that *p*-aminobenzoic acid in some way protects rats against excessive doses of carbarsone. All rats were maintained on the same adequate diet.

To test the validity of these ideas, further experiments were designed with carbarsone and also with such other pentavalent arsenicals as "Tryparsamide" (sodium N. phenylglycinamide-*p*-arsonate), arsanilic acid or atoxyl (*p*-amino phenyl arsenic acid) and acetarsone (*m*-acetylamino *p*-hydroxyphenylarsonic acid). In order to subject our presumptive conclusions to the most rigorous proof, the arsenicals were administered to heterogeneous groups of rats by various routes in dosages that previous experience had shown to be well above their respective minimal lethal ranges. *p*-aminobenzoic acid in the form of its soluble sodium salt was given by various routes in dosages well below the lethal range established by Scott *et al.*[6] All surviving rats were observed for at least ten days before being discarded. Results of a few typical experiments representative of the several drugs used are given in Table 1.

## Discussion

Conclusive evidence of the absence of any inhibitory action of *p*-aminobenzoic acid on the trypanocidal potency of the various arsenicals has been obtained. This evidence, together with a discussion of the theoretical implications of the finding, will be presented elsewhere. In the present communication, attention is drawn specifically to the detoxicating action of *p*-aminobenzoic acid against massive doses of the various pentavalent arsenicals used. The protective action is dramatically demonstrated within 24 hours when, as in the case of high intravenous doses of acetarsone, nearly all the control rats are already dead or *in extremis*. On lower but still relatively high doses, particularly when administered by the oral route, some control rats linger on for four or five days, an occasional rat even surviving the experiment. In the earlier stages of the experiment, all the classical signs of pentavalent arsenic poisoning in rats, namely, tremors, gyrations, head tic, incoordination and progressive emaciation, are exhibited by the controls. The number of rats receiving adequate doses of *p*-aminobenzoic acid that show these stigmata is relatively small. Furthermore, some of the treated rats that develop central nervous system disturbances are apparently relieved of their symptoms by the continued administration of *p*-aminobenzoic acid.

In addition to work on this important point, further studies now in progress are designed to determine what pathological lesions due to the arsenical drugs are inhibited by the administration of *p*-aminobenzoic acid. Additional studies of the clinical applications of these findings in man, especially in connection with the arsenical treatment of neurosyphilis, are being undertaken.

[6] C. C. Scott and E. B. Robbins, *Proc. Soc. Exp. Biol. and Med.*, 49: 184, 1942.

## Summary

*p*-aminobenzoic acid has been found highly effective as a detoxicant for high lethal doses of carbarsone and certain other phenyl arsonates in rats.

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## ADSORPTIVE FORCES ACTIVE THROUGH GLASS[1]

A major problem in parts of some oil fields is getting the remaining crude petroleum out of sands that have been water flooded. While sand adheres to water in preference to oil and oil may be driven from an oil-saturated sand by water, if considerable water is already present with the oil in the sand, the usual water drive is not effective. The problem of recovering the remaining oil requires a study of the effect of one liquid in causing a solid particle to adhere more firmly to another liquid.

That sand partly wet with water holds crude oil much more firmly than when no water is present with the oil is easily demonstrated in the laboratory with either loose sand or disks of sandstone. The phenomenon is familiar in many aspects; froth flotation, three component emulsions, the flotation of sand grains at the interface between gasoline and water and between water and air and many others. But how can it be measured quantitatively?

A number of methods were tried and discarded in preliminary work. There appears to be no way to get oil and water to lie side by side in alternate strips on the same glass surface. The method finally adopted was to have oil and water on opposite sides of a very thin glass wall. An easily measurable effect of one fluid through the glass on the other fluid was obtained for a few of dozens of fluid combinations tried.

The method used was simply to flow the oil or other fluid through a long, very thin-walled glass capillary, then repeat with water or other fluid in the water jacket outside the capillary at precisely the same temperature. The ratio of times of flow gives the ratio of the fourth powers of the radii of the capillary and hence the thickness of the adsorbed layer. The capillary tube was drawn from ordinary 8 mm tubing (the fusible type of hard glass worked best) and was about 0.8 mm diameter and a meter long with wall thickness about 0.1 mm. It was mounted vertically by means of the undrawn ends, the upper of which was provided with two scratches to define the volume flowed. 100

[1] Published by permission of the Director, Geological Survey, U. S. Department of the Interior.

seconds is a suitable time of flow, but this varied from 50 to over 500. Both inner and outer surfaces of the capillary must be very clean and dry. If a tube was to be used a second time it was cleaned with strong chromic acid solution. Fluids to be used were left over night close to the apparatus in a room where the temperature seldom varied more than one degree.

Several runs with a sulfur-free crude oil gave a time of flow of 110 seconds with and 103 seconds without water jacket, the ratio $110:103=1.068$, of which the fourth root is 1.0166. The tube radius 0.4 mm divided by 1.0166 is .3935; or the radius is decreased by .0065 mm = 6.5 microns or $6.5\times10^{-4}$ cm. If the adsorbed portion of the oil consists of the larger polar molecules, $6\times10^{-6}$ cm is a reasonable estimate of their length. A layer of these $6.5\times10^{-4}$ cm thick would therefore be about 100 molecules deep. The adsorptive force of silica for water is known to die out after the adsorbed layer becomes about 100 molecules deep ($4\times10^{-6}$ cm). This is of course far too small to be observed by flow methods.

Of over thirty combinations tested, about half showed no observable increase in time of flow due to an exterior liquid and none showed an effect exceeding that of the crude oil-water pair. Fresh soap solution gives an effect nearly as large, but sineresis (and aging) soon bring it below an observable limit. Apparently the long molecules lie parallel to the glass surface. Stearic acid dissolved in chlorex gave 5.8 microns, myristic acid 4.0. With sodium carbonate solution outside, the latter showed the same adsorption (4.0) as with pure water in the water jacket. Apparently the strongly basic sodium ions did not add to the effect of the water. A benzene extract of used fuller's earth showed no observable effect of a water envelope, but an ethylene glycol extract did, 2.1 microns decrease. Triethanolamine and 1 per cent. gelatine solution gave no effect, but crepe rubber in benzene gave 2.8 microns decrease.

In flowing crude oil through a fresh tube, the time of flow became constant only after about five minutes, the first run being about 3 per cent. faster than in the steady state as though several minutes were required to complete the adsorbed film. After application of the water outside, there was a similar delay in coming to an equilibrium. The observed effect is not due to compression for many liquids failed to show it.

There seems to be no question that the adsorption of hydroxyl or other anions on one side of a thin glass wall can enhance the adsorption of electropositive material on the opposite side by a measurable amount through at least 0.1 mm of glass. The action of the glass electrode in measuring pH appears to be of a similar nature. Although cell walls are not impermeable, attractive effects through them may well alter the Donnan equilibrium.

P. G. NUTTING

U. S. GEOLOGICAL SURVEY

# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## THE SEPARATION AND CONCENTRATION OF THE ISOHEMAGGLUTININS FROM HUMAN SERUMS[1]

THE difficulty of obtaining donors who possess high-titred isoagglutinins in their serums made it desirable at the Army Medical School to prepare from random A and B donors human isohemagglutinating serums of uniformly high titer and constant potency. During the course of this study, it was discovered that proteins containing isoagglutinins can be separated from both Group A and B serums with little or no loss of hemagglutinin activity by precipitation with methanol and that after this separation, they can be concentrated to the desired isoagglutinin titer.

The method is simple enough for general use and may be easily applied to large-scale production. The conditions under which separation and concentration is obtained follow.

Ten volumes of pooled group specific human serum obtained from fasting donors are added to 5 volumes of an acetate buffer of ionic strength 0.4[2] and pH 5.4 and to 7.5 volumes of distilled water. The solution is mixed and chilled to 1° C., and this temperature is maintained during the preparation of the concentrated isoagglutinins. Seven and five tenths (7.5) volumes of C.P. methanol (previously chilled to 1° C.) is slowly added with gentle stirring through a capillary tube at the rate of 0.25 volumes per minute to the protein buffer mixture. The final concentration of methanol is 25 per cent., the pH near 6.5 and the final ionic strength about 0.13. The mixture is allowed to stand at 1° C. for one hour. The proteins separating under these conditions are removed by centrifugation in the cold room or in a refrigerated centrifuge at 2,500 r.p.m. for 30 minutes. The precipitated proteins are washed once with 25 per cent. methanol (optional) and as much as possible of the excess methanol solution allowed to drain off by inverting the centrifuge tubes on filter paper.[3]

The separated material is then dissolved in the desired amount of M/15 phosphate buffer of pH 7.8[4]

[1] From the Blood Research Division, Army Medical School, Washington, D. C.

[2] A. A. Green, *Jour. Am. Chem. Soc.*, 55: 2331, 1933.

[3] The precipitate can also be freed of methanol by the application of vacuum.

[4] W. M. Clark, "Determination of Hydrogen Ions," Williams and Wilkins, Baltimore, 1928.