The experiment in plant physiology dealing with adsorption brought to my mind this fundamental question when the experiment was being discussed with the students. If ethyl alcohol reduces the adsorptive capacity of charcoal particles for methylene blue, will ether, chloroform, barbital and sulfanilamide have the same effect? Preliminary tests did show that each drug separately reduced the adsorptive capacity of charcoal particles for methylene blue.

Other tests to determine the threshold value of each drug followed. The threshold value is the maximum amount of drug which will not produce any decrease in adsorption. Less amounts have no apparent effect and larger amounts affect the adsorption progressively more. One gram of the finely ground charcoal[2] on hand in the botany laboratory was found to adsorb all the methylene blue from 13 ml of a 1 per cent. aqueous solution but no more. With this set-up it was rather simple to determine the threshold value for each drug. A standard mixture of one gram of charcoal particles and 13 ml of the 1 per cent. aqueous methylene blue with the addition of slightly more than the threshold value of a certain drug would produce a blue filtrate upon filtration.

The threshold values for a number of narcotics are given in Table 1. The concentration value is based on the total amount of mixture, which in every case was 100 ml. The temperature was close to 25° C.

TABLE 1

THE THRESHOLD VALUES FOR SOME NARCOTICS WHICH AFFECT THE ADSORPTIVE POTENCY OF ACTIVATED CHARCOAL PARTICLES FOR METHYLENE BLUE

Narcotic	Threshold value based on amount in total mixture (100 ml)
Ethyl alcohol	8 per cent.
Ether	1 " "
Chloroform	0.5 " "
Sodium barbital	0.1 " "
Sulfanilamide	0.025 " "
Saponin	0.06 " "

Table 2 presents data dealing with the effect of the narcotics on the action of diastase. In every case 10 ml of 1 per cent. soluble starch, 1 ml of a 1 per cent. diastase solution and enough narcotic to give the stated concentration were diluted to 20 ml. The concentration for each narcotic is based on the final solution volume of 20 ml. The temperature was close to 25° C.

TABLE 2

THE EFFECT OF SOME NARCOTICS ON THE DIGESTIVE ACTION OF DIASTASE

Narcotic and its concentration	Minimum time for soluble starch solution to be digested past the last iodine staining stage
Control (no narcotic added)	15 minutes
Ethyl alcohol 25 per cent.	30 "
Chloroform 25 per cent.	25 "
Saponin 0.25 per cent.	25–30 "
Sodium barbital 0.25 per cent.	180 "[a]
Sodium barbital 0.10 per cent.	70 "
Sulfanilamide 0.25 per cent.	20 "[b]

a. Retarded 165 minutes; b. Retarded 5 minutes.

It is most interesting to note the differential effects of sulfanilamide on charcoal particles and diastase. While sulfanilamide is extremely potent in its effect on the adsorption of methylene blue by charcoal particles, it is only mildly effective in arresting the digestive action of diastase on soluble starch. This difference may be fundamental in explaining why sulfanilamide is so effective in combating body pathogens without critically and dangerously upsetting the regular essential metabolic activities of the host's body. To be exact, sulfanilamide is about four times as potent as sodium barbital in reducing the adsorptive capacity of charcoal particles for methylene blue, but only one thirty-third as drastic as sodium barbital in its retardation of diastatic action on soluble starch.

That enzyme action is adsorptive becomes even more certain when one finds that the effect of ether, alcohol and chloroform on the adsorptive potency of charcoal particles for methylene blue is canceled wholly or in part by the application of hydrostatic pressure. This is exactly what Johnson, Brown and Marsland reported for luciferase in luminous bacteria.

The action of narcotics on diastase appears to be the same as that on charcoal particles, which unquestionably had their adsorptive capacity for methylene blue reduced thereby. It may, therefore, be concluded that enzyme action is fundamentally an adsorptive process. The doubt in the minds of Johnson, Brown and Marsland as to whether the effect of barbital, sulfanilamide and p-aminobenzoic acid was chemical or adsorptive can now be removed.

H. C. EYSTER

DEPARTMENT OF BOTANY,
UNIVERSITY OF SOUTH DAKOTA

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE CHEMICAL COMPOSITION OF LIVER PREPARATIONS

SINCE the discovery, about fifteen years ago, of the curative action of liver in pernicious anemia, much has been learned of the nature of the active substance and the procedures for extraction.

Methods for extraction and analytical results, including my own studies, have been published else-

[2] Charcoal, activated, for decolorizing, about 80 mesh. Will Corporation, Rochester, N. Y.

where.[1] From one kilogram of fresh liver I obtained 5.8 grams of yellowish brown powder as a silver salt. From the analytical results the presence of three free COOH groups, eighteen –CO–NH– groups and a molecular weight of approximately 10,000 was deduced.

Dakin and West[2] separated the inactive part with ammonium sulfate, magnesium sulfate and sodium chloride, and finally precipitated the active part with Reinecke salt. In this way they obtained a compound constituting 1 per cent. of the original dried weight and with a nitrogen content of 15.3 per cent., which they treated as a polypeptide and glucosamine. In a later publication Dakin, Ungley and West,[3] by a more refined method of extraction, obtained a fraction with 16.2 per cent. nitrogen which is characteristic of an albumose type, without glucosamine, and established a molecular weight of 3–5000. Sladek, Savczycka and Lipschuetz[4] demonstrated the old thesis that free amino groups derived from amino acids exist. Subbarow, Jacobsen and Prochownick[5] have isolated 2 milligrams of a crystalline sulfate from 100 grams of liver, a product which seems to be identical with that described by Lalund and Klemm.[6] Karrer, Frei and Fritsche[7] found in the active fraction a pentose and adenin, and believe that the activity is proportional to the phosphorus content, obtaining a maximum of 3.8 per cent. P.

In 1939 I began some new experiments. The extraction and purification methods were simplified. Each kilogram of milled liver was extracted with one-third volume of water and 6 cc of 20 per cent. sulfuric acid at 35° C. After pressing, this process was repeated at 50° C, 60° C and 70° C. The expressed liquids were mixed together and treated with $Ba(OH)_2$ at 50° C until a pH of 6.5–6.7 was obtained, warmed to 60° C and filtered. After concentrating in vacuum to one seventh of its volume at 40° C, 99 per cent. alcohol was added until an alcohol concentration of 70 per cent. was reached. After filtering and evaporating the alcohol (in vacuum at 40° C) the liquid was concentrated to half its volume, filtered and precipitated with $AgNO_3$. This salt was decomposed with HCl, filtered, and the solution precipitated with alcohol. The precipitate was dissolved in N/10 NaOH to pH 7.2 and with silver nitrate a new silver salt is isolated which has the following composition:

C	67.50	per cent.
H	6.41	" "
O	4.60	" "
N total	14.40	" "
N amino	1.40	" "
S	0.99	" "
P	1.06	" "
Ag	5.04	" "

The acid part of the substance had a molecular weight of 6,000. It contained three free COOH groups, as did the product obtained several years ago. Remaining are six free amino groups, this number increasing to eighteen after hydrolysis (5 hours of ebullition with HCl or 25 per cent.). From this the presence of twelve bonds of –CO–NH– is deduced. One kilogram of fresh liver gave 2.09 grams of this substance which was extraordinarily active in clinical tests.

For an approximate estimation of the potency of liver extracts the combination of the chemical method (fractional precipitation with alcohol) of Schales[8] and my biological test,[9] based on the influence of phenylhydrazine anemia, was found satisfactory. The results were confirmed by the clinical test of the reticulocyte response.

SUMMARY

For the present we can entertain the following ideas regarding the chemical structure of the active fraction of liver in pernicious anemia: it is an amino acid complex with three free COOH groups, it contains sulfur and phosphorus, is soluble in water, acids and bases, precipitates in alcohol at concentration greater than 87 per cent., and has a molecular weight of 6,000.

JOSÉ ERDOS

THE NATIONAL POLYTECHNICAL INSTITUTE,
MEXICO, D. F.

[1] Erdos, *Biochem. Zeitschr.*, 277: 337, 1935.
[2] Dakin and West, *Jour. Biol. Chem.*, 109: 489, 1935.
[3] Dakin, Ungley and West, *Jour. Biol. Chem.*, 115: 771, 1936.
[4] Sladek, Savczycka and Lipschuetz, III. Kongr. Slovenskih Aptekar. Jugoslaviji, Prague, page 266, 1935.
[5] Subbarow, Jacobsen and Prochownick, *Jour. Am. Chem. Soc.*, 58: 2234, 1936.
[6] Lalund and Klemm, *Acte med. Scand.*, 88: 620, 1936.
[7] Karrer, Frei and Fritsche, *Helv. Chim. Acta*, 20: 622, 1937.
[8] Schales, *Klin. Wochenschr.*, 16: 277, 1937.
[9] Erdos, *Biochem. Zeitschr.*, 277: 342, 1935.

BOOKS RECEIVED

Biological Action of the Vitamins. Edited by E. A. EVANS, JR. Pp. ix + 227. Illustrated. The University of Chicago Press. $3.00.

HAYNES, WILLIAMS. *The Stone that Burns.* Pp. xii + 345. Illustrated. D. Van Nostrand Company, Inc. $3.75.

HOLMES, HARRY N. *Strategic Materials and National Strength.* Pp. 106. Illustrated. Macmillan. $1.75.

MOORE, JUSTIN H. and JULIO A. MIRA. *The Gist of Mathematics.* Pp. xii + 726. Illustrated. Prentice-Hall. $4.00.

MORRIS, MAX and ORLEY A. BROWN. *Differential Equations.* Pp. xi + 355. Prentice-Hall. $3.00.

ROJAHN, C. A. and F. GIRAL. *Preparacion de productos quimicos y quimico-farmaceuticos.* Two volumes. Pp. xxxix + 1002. Editorial Atlante, S.A., Mexico, D.F.