equivalent conductivities of the poisons at various concentrations were determined at 37° , and these results were plotted against X/M of the corresponding solutions. The curves so obtained are shown in Fig. 2. It is evident that the curves for lead acetate and arsenious oxide are both hyperbolic, and that for each concentration of the poison

$$A(C - P) = \text{Constant},$$

where $A = X/M$,

C = Equivalent conductivity at 37° .

P = a constant depending upon the nature of the poison.

It also appears that poisoning is a function of the adsorption and the degree of dissociation of any particular poison. It is further clear from Fig. 2 that with increasing concentrations of the poison the adsorption (per gram of the adsorbent) increases, while the equivalent conductivity of the corresponding solution decreases and consequently the enhanced poisonous effect due to greater adsorption of the poison is partly neutralised by the lowering of the dissociation. These facts clearly point out that there is a particular concentration at which the combined poisonous effect due to adsorption and dissociation is the maximum.

Work in this direction is in progress and the results are expected to show that merely the quantity of a poison, independent of its concentration, is not a sufficient indication of the minimum fatal dose of that poison. It is expected that there will be one particular concentration for each poison at which the

lethal dose will be most effective. On the basis of such results it may also be possible to establish certain mathematical relationships between adsorption, concentration and the lethal dose of a poison.

Copper Sulphate.

Aqueous solutions of pure Copper sulphate, of concentrations approximating to $N/1$, $N/5$, $N/10$ and $N/100$ were employed and 40 cc. of the solution of each concentration were used for each experiment. The general procedure adopted during the course of these experiments was the same as described before (p. 615), with the exception that the experiments were conducted at 28° and the extent of sorption was determined after one-fourth, one and three hours. Copper sulphate was estimated volumetrically by determining the amount of iodine liberated by it from a solution of potassium iodide. A standard solution of sodium thiosulphate was used for estimating the iodine thus liberated.

Fig. 2.

The results of the sorption of copper sulphate by caseinogen, calculated in terms of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ are given in Table III.

Table III.

Concentration of original solution (in terms of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per litre)	After sorption for one-fourth hour		After sorption for one hour		After sorption for three hours	
	X/M	Volume concentration	X/M	Volume concentration	X/M	Volume concentration
122.7 g.	0.02873	0.1220	0.04357	0.1216	0.04696	0.1215
24.82	0.02572	0.0242	0.03803	0.0239	0.04473	0.0237
12.26	0.01823	0.0118	0.03202	0.0115	0.03702	0.0113
1.207	0.00549	0.0011	0.00859	0.0010	0.00875	0.0010

If curves are drawn from the above data they are seen to be typical "Adsorption Isotherms."

Copper Sulphate and Saliva.

A set of sorption experiments was conducted with approximately $N/1$, $N/5$, $N/10$ and $N/100$ solutions of copper sulphate, using human saliva as the sorbent. A few hours after meals, the salivary glands were excited by chewing some black pepper, and after thorough rinsing of the mouth with water, saliva was collected in a clean beaker. 5 cc. of this saliva were used for each experiment. All other conditions of experiment were the same as described before (p. 615). The extent of sorption of copper sulphate was determined after one-fourth, one, and twenty-four hours. The results, calculated in terms of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, sorbed per gram of saliva, are given in Table IV.

Table IV.

Concentration of original solution (in terms of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per litre)	After sorption for one-fourth hour		After sorption for one hour		After sorption for twenty-four hours	
	X/M	Volume concentration	X/M	Volume concentration	X/M	Volume concentration
123.492 g.	0.00505	0.12287	0.00505	0.12287	0.00505	0.12287
24.81	0.00493	0.02420	0.00493	0.02420	0.00523	0.02417
12.26	0.00395	0.01177	0.00417	0.01174	0.00417	0.01174
1.207	0.00017	0.00119	0.00088	0.00110	0.00088	0.00110

By plotting the data given in Table IV, typical adsorption curves are produced, even in the case of saliva.

While experimenting with saliva it was observed that a precipitate was formed immediately on adding saliva to a solution of copper sulphate. Later it was found that similar observations had been recorded before in the case of other albuminous substances (*Practical Biological Chemistry*, by G. Bertrand and P. Thomas). However, it was considered worth while to study the nature of the precipitates thus obtained in order to determine their composition and the probable mechanism of their formation. With this end in view, equal amounts of saliva were added to equal volumes of copper sulphate solutions of various concentrations, and the copper-content of the precipitates thus formed, was determined after one-fourth hour and twenty-four hours. These results show that the copper-content of these precipitates is almost the same after fifteen minutes of sorption as it is after contact with the copper sulphate solution for twenty-four hours.

The facts that a precipitate is formed immediately on adding saliva to a solution of copper sulphate and that the maximum sorption takes place almost instantaneously, point to the existence of some close union between saliva and copper sulphate. Again, the amount of copper sulphate adsorbed per gram of saliva was found to vary with the concentration of the copper sulphate solution used, indicating thereby the absence of a purely chemical combination between the two substances. The only other alternative is to assume the formation of certain chemico-adsorption-compounds by the interaction of copper sulphate solution and saliva. This view is further supported

by a fact which has been clearly brought out in this work, namely, that the principal reactions between aqueous copper sulphate and saliva bear a close analogy to the phenomenon of adsorption.

Sorption of Copper Sulphate by Blood-serum and Bile.

In one set of experiments serum prepared from fresh goat's blood was used as a sorbent for copper sulphate and another set of experiments was conducted in which fresh goat's bile was employed as the sorbent. Approximately $N/1$, $N/5$, $N/10$ and $N/30$ solutions of copper sulphate were used in both the cases. 5 cc. of blood-serum or bile were employed for each experiment. 40 cc. of copper sulphate solution of each concentration were used with blood-serum, while in the case of all experiments with bile, only 20 cc. of the poison were employed each time. All other conditions of experiment were identical with those described before (p. 615). Some of the results obtained in the case of blood-serum are given in Table V.

Table V.

Concentration of original solution (in terms of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per litre)	After sorption for one-fourth hour		After sorption for twenty-four hours	
	X/M	Volume concentration	X/M	Volume concentration
124.815 g.	0.00447	0.12425	0.00594	0.12406
24.963	0.00287	0.02460	0.00402	0.02445
12.4815	0.00173	0.01226	0.00411	0.01198
4.1605	0.00039	0.00367	0.00062	0.00337

Results very similar in nature to those given in Table V were obtained in the case of bile also. Curves plotted from these data reveal the fact that bile and blood-serum behave exactly like caseinogen and saliva in the matter of adsorbing copper sulphate from its aqueous solutions.

It may be mentioned here that the phenomenon of the formation of chemico-adsorption-compounds was observed in the case of blood-serum also—a fact which was verified in a manner similar to the one employed in the case of saliva.

CONCLUSIONS.

The results of this investigation show that adsorption is the most predominant feature in the interaction between poisons like lead acetate, arsenious oxide and copper sulphate and substances of the type of caseinogen and blood-serum, etc. In a number of cases, the formation of chemico-adsorption-compounds has also been pointed out. These conclusions naturally lead one to think that the more complex phenomenon of poisoning in the living animal is also probably governed, at least in its initial stages, by laws essentially similar to the ones that guide and control the ordinary laboratory reactions of the type indicated in this paper. If the results indicated here represent, even in some degree, the trend of action of such poisons in the living organism, it is clear that adsorption forms the first step in the very complicated process of poisoning due to irritant poisons. It cannot be said at this stage whether all other

poisons act in a manner similar to the one in which irritant poisons appear to behave. Further work is necessary in this direction and it is hoped that the extended investigation may throw some more light on this obscure yet extremely interesting phenomenon of the mechanism of poisoning.

My best thanks are due to Dr S. S. Bhatnagar, for his suggestion of the problem and for the keen interest he has taken in it throughout.

REFERENCES.

- Blake (1839). *Compt. Rend. Acad. Sci.*
— (1881). *Ber. deutsch. chem. Ges.* **14**, 394.
Dreser (1893). *Arch. exp. Path. Pharm.* 456.
Ehrlich (1909). *Ber. deutsch. chem. Ges.* **42**, 17.
Kahlenberg (1900). *J. Phys. Chem.* **4**, 553.
Karrer (1918). *Chem. Zeitung.* **42**, 521.
Paul and Krönig (1896). *Z. physikal. Chem.* **21**, 414.
Loeb (1897). *Pflüger's Arch.* **69**, 1.
— (1898). *Pflüger's Arch.* **71**, 457.
Loew (1893). *Ein natürliches System der Giftwirkungen*, Stuttgart.
McBain (1919). *Trans. Faraday Soc.* 202.
Moore and Roaf (1904). *Proc. Roy. Soc. London*, **73**, 382.
— — (1906). *Proc. Roy. Soc. London*, B, **77**, 86.