clusion may therefore be drawn (at least in respect to uracil), that, in the metabolism of yeast nucleic acid before the pyrimidine is liberated and even before the nucleoside is formed, the pyrimidine is altered in such a way that it may be further broken down and its nitrogen converted into urea.

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The variable acidity of hemoglobin and the distribution of chlorides in the blood.

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We have undertaken an investigation of the shift of chlorides between the serum and corpuscles of the blood described by Koeppe and by Hamburger, and have studied this phenomenon particularly in its relation to the heterogeneous acid-base equilibrium between hemoglobin, oxygen, carbon dioxide, bicarbonate, and the concentration of hydrogen ions.

Such a shift in chlorides may be easily produced, in vitro, by disturbing, in any way, the acid-base equilibrium, and is observed, under physiological conditions, between arterial and venous blood.

For the purposes of the investigation we have used fresh defibrinated ox blood, expelling the oxygen from combination with hemoglobin by first passing through carbon dioxide at 38° and then by boiling in vacuo at the same temperature. This can be accomplished with only very slight hemolysis. The blood has then been brought into equilibrium, at constant temperature and at atmospheric pressure, with various tensions of carbon dioxide, first in an atmosphere free from oxygen and then in an atmosphere with oxygen present at the tension at which it is present in atmospheric air. The whole blood has then been analyzed for oxygen and carbon dioxide, free and combined, and the serum, obtained by immediate centrifugalization under oil, analyzed for carbon dioxide and chlorides. The atmosphere with which the blood has been brought into equilibrium has in each instance been analyzed for carbon dioxide and oxygen after equilibrium has been reached.

From the data obtained the hydrogen ion concentration has been calculated from the ratio of free dissolved carbonic acid to bicarbonate. When the concentration of bicarbonate has been plotted against the hydrogen ion concentrations, curves similar to those given by L. J. Henderson in a recent paper[1] have been obtained, showing an isohydric shift of base between hemoglobin and the other constituents of the blood, according to whether the hemoglobin was oxygenated or reduced. When the bicarbonate was plotted against the logarithm of the figure obtained for the hydrogen ion concentration a linear relationship was found.

On plotting the serum chlorides against the hydrogen ion concentration an isohydric shift of chlorides has been noted in every experiment—*i.e.*, at the same hydrogen ion concentration the concentation of chlorides in the serum is higher in the case of oxygenated blood than in the case of reduced blood, which is the reverse of the condition in the case of bicarbonate. The total isohydric shift of chloride amounts to about two thirds that of base under the same conditions.

On plotting the serum bicarbonate against the serum chlorides it was found that they have a linear relationship, in the case of both oxygenated and of reduced blood, and that an increase in the concentration of bicarbonate in the serum is accompanied by a decrease of chlorides, corresponding to somewhat more than one half of the increase of bicarbonate. These curves appear to be straight lines, and are apparently parallel, indicating a constant difference between oxygenated and reduced blood at all hydrogen ion concentrations. This constant difference, accompanying the change in the state of hemoglobin, and not dependent on the change in concentration of bicarbonate, we are inclined to attribute to the change known to occur in the relative volumes of the red cells and serum. Of the total shift of chlorides at an isohydric point, this constant difference makes up only a small part.

Since the shift of chlorides, from or to the cells, accounts for

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[1] L. J. Henderson, "The Equilibrium between Oxygen and Carbonic Acid in Blood," *J. Biol. Chem.*, 1920, XLI, 401.

two thirds of the shift of base, and since other anions, chiefly HCO_3' but also SO_4'', shift in the same direction as Cl', it seems improbable that there is any considerable migration of kations in and out of the cells, with the exception of hydrogen ions. This is in accord with the older ideas on this subject. We are at present investigating also the extent of migration of HCO_3' ions.

As a general conclusion it may be stated that when the heterogeneous acid-base equilibrium is disturbed, from any cause, the new equilibrium is established by the migration of acids in and out of the cells, and that about two thirds of this acid is hydrochloric acid.

Further confirmation of the mechanism of the chloride shift is obtained from the fact that the shift is produced by varying the tension of oxygen as well as by varying the tension of carbon dioxide. The fact that the chloride shift occurs isohydrically provides additional evidence as to the change in the acidity of hemoglobin at varying tensions of oxygen.

In all of our experiments we have obtained data pointing to an extremely constant relationship between the various factors studied—*i.e.*, hemoglobin, oxygen, carbon dioxide, bicarbonate, chlorides, and hydrogen ion concentration—for different samples of ox blood. We are proceeding with the investigation in the hope of reducing these relationships to precise mathematical form.

We have obtained evidence to the effect that the buffer action of the serum under physiological conditions as compared with isolated serum is increased at least ten times by the change in acidity of hemoglobin.

It should be noted that we have studied the hydrogen ion concentration on the alkaline side of the isoelectric points of hemoglobin and serum proteins. At these hydrogen concentrations, as has been shown by Loeb,[1] the amphoteric colloids dissociate entirely, or almost entirely, as acids, so that we have disregarded, for the present, the possibility of combination of chloride and other anions with the colloid substances present.

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[1] Jacques Loeb, "Emphoteric colloids. I. Chemical influence of the hydrogen ion concentration," *J. General Physiol.*, 1918, I, 39.