used. The "floating clot" method has the advantage of providing a large mass of proliferating cells, which provide a great yield of virus. In view of this, experiments are being performed to determine whether these cultures can be employed as a vaccine.

HARRY PLOTZ
REGINALD REAGAN

ARMY MEDICAL SCHOOL,
WASHINGTON, D. C.

MECHANISM OF P-AMINOBENZOIC ACID ACTION AND THE PARALLEL EFFECTS OF ETHYL CARBAMATE (URETHANE)*

IN seeking a theoretical basis for the bacteriostatic effects of sulfanilamide, Woods and Fildes[1,2] first postulated that the drug competed with a structurally related molecule, para aminobenzoic acid (PAB), which was thereby presumed to occupy some essential rôle in the normal growth and metabolism of micro-organisms. The latter compound thus has a dual interest: as a possible intermediary in ordinary metabolism and as a possible site of sulfanilamide inhibitions. Numerous investigations seem to have provided evidence supporting both aspects of the original hypothesis, and to have greatly extended the biological significance of PAB.

It now appears widely accepted that PAB is not only a naturally occurring "essential metabolite,"[3] but an anti-sulfanilamide or a growth factor for diverse organisms, including chicks,[4] dermatophytes[5] and even autotrophic plants, *e.g.*, diatoms.[6] The same compound is thought to be concerned in lactation[7] and in pigmentation of hair.[4] Its anti-sulfanilamide effects on the growth of bacteria have been demonstrated *in vivo*[8] as well as *in vitro*.[1] Doubt has been expressed, however, that this anti-sulfanilamide effect is in the nature of a competitive action of the two molecules for the same receptor in the organism, since 1 molecule of PAB may antagonize 23,000 molecules of sulfanilamide. Before the above-mentioned interpretations become too deeply entrenched in the scientific literature and thought as fully correct, an alternative explanation for the mode of sulfanilamide action, as well as the stimulatory effects of PAB, should be considered.

Recent experiments in this laboratory have shown that *ethyl carbamate* (*urethane*) *as well as PAB may exert anti-sulfanilamide effects* on luminous bacteria. The results are more striking in relation to luminescence than to growth, although both are influenced. The structural similarities between the molecules of urethane and sulfanilamide are so remote as to rule out competitive action, and urethane could hardly be considered an "essential metabolite." It is a familiar principle, however, that narcotics and, indeed, poisons of many sorts, have stimulatory effects in low, and inhibitory effects in high concentration. All three of the above compounds—urethane, PAB and sulfanilamide—act in the manner of narcotics on luminous bacteria, stimulating growth and luminescence in low, while inhibiting in high concentrations.

Further evidence of the fundamentally narcotic action of PAB, sulfanilamide and urethane, quite apart from growing cultures, is found in their effects on washed cell suspensions. The intensity of luminescence is readily and reversibly reduced on the addition of any one of these or a host of other narcotics. Experiments with the luminescent luciferin-luciferase system, which can not be extracted yet from bacteria but can be obtained in purified preparations from *Cypridina*[9] have shown that the velocity constant of the reaction *in vitro* is retarded by urethane, PAB, sulfanilamide, sulfapyridine and sulfathiazol. The action is reversible and clearly on the enzyme, luciferase. Over a wide range it is independent of the substrate (luciferin) concentration.[10] Thus, the inhibitory effects of PAB, urethane and sulfonamides appear to be definitely related to those of narcotics in general. Recent work with hydrostatic pressure and temperature[11] has opened a new approach to the study of the basic mechanism involved.

The stimulatory action of narcotics in low concentration is not easy to explain. In the present connection, the point to be emphasized is that the stimulatory effects of one narcotic may antagonize or completely overcome the inhibitory effects of another that is simultaneously present. If the inhibitant is sulfanilamide, the antagonist is naturally looked upon as "anti-sulfanilamide." The anti-sulfanilamide action of both urethane and PAB might well belong in this category. The molecular structure of the antagonistic narcotics need not be closely related, as would be required for competitive inhibition in the physicochemical sense. The action of urethane and of nembutal in preventing death from sulfonamide overdosage of animals[12] lends support to the view ex-

* The studies in this laboratory have been aided, in part, by a grant from the Penrose Fund of the American Philosophical Society.

1 D. D. Woods and P. Fildes, *Chem. Ind.*, 59: 133, 1940.
2 D. D. Woods, *Brit. Jour. Exp. Path.*, 21: 74–90, 1940.
3 S. D. Rubbo and J. M. Gillespie, *Nature*, 146: 838, 1940.
4 S. Ansbacher, SCIENCE, 93: 164, 1941.
5 N. S. Dimond, SCIENCE, 94: 420, 1941.
6 S. Wiedling, SCIENCE, 94: 389, 1941.
7 B. Sure, SCIENCE, 94: 167, 1941.
8 G. M. Findlay, *Brit. Jour. Exp. Path.*, 21: 356, 1940; F. R. Selbie, *ibid.*, 21: 90, 1940.
9 E. N. Harvey, *Erg. d. Enzymforsch.*, 4: 365, 1935; R. S. Anderson, *Jour. Cell. Comp. Physiol.*, 8: 251, 1936.
10 F. H. Johnson and A. M. Chase, *ibid.*, in press.
11 F. H. Johnson, D. E. S. Brown and D. A. Marsland, to be published in the near future.
12 R. K. Richards, *Jour. Lab. Clin. Med.*, 26: 1256, 1941.

pressed above, although its possible significance in relation to the mechanism of sulfanilamide and PAB effects has apparently been overlooked. Other examples of antagonisms among narcotics could be cited. The whole problem needs further study.

In summary, both the stimulatory and inhibitory effects of PAB and sulfanilamide, as well as urethane, appear to be fundamentally related to the general problem of narcotic action, which does not necessarily involve a structural similarity between the molecules of the inhibitor and an intermediary of normal metabolism in the cell. This interpretation has some interesting implications with respect to the various effects of PAB in different organisms referred to above. Further study from the point of view discussed would appear justified on the basis of the evidence at hand.

FRANK H. JOHNSON

PRINCETON UNIVERSITY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A HEAD HOLDER FOR INTRACRANIAL OPERATIONS ON THE MONKEY

THE advantages of fixation of the head, apparent to any one undertaking intracranial operative procedures in experimental animals, are readily secured in carnivores by use of the Czermak type head holder. Adaptation of the Czermak holder for employment with monkeys has not proved satisfactory in our hands, and we have thought it worth while briefly to describe the apparatus devised for that purpose in this laboratory.

The essential instrument is the head holder (A) invented some years ago by Dr. A. R. Buchanan for use with the Horsley-Clarke machine on guinea pigs (Fig. 1). This consists of a cylindrical cross-bar (1) and two side arms (2) which slide onto the bar and can be tightened in place by set screws (3). The cross-bar is slotted and an interlocking piece fitted on the interior of the base of each arm, in order that the arms be aligned in the same plane. When employed with the stereotaxic instrument on the guinea pig, the Buchanan holder is applied by approximating the two arms until the shaped pins (4) fit into the meatuses. Finally the ear bars of the Horsley-Clarke machine are seated in the openings (5). When the Buchanan holder is used to fix a monkey's head, the ear plugs (D) are firmly inserted into the meatuses; these are the short, straight plugs described by Harrison.[1] The side arms are then approximated until the shaped pins are solidly set in the open ends of the ear plugs, and the arms held in place by tightening the set screws. Dorso-ventral rotation of the animal's head is prevented by introducing into the opened mouth a straight bar covered with rubber tubing (E), and making this fast on the side arms by the use of two common right angle clamps (C), as illustrated in the lower figure.

As shown by the sketch of the apparatus set up for operation, the Buchanan holder can be attached to a vertical bar (F), arising from the table, and adjusted to a convenient height by any suitable clamp. We have employed a universal clamp (G) to allow tilting to either side. For approaches through the convexity of the calvarium no further fixation of the head is needed. In lateral approaches involving exposures down to the zygoma, the distal part of the side arm forms an inconvenient bulge beneath the drapes.

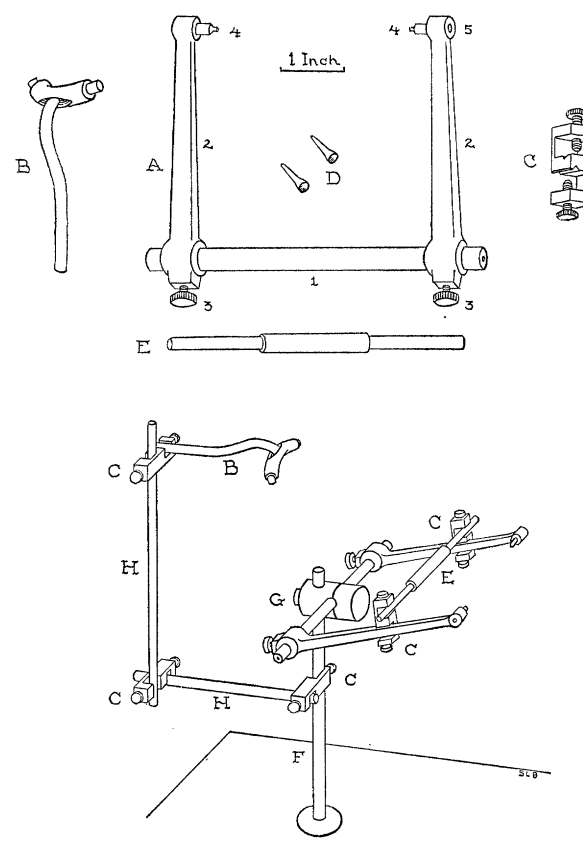

FIG. 1.

This might be obviated by constructing a side arm containing a right angle or one made so that it would lie flush with the ear.

For approach to the posterior fossa through the enlarged foramen magnum, we have found it necessary further to stabilize the head to prevent its dorsiflexion. For this purpose a simple nose piece (B) was contrived, the cross-bar of which, covered with

[1] F. Harrison, *Arch. Neurol. Psychiat.*, 40: 563, 1938.