sion des Farbstoffsoles". As evidence for this, the author gives the results of measurements of the binding capacity of albumin and fibrinogen for five dyes. However, a close critical examination of the method followed and of the results fails to substantiate the claim made by the author. In the first place, it is difficult to see how some of the dye in the Gibbs' adsorption layer could be prevented from adhering to the protein when the latter is transferred to the glass slide. The removal of the adhering solution by means of filter paper does not diminish the uncertainty introduced by the method of removing the protein film. On the contrary it introduces a further complication since the equilibrium between solution and protein may be disturbed by the presence of the cellulose of the paper. But even if we neglect these shortcomings in the method, the results themselves are inconclusive. The author seems to base his claim merely on the fact that Congo Red, known to be associated in solution, shows the highest binding power of the dyes tested when expressed as molecules of dye bound per molecule of protein whereas the highly dispersed Naphthol Yellow shows so little affinity that it was not measurable.

Since the structure of Naphthol Yellow is very different from that of Congo Red, the claim that the association of the molecules of Congo Red rather than its structure is responsible for its higher affinity is certainly not justifiable. As to the other dyes tested, the author fails to quote any data on their degree of dispersion and his argument is therefore not very forceful. Moreover he is not on very safe ground when he bases his conclusions on the number of molecules of dye bound by one molecule of protein. The binding of the dyes tested takes place in all probability through the sulphonic groups; that is the dyes are bound to the protein in the form of anions. Therefore the number of equivalents rather than the number of molecules of the dye bound by a given weight of the protein should be the criterion used in comparing affinities. If the data in the article under discussion is recalculated on that basis one obtains the following:

	Milliequivalents of dye bound by 1 g of protein	
	Albumin	Fibrinogen
Evans Blue	0·068	0·110
Trypan Red	0·173	0·255
Trypan Blue.	0·228	0·298
Congo Red	0·130	0·191

When expressed in this way, Congo Red does not give the highest result but it is second in order of increasing affinity. Of the dyes mentioned in the article, two have been studied by RAWSON[1] and it may be noted that she obtained results at variance with those of WUNDERLY. By other methods, RAWSON found that the affinity of Evans Blue for plasma albumin was appreciably higher than that of Trypan Blue. WUNDERLY also remarks that as the acidity of the solution is increased, the binding capacity of the protein for the dye increases. He advances this as another indication that the affinity is determined mainly by the degree of dispersion of the dye. However, it is well known that increasing the acidity of solutions containing anions capable of being bound by the protein will increase the amount of anions so bound. It is not surprising, therefore, that WUNDERLY noted an increased affinity as the pH of the solution was lowered.

P. LAROSE

Division of Chemistry, National Research Council, Ottawa, Canada, October 18, 1951.

[1] RUTH A. RAWSON, Amer. J. Physiol. *138*, 708 (1943).

Über die Farbstoffbindung durch monomolekulare Proteinschichten[1]

Erwiderung auf die vorstehenden Bemerkungen

VON P. LAROSE

Wir bedauern, wenn unsere Mitteilung den Eindruck erweckte, wir wollten mit den paar Messungen die komplexe Frage nach der Eiweiss-Farbstoffbindung abschliessend behandeln. Unsere Absicht war, einen Weg zu zeigen, wie man die Farbstoffbindung an gespreizte Proteinfilme messen kann. Vier der verwendeten sauren Azofarbstoffe gehen als Kolloidelektrolyte in Lösung und zeigen in derselben eine zunehmende Dispersion in der Reihenfolge Kongorot < Trypanblau < Trypanrot < Evans-Blue, wobei beträchtliche Teile der beiden zuletzt genannten Farbstoffe molekulardispers in Lösung gehen (vgl. WUNDERLY[2]); der Nitrofarbstoff Naphtholgelb geht molekulardispers in Lösung (vgl. BENNHOLD[3]). Unsere Versuche ergeben eine Abnahme der Bindung an das gespreizte Protein in der Reihenfolge Kongorot > Trypanblau > Trypanrot > Evans-Blue > Naphtholgelb, wobei die Bindung von Trypanblau zu Trypanrot besonders stark abnimmt (40%) und Naphtholgelb nicht messbar gebunden wird. Stellen wir dieser kolloidchemischen Betrachtung die strukturchemische gegenüber, so ergeben die von P. LAROSE auf Grund der Sulfogruppen errechneten Milliäquivalente, in der Reihenfolge abnehmender Bindung, das Folgende: Trypanblau (4 Sulfogruppen) > Trypanrot (5) > Kongorot (2) > Evans-Blue (4, isomer mit Trypanblau). Da diese Reihenfolge offensichtlich stark abweicht von der experimentell gefundenen (siehe oben), müssen wir daran festhalten, dass unsere Resultate durch kolloidchemische Überlegungen besser erklärt werden als durch strukturchemische. Unser ursprünglicher Text zeigte, dass wir keinesfalls die Wichtigkeit der Farbstoffstruktur verkennen, nur ist diese mit unseren Ergebnissen nicht in einen kausalen Zusammenhang zu bringen.

CH. WUNDERLY

Medizinische Universitätsklinik, Kantonsspital Zürich, den 8. Dezember 1951.

[1] CH. WUNDERLY, Exper. *7*, 296 (1951).
[2] CH. WUNDERLY, Z. ges. Exp. Med. *110*, 274 (1942).
[3] H. BENNHOLD, E. KYLIN und ST. RUSNYAK, *Die Eiweisskörper des Blutplasmas* (Th. Steinkopff, Dresden 1938), S. 233.

Model Experiments for the Production of Gastric Hydrochloric Acid

Under this title SZABÓ, ORSÓS, and CSÁNYI[1] have recently put forward a hypothesis for the production of gastric hydrochloric acid from a weak acid[1]. This depends on the properties shown by ion-exchange resins. The views that the double decomposition of sodium

[1] Z. G. SZABÓ, S. ORSÓS, and L. CSÁNYI, Exper. *7*, 297 (1951).

chloride and a weak acid, or the splitting of a protein hydrochloride by carbonic acid, could lead to the formation of the acid in gastric juice have been put forward on many occasions in the last 100 years (see review by DAVIES[1]) and can be ruled out as impossible. Primary oxyntic cell secretion can be produced continuously, contains about 0·16 *M*-hydrochloric acid and is isotonic with blood. To form the acid at this strength from carbonic acid directly in oxyntic cells would require the impossibly high concentration of 17 *M* (DAVENPORT[2]). Now, although the ion-exchange system of SZABÓ *et al.*[3] apparently by-passes the difficulty of this high concentration, the laws of thermodynamics require that the same energy as would be needed to produce such a concentration must be supplied at some stage in any alternative route from the carbonic acid of the cells to the acid of the gastric juice. This difficulty is veiled in the model presented by SZABÓ *et al.*[3], but it is here that the weakness of their case lies. Not less than about 10,000 cal. are required per gram molecular weight of gastric hydrochloric acid as secreted (for discussion see DAVIES and OGSTON[4]). The process must therefore be coupled with energy-giving reactions in the cell and cannot occur spontaneously.

Since the pH of 0·1 *n*-succinic acid is only 2·7, it is of interest that SZABÓ *et al.*[3] (Table III) obtained a solution of pH 1·23 (0·06 *M*-H^+) from 0·1 *n*-succinic acid, 2% sodium chloride and Amberlite IR-105. This was possible because the resin had been turned from the sodium to the hydrogen form by the successive application of 0·1 *n*-succinic acid. The hydrogen ions were then liberated at a higher concentration from the resin by applying a stronger solution of sodium chloride (2%, 0·34 *M*). Still higher acidities could be obtained by the use of even more concentrated sodium chloride solutions. It is important to realize that the energy needed to make this higher concentration of hydrogen ions came from that originally required to make the 2% sodium chloride used in the experiments of SZABÓ *et al.*[3].

For such a process to be operative continuously in the stomach the oxyntic cells would therefore have to be able to do secretory work on sodium chloride to make the very strong and very weak ("distilled water") solutions required. Whilst the stomach can certainly secrete hydrogen ions and must therefore contain a structure capable of liberating these ions into solution, this liberation is much more likely to follow from oxidation-reduction changes (DAVIES[5]) than from, for example, the alternating association of hydrogen and sodium ions with some hypothetical insoluble acid radicles. A coherent theory has been based on the first possibility but the second requires a mechanism for concentrating sodium ions, which presents at least as difficult a problem as the concentration of the hydrogen ions themselves (DAVIES and OGSTON[6]).

Thus whilst it is conceivable that ion exchange may play a part in gastric acid secretion, it is impossible for processes of the type described by SZABÓ *et al.*[3] to occur in the stomach without a continuous energy supply. Since these workers do not specify or consider such an energy supply their experiments are not acceptable models for the production of gastric hydrochloric acid.

R. E. DAVIES and P. A. H. WYATT

Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, and Department of Physical Chemistry, University of Sheffield, England, October 5, 1951.

[1] R. E. DAVIES, Biol. Rev. *26*, 87 (1951).
[2] H. W. DAVENPORT, J. Physiol. *97*, 32 (1939).
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[4] R. E. DAVIES and A. G. OGSTON, Biochem. J. *46*, 324 (1950).
[5] R. E. DAVIES, Biol. Rev. *26*, 87 (1951).
[6] R. E. DAVIES and A. G. OGSTON, Biochem. J. *46*, 324 (1950).

Ion Exchange and Permeability

Reply to the preceding remarks of

R. E. DAVIES and P. A. H. WYATT

In a short communication[1] we have reported on double decompositions carried out upon ion-exchange resins which can be considered as a model of the gastric hydrochloric acid formation in relation to the separation of ions from reaction mixture.

In our considerations we have omitted the energetics, being only interested in the kinetics, the mechanism of the process. DAVIES estimated the energy needed for this endothermic reaction to be about 10,000 cal. This work could be supplied in the case of our model by the activation energy which proved to be just as great in other experiments[2]. In the organism the cell metabolism can furnish this energy simultaneously.

In the exchange reactions—in our model experiments—the concentration ratios differ appreciably from those in the oxyntic cells. However, the remark of DAVIES referring to the data of DAVENPORT is not valid concerning the formation of 0·16 *M* hydrochloric acid requiring a carbonic acid concentration of 17 *M* in the oxyntic cells, because there is no equilibrium in the stomach but only a stationary state. In this case, however, the ion-exchange substances are especially appropriate systems for the development of concentrations differing from the equilibrium values.

In ion-exchange processes the quantity of the ion exchanged pro unit of time is a function of the capacity, whilst the maximal concentration developed depends on the exchange potential.

The ion-exchange substances may play their part concerning the separation of the ions governed by the exchange potential on the basis of their operation being. Without assuming this valve-like function the permeability processes of the organism can hardly be interpreted, and—in our opinion—this is true also in the case of the oxydation-reduction changes of DAVIES. Ion exchange processes in the substances of the organism are possible, as the recently published experiments of HUDSON and SCHMEICHLER[2] suggest.

Z. G. SZABÓ and L. CSÁNYI

Department of Chemistry, University of Szeged, Hungary, November 23, 1951.

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[2] R. F. HUDSON and G. A. SCHMEICHLER, J. Phys. Coll. Chem. *55*, 1120 (1951).