Experimental Argyrosis: II. Treatment of Rats Receiving Silver With 2,3-Dimercaptopropanol (BAL)

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In a previous communication (*3*) it has been reported that silver pigment is deposited in the experimental rat in a way essentially similar to that in which it is deposited in man. In view of the efficacy of 2,3-dimercaptopropanol (BAL) in the treatment of poisoning with arsenic (*1, 4*) and mercury (*2*), it appeared desirable to study the effect of this agent on the experimental argyrosis of the rat.

The experiment was carried out on four rats, grouped in pairs and placed on a diet of dog pellets. The first pair received a solution of 1:1,000 silver nitrate in place of drinking water for a period of 456 days. During this time a total amount of 23.2 grams of silver nitrate was consumed by the two animals, or an average of 11.6 grams each. On the 457th day the silver nitrate solution was discontinued and replaced by water. At this time the eyes of both rats were distinctly pigmented, one slightly more so than the other. Eighteen days later the more deeply stained rat was started on treatment with intramuscular BAL. The BAL was given in a 1:50 dilution in cottonseed oil. A total of nine injections was given on alternate days, covering a period of 18 days. Each single dose was 0.2 mM/kg. (10 times the minimal effective dose for the treatment of acute arsenic poisoning in the cat). The other rat was maintained as a control. The treated animal showed a weight loss of 30 grams over the period of therapy, but otherwise appeared healthy. The control animal lost 6 grams. Both animals were sacrificed on the 21st day, at which time the eyes of the treated rat were still darker than those of the control.

On histological examination, the eyes, thyroid, liver, pancreas, spleen, and kidneys contained an apparently identical amount of silver deposit. No lesions were found in either rat.

The second pair of rats received for a period of 514 days a 1:1,000 solution of silver chloride with added sodium thiosulfate (approximately 1:300) in place of drinking water. The total average silver chloride intake for each of the two animals was 12.9 grams. On the 515th day the silver chloride solution was replaced by drinking water. The eyes of the pair were also distinctly pigmented. Eighteen days later the more deeply stained rat was started on BAL therapy. The dose and manner of administration was as described above, with the exception that a total of 18 injections of BAL was given, covering a period of 38 days. The cage mate was kept as a control. During the period of treatment the treated animal lost 25 grams in weight, but otherwise appeared healthy; the control showed a 3-gram gain. On the 42nd day both were sacrificed, at which time the eyes of the treated rat still appeared darker than those of the control.

On histological examination, there were apparently identical amounts of silver deposits in the thyroid, kidneys, eyes, and choroid plexus of each rat. There were no lesions indicating any toxic effect in either rat.

It appears from these observations on a limited number of animals that BAL is incapable of mobilizing silver, which is deposited in the tissues as metallic silver or silver oxide. It seems likely, therefore, that BAL should prove of little or no value in the treatment of argyria in man. It is interesting to note the marked resistance of the rat to poisoning by BAL. No acute symptoms resulted from the injection of 0.2 mM/kg., and there were no chronic effects from the repeated administration of this quantity of BAL.

References

1. EAGLE, H., MAGNUSON, H. J., and FLEISCHMAN, R. The systemic treatment of experimental arsenic poisoning with 2,3-dimercaptopropanol (BAL). (To be published.)
2. LONGCOPE, W. T., and LUETSCHER, J. A., JR. *J. clin. Invest.*, in press.
3. OLCOTT, C. T. *Experimental argyrosis.* New York: New York Pathological Society, 1944.
4. RIKER, W. F. *J. Pharm. exp. Therap.*, 1946, **87**, 66.

The Movement of Substances Through a Two-phased Solution System[1]

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A thermodynamically sound treatment is essential to an understanding of the movement of substances through solutions. Manifestations of flux are common in aqueous biological systems. These phenomena are logically expressed on the basis of the concept of escaping tendency or free energy of a constituent component. Certain fundamentals of the process are presented in the hope that the free-energy concept, as it applies to this movement, will be adopted generally by biologists. The tendencies for movement of water will be discussed first, followed by that of a solute in an aqueous solution system.

WATER MOVEMENT

Pure water possesses an internal energy representing the sum of its internal kinetic and potential energies. At thermodynamic equilibrium, a steady state, the free energy of the water is equal throughout the phase. The free energy or escaping tendency of the water molecules may be modified by the application of certain chemical or physical influences. The addition of a solute to water lowers the free energy of the solvent in the resultant solution. The application of a pressure to water increases its free energy. At thermodynamic equilibrium within a solution consisting of two components,

[1] Detailed treatises will be printed in *Botanical Review* (1947), comprehending the movement of water and of a solute. In these, references are made to earlier publications pertinent to the subject.

Symbols used are defined as follows: p is an applied pressure, in a given state of the system, necessary to make $\bar{f}$ equal to $\bar{f}^{o}$; p^{o} is an applied pressure, in a reference or standard state of the system; $\bar{f}$ is the partial molal free energy of a constituent component of a solution in a given state; $\bar{f}^{o}$ is the partial molal free energy of a constituent component of a solution in a reference or standard state—here, at infinite dilution; $\bar{v}^{o}$ is the partial molal volume of a constituent component of a solution in a reference or standard state—here, at infinite dilution; F is a specific (volumed) free energy of a constituent component of a solution in a given state; subscripts i and e refer to the separate phases of a two-phased solution system—here, internal and external, respectively; and subscripts (Δf) or ($-\Delta f$) refer to influences in or on a system, which increase or decrease, respectively, the free energy of a constituent component of a solution from that in a reference or standard state to that in a given state.

solvent water and a single solute, the free energy of each component is equal throughout the phase. If each component, the water or the constituent solute, is not at thermodynamic equilibrium throughout the phase, it will work itself through the solution toward equality of escaping tendency, in accordance with the difference in free energy. Here, this process is called a flux, the term diffusion being employed where migration is restricted to a response to a difference of concentration or activity of a constituent component in solution within a system.

In practice, the free energies themselves are not measured, but the *difference* in free energy of the component between that in the given state and that in a reference or standard state is determined. Gauging the pressure difference which would be necessary to adjust the free energy of the water from that in the given state to that in a reference state serves as a measure of the free-energy difference under the two conditions. In dilute solutions, where the free-energy difference of the water is due only to the presence of solute in the solution, these pressures and free energies are equated approximately through the relation,

$$p - p^0 = \frac{-(\bar{f} - \bar{f}^0)}{v^0} = F. \qquad (1)$$

Where pressure is imposed on the medium, as a single factor contributing to the free-energy difference of the water, the equation takes the form,

$$-(p - p^0) = \frac{(\bar{f} - \bar{f}^0)}{v^0} = F. \qquad (2)$$

The only difference between Equations 1 and 2 is that of algebraic sign. The net effect of those influences which decrease the free energy of water and those which increase the free energy in a single phase may be expressed by the equation,

$$\Sigma F_{(-\Delta f)} - \Sigma F_{(\Delta f)} = \frac{-(\bar{f} - \bar{f}^0)}{\bar{v}^0}. \qquad (3)$$

The pressure which must be applied to make the free energy of water in a given state equal to that in a reference state may be conveniently observed by a two-phased system in which a semipermeable membrane, permeable to the water, is interposed. The difference in pressure which must be applied to the two phases to bring about thermodynamic equilibrium for water across the interposed membrane is a measure of the free-energy difference of the water between the two phases. The specific (volumed) free energy (F), numerically equal to the partial molal free-energy difference divided by the partial molal volume of the water, is likewise a measure of the free-energy difference. Moreover, it expresses the thermodynamic viewpoint of the escaping tendency of the water from one phase to the other in physically measurable dimensions, namely, pressures. These may be converted to dimensions of energy by means of a coefficient, namely, the partial molal volume of the constituent component at infinite dilution of the solution.

It is of interest to know whether water will tend to migrate from one phase (external) to the other (internal) due to a possible difference in free energy of the water, schematically represented by the formula,

$$H_2O \text{ (external)} \xrightarrow{\text{influx}} H_2O \text{ (internal)}. \qquad (4)$$

The measure of the tendency for water to move is given by the difference between the free energy of the water in the internal phase and that in the external phase of the osmoscope, namely, $(\bar{f}_i - \bar{f}_e)$. If this difference is negative in sign, water will tend to migrate inward, as written in the previously cited formula (4). If the quantities $(\bar{f}_i - \bar{f}^0)$ and $(\bar{f}_e - \bar{f}^0)$ are determined, then the difference between these values is $(\bar{f}_i - \bar{f}_e)$.

For a single phase, the net effect of those influences which decrease the free energy of water and those which increase the free energy was given by the relation,

$$\Sigma F_{(-\Delta f)} - \Sigma F_{(\Delta f)} = \frac{-(\bar{f} - \bar{f}^0)}{\bar{v}^0}. \qquad (3)$$

Then, for the two phases, the net influx specific free energy (NIF) is expressed by the equation,

$$(\Sigma F_{(-\Delta f)} - \Sigma F_{(\Delta f)})_i - (\Sigma F_{(-\Delta f)} - \Sigma F_{(\Delta f)})_e = \frac{-(\bar{f}_i - \bar{f}_e)}{\bar{v}^0} = NIF. \qquad (5)$$

Instead of expressing the specific free energies in groups relating to the two phases, i and e, respectively, they may be rearranged into categories representing the sums of the specific free energies tending to cause the water to move inward (influx, IF) and outward (efflux, EF) across the membrane. The net influx specific free-energy equation for water then becomes

$$NIF = \Sigma IF - \Sigma EF \qquad (6)$$

or, in more detailed form,

$$NIF = (\Sigma F_{(-\Delta f)i} + \Sigma F_{(\Delta f)e}) - (\Sigma F_{(-\Delta f)e} + \Sigma F_{(\Delta f)i}). \qquad (7)$$

The latter mode of expression has certain advantages when used in connection with graphic presentations of the specific free-energy and volume relations of an osmometer.

Solute Movement

By similar reasoning it may be shown that the fundamental principles of the tendencies for movement of solute are analogous to those discussed for water. The only differences in application of the general formulas relate specifically to the influence of the solute concentration of a solution on the specific free energy of a component. In the discussion of water movement the specific free energies referred to the solvent component. There, an *increase* in concentration of solute in a solution is accompanied by a corresponding *decrease* of the partial molal free energy of the water within the phase. For solute migration, the specific free energies refer to a constituent solute species in solution. In contrast, therefore, an *increase* in concentration of solute in a solution is accompanied by a corresponding *increase* of the partial molal free energy of the solute within the phase. In other words, the effect of a solute in solution is included here, in $F_{(\Delta f)}$ rather than in

$F_{(-\Delta f)}$ of Equations 3, 5, and 7. In this case, the symbol $\bar{v}^o$ refers to the partial molal volume of a constituent solute in solution.

SUMMARY

The tendencies for movement of either solvent or solute in solution through a two-phased system are expressed in terms of specific (volumed) free energies. These are based on the concept of escaping tendency or free energy of a constituent component of a solution. This scheme is particularly useful to the biologist for evaluating the movement of water and solutes into cells or organs.

The Effect of Streptomycin on the Oxygen Uptake of *Eberthella typhosa*[1,2]

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During the course of investigations into the mode of action of streptomycin, it was found that the oxygen uptake of suspensions of washed cells of *Eberthella typhosa* was increased in the presence of streptomycin.

In 1934, Clowes and Krall (*2*) noted that dinitro compounds increased the oxygen uptake of sea urchin eggs, while apparently inhibiting cell division. In 1937, Clifton (*1*) found other substances which appeared to produce similar results in suspensions of bacteria and yeasts. Clifton took the view that such substances, which increased oxygen uptake and also interfered with certain cell functions, exerted their effects by inhibiting normal synthesis of cell components from the available substrate. In addition, he suggested that these substances might also favor oxidation of materials which had already been stored by the cell.

In this investigation oxygen uptake and respiratory quotients were measured in the Barcroft-Warburg apparatus. The phenomena which were observed resembled, at least superficially, those noted by Clowes and Krall and by Clifton. While the Hopkins strain of *E. typhosa* was used throughout most of this work, preliminary studies indicate that the results apply in general to other strains of *E. typhosa*, as well as to some other species of bacteria. With suspensions of the density used in this work it was found that streptomycin in a concentration greater than 500 units/ml. produced a change in oxygen uptake which was readily measurable and reproducible. Since the dry weight of bacteria per milliliter of suspension was approximately 3 mg., such a concentration of streptomycin is not unduly high.

Streptomycin, sufficient to make a final concentration of 1,000 units/ml., was added to a system in which endogenous respiration was proceeding at 37°C. in a phosphate buffer of pH 7.40. The result was an immediate and rather marked increase in the rate of oxygen uptake. After two hours had passed, the rate of uptake decreased until at six hours it was less than that of the controls, in which the rate of uptake remained constant for many hours.

The addition of glucose in a concentration of 0.01 per cent to a similar system also produced, of course, an increased rate of oxygen uptake. By the time that sufficient oxygen had been taken up to account for oxidation of about 65 per cent of the glucose as well as for endogenous oxidation, the rate of uptake had dropped to that of the controls. Further oxygen uptake occurred at this rate for many hours.

During the same time, when streptomycin in a concentration of 1,000 units/ml. was present as well as the glucose, sufficient oxygen was taken up to account for complete substrate oxidation as well as for oxidation due to the presence of a similar concentration of streptomycin in the system, which contained no available substrate. After this had occurred, oxygen uptake continued at a decreasing rate. When six hours had passed, the rate of uptake was less than that of the controls.

The evidence so far obtained indicates that the increase in oxygen uptake in the presence of streptomycin is not explainable on the grounds that the bacteria are bringing about the oxidation of the streptomycin or of impurities in it. Respiratory quotients are apparently increased 25 per cent by the addition of streptomycin to the previously described system, in which glucose is undergoing oxidation. In general, similar effects occur where other simple substrates are oxidized in the presence of streptomycin by the Hopkins strain of *E. typhosa*.

The effect of streptomycin on the oxygen uptake of a streptomycin-resistant variant of the Hopkins strain is of particular interest. This variant was produced by passing a subculture of the Hopkins strain through peptone water and increasing concentrations of streptomycin until it grew well in the presence of 1,100 units of streptomycin/ml. of peptone water. When glucose and streptomycin, the latter in a concentration of 500 units/ml., were both added to a suspension of the variant, the oxygen uptake was actually less than when glucose alone was added. When the streptomycin concentration was increased to 2,000 units/ml. under similar conditions, the oxygen uptake considerably exceeded the uptake which occurred when glucose alone was added.

When 500 units of streptomycin/ml. were added to suspensions of the variant in the absence of available substrate, no significant change in oxygen uptake occurred, but when the streptomycin concentration was increased to 2,000 units/ml., there was a considerable increase in oxygen uptake.

Preliminary studies of the biochemical changes produced in simple substrates by *E. typhosa* in the presence of streptomycin indicate that the presence of the antibiotic stimulates the production of changes which are compatible with the results observed in oxygen uptake studies. The utilization of carbohydrate substrate appears to be more complete and more rapid when streptomycin is present than when it is absent. It was found that streptomycin interfered with many of the commonly used procedures for the identification and quantitative estimation of the by-products of metabolic activity.

The investigation is in progress and will be reported in detail in a subsequent paper.

[1] The work described in this report was done under a contract, recommended by the Committee on Medical Research, between the office of Scientific Research and Development and the University of Chicago.

[2] The streptomycin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for experimental investigations recommended by the Committee on Chemotherapeutics and Other Agents, National Research Council.

References

1. CLIFTON, C. E. *Enzymologia*, 1937, **4**, 246–247.
2. CLOWES, G. H. A., and KRALL, M. E. *Science*, 1934, **80**, 384–385.