27 minutes, whereas six control dogs receiving the same dose of barbital sodium, but not cross-circulated, recovered in an average time of 29 hours.

Two dogs received 500 mg of sodium barbital per kgm of body weight and following the period of one to two hours were similarly cross-circulated to etherized partners, the pairs being of approximately equal weights. After an hour the partners were separated and both survived and were asleep for over 24 hours. Six dogs were given 500 mg of sodium barbital and they all died; the survival periods for five dogs were 127 to 235 minutes and one died in 45 minutes.

These experiments substantiate the original thesis of Koppanyi concerning the establishment of dynamic equilibrium of barbiturates in the body and show that they are not fixed in the tissues and that they may be mobilized at any time. This method of cross-circulation is proposed to study the fixation or alleged fixation of drugs, the criterions varying, of course, from drug to drug. Opiates and digitalis principles would seem to offer a particularly fruitful field for this line of study. The authors believe that few drugs if any will not be removed by cross-circulation.

The cross-circulation obviously is a method which has given excellent results in the treatment of experimental barbiturate poisoning and probably an apparatus could be constructed to provide for slow, continuous bleeding of the poisoned individuals and to replace the drawn blood at the same rate with normal plasma or compatible whole blood.

THEODORE KOPPANYI
CHARLES R. LINEGAR
DEPARTMENT OF PHARMACOLOGY
AND MATERIA MEDICA,
GEORGETOWN UNIVERSITY, SCHOOL OF MEDICINE

THE ACTION OF SULFANILAMIDE COMPOUNDS ON THE LETHAL FACTOR OF BACTERIAL TOXINS

IN most gram negative bacteria the toxic factor appears to form a part of the complex O antigen,[1] and although earlier work suggested that the antigenic, and presumably the toxic portion of the endotoxin was associated with its "polypeptide" component, more recent studies[2] indicate that this component is a protein. Our studies[3] of the property which such bacterial products have of inducing hemorrhage[4] in implanted mouse tumors led to an investigation of the mode of detoxication of these antigens when introduced parenterally.

While it is generally assumed that the action of sulfanilamide compounds is one of bacteriostasis ensuing from interference with the utilization of *p*-aminobenzoic acid, our results confirm previous findings,[5,6] that sulfanilamide also markedly increases the resistance of mice to certain preformed bacterial toxins.

The toxin used in this study was prepared by growing *Salmonella typhimurium* in a medium containing citrate and dextrose as sole organic constituents. The profuse growth was killed with 2 per cent. phenol, and the suspension transferred to regenerated cellulose tubing. The contents of the tubes were simultaneously dialyzed against water and reduced to a small volume by pervaporation in a current of warm air. The phenol-free toxin was precipitated in 80 per cent. acetone, the precipitate dried with alcohol and ether, and then taken up again in water. Injected intraperitoneally into male mice weighing 20 g, 1.3 mgm of this preparation killed 50 per cent. of the animals within twenty-four hours. This amount of toxin was therefore designated as one minimum lethal dose. It was found that 2.0 MLD killed 92 per cent. of the mice, and 10.0 and 20.0 MLD killed 100 per cent.

Compounds assayed for protective effects were administered in neutral aqueous suspension by stomach tube to mice receiving simultaneously an intraperitoneal dose of toxin. In Table 1 it is seen that sulfanilamide affords almost complete protection from 2.0 MLD of the toxin, and 33 per cent. of the mice survived 10.0 MLD. Sulfathiazole and sulfapyridine were somewhat less effective. Also, if sulfanilamide is administered one hour before the toxin is injected and is followed by supplementary doses of sulfanilamide, the degree of protection against the toxin is somewhat increased.

Animals receiving toxin together with adequately protective amounts of sulfanilamide were subcutaneously given small amounts of *p*-aminobenzoic acid. As seen in the table, the effect of *p*-aminobenzoic acid in reducing the action of the sulfanilamide and thus allowing the toxin to exert its lethal effect was quite striking, and suggests that sulfanilamide and *p*-aminobenzoic acid compete for the enzymes concerned in the detoxication effect in a manner comparable to that described for bacteriostasis.

[1] A. Boivin, *Rev. d'Immun.*, 6: 86–115, 1940.
[2] W. T. J. Morgan and S. M. Partridge, *Biochem. Jour.*, 35: 1140–63, 1941.
[3] P. A. Zahl, S. H. Hutner, S. Spitz, K. Sigiura and F. S. Cooper, *Am. Jour. Hyg.*, 36: 224–42, 1942.
[4] Experiments in progress indicate a protective action by sulfa drugs against the hemorrhage inducing effects of this antigen.
[5] C. M. Carpenter, P. L. Hawley and G. M. Barbour, SCIENCE, 88: 530–1, 1938; C. M. Carpenter and G. M. Barbour, *Proc. Soc. Exp. Biol. and Med.*, 41: 255–9, 1939; C. M. Carpenter, *Proc. Soc. Exp. Biol. and Med.*, 41: 354–7, 1939.
[6] C. Levaditi and A. Vaisman, *C. R. Soc. Biol.*, 128: 463–5; C. Levaditi, A. Vaisman and L. Reinié, *Ann. Inst. Pasteur*, 61: 635–61, 1938.

TABLE 1

Toxin	Sulfanilamide	*p*-aminobenzoic acid	Per cent. survival	No. of animals
1 M.L.D.	.....	.....	52	48
2 M.L.D.	.....	.....	8	36
10 M.L.D.	.....	.....	0	38
.....	20 mgm (oral)	.....	95	20
2 M.L.D.	20 mgm (subc.)	.....	46	13
2 M.L.D.	20 mgm (oral)	.....	94	38
10 M.L.D.	20 mgm (oral)	.....	33	30
10 M.L.D.	20 mgm one hour prior to toxin injection plus two doses of 10 mgm each at four hour intervals (oral)	.....	45	20
20 M.L.D.	20 mgm (oral)	.....	0	9
.....	20 mgm (oral)	10 mgm (subc.)	100	11
2 M.L.D.	.....	10 mgm (subc.)	0	5
2 M.L.D.	20 mgm (oral)	10 mgm (subc.)	17	35
2 M.L.D.	Sulfathiazole 20 mgm (oral)	.....	50	20
10 M.L.D.	Sulfathiazole 20 mgm (oral)	.....	0	18
2 M.L.D.	Sulfapyridine 20 mgm (oral)	.....	75	16

1 M.L.D. is designated as the amount of antigenic material required to kill within 24 hours 50% of mice injected intraperitoneally with an aqueous solution of the antigen. By dry weight 1 M.L.D. equals 1.3 mgm of toxic material.

Since sulfanilamide is not in all probability a naturally occurring substance the question arises as to whether the protective effect of sulfanilamide results from increasing the general resistance of the body to the toxin, or whether sulfanilamide or one of the products into which it is converted by the body is utilized more specifically for a detoxication process. The first hypothesis is weakened by the finding of Carpenter and associates who observed that sulfanilamide protected mice against toxins produced by such gram positive organisms as *Staphylococcus aureus* and *Clostridium welchii*, both of which differ markedly in pathogenesis from the toxins of gram-negative organisms, particularly *Salmonella* studied by us and by Levaditi, and the *Neisseria* toxins studied by Carpenter and by Levaditi.

A specific detoxication mechanism for toxic proteins, aside from hydrolysis, has never to our knowledge been proposed. The experiments of Morgan[7] showing that the toxicity of typhoid antigen is not destroyed by its homologous antibody (in contrast to the neutralizing action in other toxin-antitoxin reaction mixtures, *e.g.*, diphtheria and tetanus) may suggest that some other means, presumably non-immunological, within the body is called upon to detoxify this antigen.

This action of sulfanilamide, if it is a detoxication of bacterial toxins, may represent a special instance of the enhancement of the general detoxication of proteins within the body.

[7] H. R. Morgan, *Jour. Immunol.*, 41: 161–80, 1941.

SUMMARY

Sulfanilamide compounds protect mice against multiple lethal doses of purified *Salmonella* endotoxin. This protective action of sulfanilamide is inhibited by *p*-aminobenzoic acid.

S. H. HUTNER
PAUL A. ZAHL

THE HASKINS LABORATORIES,
NEW YORK, N. Y.

ANTICIPATORY CARDIAC ACCELERATION DURING SLEEP

As a part of a series of studies of sleep motility[1] at the University of Virginia, recordings of the heart rate during sleep were made. The data obtained proved to be of considerable interest and served to indicate the nature of the stimuli causing sleep movements.

Johnson[2] has called attention to the fact that during sleep, when body positions are maintained for fairly long intervals, there is, among other things, an interference of circulation (stasis of the blood and body fluids in parts of the body) and an overheating of the unventilated portions of the skin. These conditions, he suggested, must become sufficiently irritating in time to lead to a change in body position. For convenience this shall be termed the congestion hypothesis.

It is a matter of common knowledge that, as Johnson mentioned, such stimuli become irritating and even painful after some minutes. Further, it has been shown that either restriction of circulation or raising of the skin temperature produces an increase in the heart rate. If these irritating stimuli were the ones causing movement, an increase in the heart rate prior to movement would be expected. According to the same reasoning, a decrease might be shown following movement, as a change in position would relieve the irritating conditions.

This reasoning led to an experiment in which the heart rate was examined in conjunction with motility. In order to rule out the possibility of experimental artifacts interfering with the normal circulation during sleep, the heart rate was determined electrically by means of a cardiotachometer. Each heart beat was recorded on a moving strip of paper along with the movements of the sleeper. A high-speed kinetograph was used to determine precisely the onset and termination of each movement. In this study only the larger movements involving a change in position of the trunk were considered. To obtain the heart rate uncomplicated by factors other than those under consideration, a given movement must be preceded and followed by several minutes of inactivity.

[1] Results of these studies are to be published.

[2] H. M. Johnson, T. J. Swan and G. E. Weigand, *Psychol. Bull.*, 27: 18, 1930.