TABLE 1

Test-tube No.		1	2	3	4	5	6	7
Inoculation per 5 ml		1	$\frac{1}{10}$	$\frac{1}{10}^2$	$\frac{1}{10}^3$	$\frac{1}{10}^4$	$\frac{1}{10}^5$	$\frac{1}{10}^6$ drop
		Growth after 43 hours at 37° C						
a	Control	+	+	+	+	+	+	–
b	p–Aminobenzamide 1.10^{-3}M	+	+	–	–	–	–	–
b′	p–Aminobenzamide 1.10^{-3}M + p–Aminobenzoic acid 1.10^{-4}M	+	+	+	+	+	+	+
c	Sulfanilamide 1.10^{-3}M	+	–	–	–	–	–	–
c′	Sulfanilamide 1.10^{-3}M + p–Aminobenzoic acid 1.10^{-4}M	+	+	+	+	+	+	–

on a synthetic medium for staphylococci.[9] Medium: 10.53 per cent. of ammonium chloride, 0.3 per cent. of glucose, 0.5 per cent. of sodium sulfate, 0.01 per cent. of magnesium chloride, phosphate buffer (M/15) pH 7.2; each 5 ml.

The bacteriostatic effect of p-aminobenzamide is almost as strong as that of equimolecular quantities of sulfanilamide; both effects are suppressed by p-aminobenzoic acid.

(2) The Antibacterial Effect of p-Aminophenylarsinic Acid (Atoxyl) and Its Inhibition by p-Aminobenzoic Acid

Atoxyl reduces the speed of bacterial growth, but no complete bacteriostasis is attained. The speed of bacterial growth in aerobic cultures has been determined by continuous measurement of the oxygen consumption in Warburg vessels.[10]

Material for inoculation: 13 hours culture of *B. coli* on a synthetic medium;[9] one drop per 30 ml was inoculated in the same medium.

TABLE 2

Culture No.	I	II	III	IV	V	VI
Addition	–	–	Atoxyl 1.10^{-4}M		Atoxyl 1.10^{-4}M + p–Aminobenzoic acid 1.10^{-4}M	
Hours after inoculation	mm^3 Oxygen consumption per ml in 30 minutes					
3 00	4	4	2	3	6	4
3 30	11	9	7	7	13	11
4 00	23	20	13	13	28	26
4 30	51	40	20	20	49	46
5 02	91	70	30	28	76	75
5 34	126	115	37	38	103	99

p-Aminophenylarsinic acid (atoxyl) acts in the same way but decidedly weaker than sulfanilamide and p-aminobenzamide. This corresponds to the lesser antibacterial efficiency of free sulfanilic acid.

Among the derivatives of p-aminobenzamide possibly a further new group of substances with chemotherapeutic effects towards bacterial infections might be found. Other substances, too, devoid of sulfo groups, but structurally related to p-aminobenzoic acid, should be tested with regard to their chemotherapeutic effects.

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[9] P. Fildes and G. M. Richardson, *Brit. Jour. Exp. Path.*, 18: 292, 1937.

[10] J. Hirsch, *Enzymologia*, 4: 94, 1937.

ENZYME ACTION

Just as the adsorptive capacity of finely ground activated charcoal for methylene blue is decreased by narcotics, so it appears that the activity of enzymes is reduced similarly by the same narcotics.

In plant physiology there is an experiment whose object is adsorption and whose procedure involves the mixing of finely ground activated charcoal with an aqueous solution of methylene blue. Under the correct proportion which varies with the concentration of methylene blue and the adsorptive potency of the finely ground activated charcoal, the filtrate is clear and devoid of methylene blue. The conclusion is that the methylene blue has been adsorbed by the finely ground activated charcoal. The experiment also directs the students to add ethyl alcohol to the residue, whereupon the filtrate becomes deep blue and the conclusion is that alcohol decreases the adsorptive capacity of the charcoal particles.

The students in plant physiology at the University of South Dakota performed this experiment in January, 1942, shortly after the meetings of the American Association for the Advancement of Science in Dallas, and when the prize-winning paper was still fresh in my mind. As most of you may recall, the prize was awarded to Johnson, Brown and Marsland for the paper entitled "The Mechanism of Temperature and Hydrostatic Pressure Reversal of Narcosis in Luminous Bacteria."[1] The research involved the action of narcotics on luciferase, the enzyme which is responsible for luminescence in luminous bacteria. The investigators found that narcotics readily reduce the intensity of luminescence, clearly indicating a decrease in enzymatic activity.

[1] Frank H. Johnson, Dugald E. S. Brown and Douglas A. Marsland, *Anat. Rec.*, 81: 4, Supplement, page 33, 1941.

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.

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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.