versible changes in the enzymes. In neither case would the added sulfhydryl compounds have any reactivating effect.

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Manuscript received August 7, 1951.

Enzymatic Hydrolysis of Dextran

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The preparation of blood volume extenders from dextran currently is based on partial acid hydrolysis of the bacterial polysaccharide, with subsequent solvent fractionation of the hydrolysate to yield products with average molecular weights approximating those of plasma albumins (*1, 2*). Ingelman (*3*) and Nordström and Hultin (*4*) have reported the presence of enzymes capable of hydrolyzing dextran in filtrates from cultures of *Cellvibrio fulva, Penicillium lilacinum, P. funiculosum,* and *Verticillium coccorum.* The main effect of these enzymes on the polysaccharide is the production of reducing sugars, with the result that they have been of no practical value in the preparation of partially degraded dextrans.

For the past year we have been inoculating dextran-salts solutions with various soil samples, and also allowing such solutions to be exposed to airborne contaminants, in an attempt to isolate organisms capable of attacking dextran. Among a number of such isolates we obtained an *Aspergillus* sp. which has proved useful for the purpose of degrading various dextrans. Filtrates from cultures of the mold in media containing dextran are highly active in splitting the polysaccharide. Only a small amount of reducing sugar is produced during the time required for the filtrates to degrade the polysaccharide into fragments with an average molecular weight in the region of 75,000. By analogy with the α-amylases (*5*), the enzyme formed by this mold probably should be termed an endo-dextranase, since it appears to have a preference for splitting glucosidic linkages remote from end groups. We have found the enzyme to be active against dextrans obtained from 4 different strains of *Leuconostoc.* Active concentrates of the enzyme have been prepared by fractional precipitation of mold filtrates with ammonium sulfate.

A particular advantage of this method of hydrolysis is that the enzyme solution can be allowed to act directly on fermented culture media containing dextran. This eliminates the preliminary precipitation of the polysaccharide with alcohol, as called for in the scheme employing acid hydrolysis (*1*). Since the enzymatic hydrolysis proceeds at room temperature, it is easy to obtain partially hydrolyzed dextrans free from objectionable color.

A typical experiment was carried out as follows: Eight liters of a 20% sucrose medium was fermented with *Leuconostoc mesenteroides* 683, yielding a solution containing dextran in a concentration of 6.1%. Eighty ml of the mold filtrate was added, and enzymatic action allowed to proceed for 40 min, during which time the viscosity of the solution decreased rapidly. The action of the enzyme was stopped by adding alkali to pH 9. The solution was then fractionated with alcohol in the usual manner (*1*), yielding 400 g of a white product with an average molecular weight of 71,000, as determined by viscometry (*6*).

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Manuscript received August 10, 1951.

Blocking of Action of Acetylcholine by Barbiturates

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The barbiturates have proved to be an important group of depressants of the central nervous system since their introduction for clinical trial in 1912 on the basis of the studies of Loewe (*1*), Juliusburger (*2*), and Impens (*3*). In anesthetic doses all the barbiturates inhibit convulsions such as occur in strychnine poisoning, tetanus, and epilepsy. Notwithstanding their continuous use in chemotherapy since their introduction in 1912, evidence of their mode of action has been scanty. Thus Schütz (*4–6*) has shown that there is a progressive decline of cholinesterase activity of human serum and in guinea pig serum, muscle, and certain nervous tissues, during prolonged treatment with a barbiturate. Heinbecker and Bartley (*7*) have shown in their studies with peripheral nerves that phenobarbital increases threshold and at the same time prolongs recovery time of neurones after impulse propagation. Eccles (*8*) showed that phenobarbital blocked two-neurone transmission in the spinal cord primarily by increasing the extent of local depolarization required for initiation of a propagated impulse by motoneurone soma. The author's interest in the

[1] The author wishes to acknowledge his indebtedness to C. L. Prosser, of the Department of Physiology, University of Illinois, Urbana, and J. H. Welsh, of the Harvard University Biological Laboratories, Cambridge, Mass., for their valuable suggestions on techniques in the use of Venus heart.

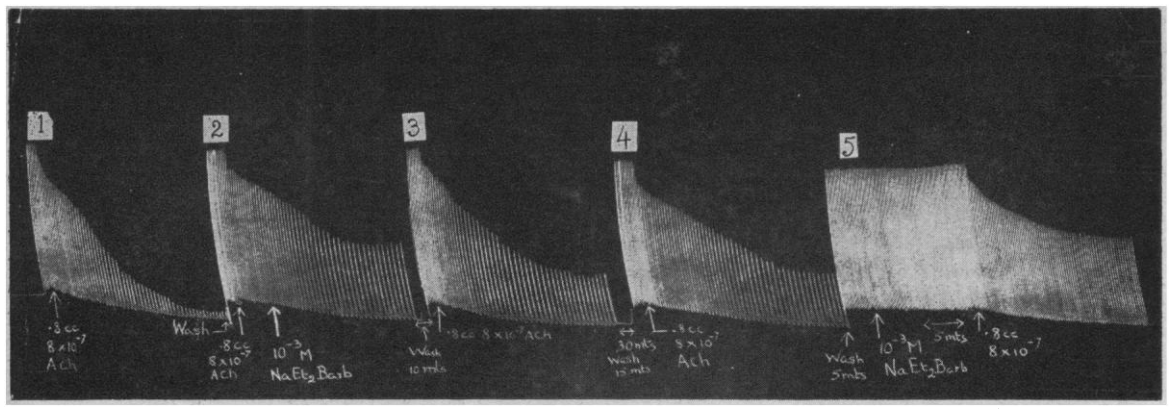

FIG. 1. Record of Venus heart showing blocking of acetylcholine by sodium diethyl barbiturate (Test 1).

barbiturates arose from the fact that these compounds are important antidotes for poisoning of both insects and mammalian subjects by the chlorinated hydrocarbon insecticides. Thus, Bodenstein (*9*) showed that symptoms of DDT poisoning (hyperactivity, tremors, convulsions) in flies (*Drosophila*) could be prevented by injection of 1% phenobarbital, whereas his reciprocal experiments demonstrated that pretreatment of flies by injection of 1% phenobarbital followed by DDT prevents the appearance of symptoms of DDT poisoning. Furthermore, phenobarbital is the only antidote recommended by the U. S. Public Health Service (*10*) for poisoning of humans and domestic animals by dieldrin, one of the chlorinated hydrocarbon insecticides. Therefore, in the course of investigations involving the changes in levels of acetylcholine-like substances in the central and peripheral nervous system of the American roach *Periplaneta americana* (L.), during poisoning by these insecticides, the author felt it might be of value to investigate the action of barbiturates on the activity of acetylcholine.

Hearts of the pelecypod mollusk *Venus mercenaria*, employed earlier for several hours of bioassay of Ach (acetylcholine)-like substances in insect nervous tissue, were used to study the action of barbiturates on the activity of Ach. The high sensitivity of this preparation to Ach was first described by Prosser (*11*), and techniques for its use in bioassay of Ach have been described by Wait (*12*) and Welsh and Taub (*13*). Sea water at 15°–18° C was the fluid used in the bath. A standard solution of Ach was prepared in a 5% solution of sodium dihydrogen phosphate, and concentrations required for tests were prepared immediately before use by dilution of aliquots in sea water. Barbiturates used in this study were the sodium salts of 5, 5-diethyl barbituric acid and 5, 5-phenyl ethyl barbituric acid. Required concentrations of the barbiturates were prepared in sea water before use. Test solutions were introduced at the bottom of the heart bath by way of a 2-ml hypodermic syringe with its needle bent at right angles. The concentrations of Ach represented on all the plates indicate the amount of Ach in g/ml added to the 20-ml heart bath, and those of the barbiturates indicate the final molar concentrations in the 20-ml bath.

Concentrations of 10^{-4} *M* sodium diethyl barbiturate had little or no effect in blocking the action of Ach on

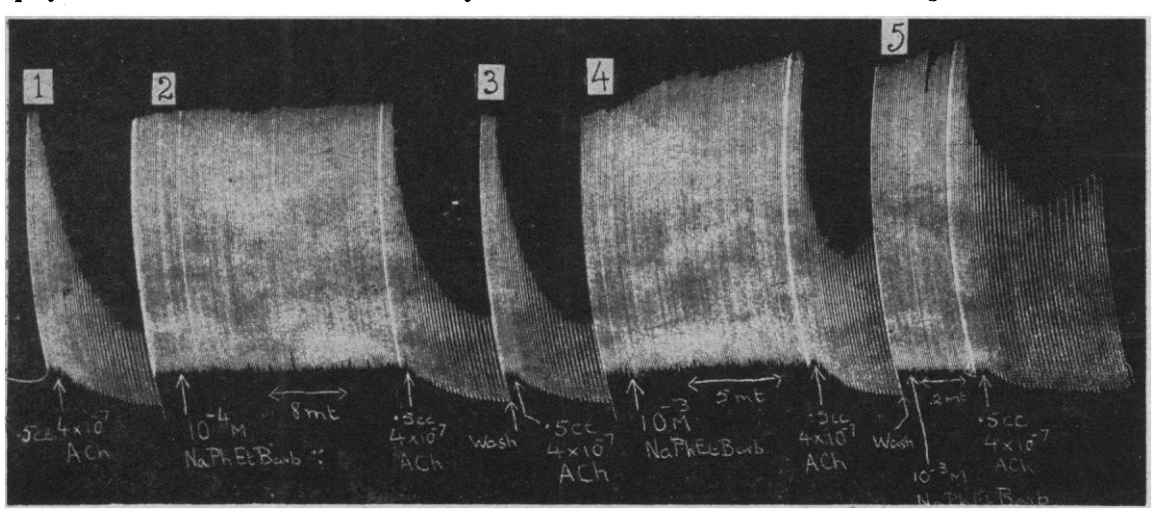

FIG. 2. Record of Venus heart showing blocking of acetylcholine by sodium phenobarbital (Test 2).

the heart. Records of tests with 10^{-3} *M* sodium diethyl barbiturate are shown in Fig. 1. In Test 1, the addition of .8 ml 8×10^{-7} Ach into the heart bath is shown to result in a 95% inhibition of the amplitude of heartbeat. In Test 2, after washing and complete recovery of the heart, .8 ml 8×10^{-7} Ach was added to the bath, followed during inhibition of beat by treatment of the heart with 10^{-3} *M* sodium diethyl barbiturate. Inhibition of the amplitude of the heartbeat was now only 50%, 10^{-3} *M* sodium diethyl barbiturate having blocked 45% of the action of the Ach. Tests 3 and 4 were designed to discover whether the Ach-blocking action of the barbiturate could be washed away, and, if this was not so, how long it lasted. These tests show that a time lapse of 40 min involving about 20 min of washing after barbiturate treatment resulted in only a small reduction of the Ach-blocking action. This makes apparent immediately the impracticability of attempting to compare the action of two or more barbiturates on the same heart. In Test 5, the heart was pretreated for 5 min with 10^{-3} *M* sodium diethyl barbiturate before addition of .8 ml 8×10^{-7} Ach. This treatment resulted in only a 37.5% inhibition of amplitude of heartbeat.

Fig. 2 shows the records of tests with sodium phenobarbital at concentrations of 10^{-4} *M* and 10^{-3} *M*. In Test 1, the addition of .5 ml 4×10^{-7} Ach is shown to result in 71.4% inhibition of amplitude of heartbeat. Test 2 indicates the effect of pretreatment of the heart for 8 min with 10^{-4} *M* sodium phenobarbital followed by the addition of .5 ml 4×10^{-7} Ach to the bath. This treatment resulted in a small blocking of the action of the Ach, for the percentage of inhibition was now 66.25. Test 3 would suggest that this low Ach-blocking action by 10^{-4} *M* sodium phenobarbital is washed away to some extent (percentage inhibition, 69.6). In Test 4, the heart was pretreated for 5 min with 10^{-3} *M* sodium phenobarbital, followed by the addition of .5 ml 4×10^{-7} Ach to the bath. Two results of treatment with phenobarbital are now apparent. First, there is a marked increase in the amplitude of heartbeat (18%), and, second, the action of Ach is blocked more significantly. The percentage of inhibition was 54.5. It was felt, however, that 5 min pretreatment of the heart with the barbiturate was not long enough. The heart was treated again for 2 min with 10^{-3} *M* sodium phenobarbital, and then .5 ml 4×10^{-7} Ach was added to the bath. This further pretreatment with the barbiturate resulted in a more marked blocking of the Ach, since a 42.8% inhibition of amplitude of heartbeat ensued.

Fig. 3 shows the effect of sodium phenobarbital in blocking Ach on a heart more sensitive to Ach than those used in the previous tests. In this series of tests a concentration of Ach was selected which was more than that required for 100% inhibition of the heartbeat. In Test 1, .5 ml 2×10^{-7} Ach is shown to cause complete inhibition of the heartbeat. Test 2 shows the effect of treatment of the heart with 10^{-3} *M* sodium phenobarbital after the heart started toward total inhibition by the addition of .5 ml 2×10^{-7} Ach. Com-

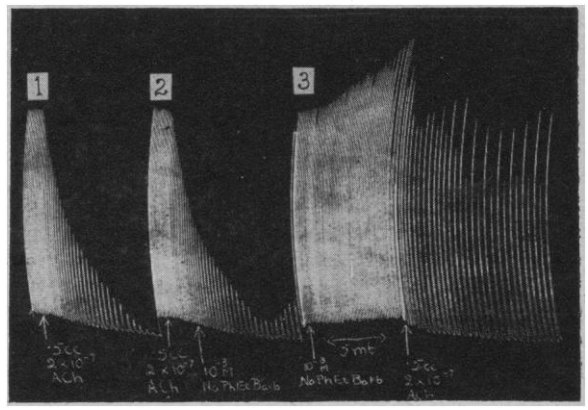

FIG. 3. Record of Venus heart showing blocking of acetylcholine by sodium phenobarbital (Test 3).

plete inhibition of the heartbeat was prevented by this treatment, and it resulted in 91.6% inhibition of amplitude of beat. In Test 3, the heart, after washing and recovery, was pretreated with 10^{-3} *M* sodium phenobarbital for 5 min, followed by the addition of .5 ml 2×10^{-7} Ach to the bath. Here, again, sodium phenobarbital caused an increase in amplitude of heartbeat (33.3%). The percentage of inhibition of amplitude of heartbeat was only 25% under these conditions.

In all tests with the two barbiturates used in this investigation, the effect of blocking the action of Ach on the Venus heart was a consistent feature. However, with preparations that normally gave an inconsistent pattern of beat, the response to barbiturate treatment, like that to Ach treatment, was erratic. Although concentrations of 10^{-3} *M* barbiturate may be regarded as high for test purposes, it must be mentioned that the hearts used in these tests were of low sensitivity to Ach (usual threshold is reported to be between 10^{-10} and 10^{-11} g/ml Ach [*13*]). It may well be possible that with hearts of higher sensitivity to Ach the barbiturates would be effective Ach-blocking agents at concentrations of 10^{-4} *M* or 10^{-5} *M*, as is suggested by a comparison of the results shown in Figs. 2 and 3.

The results obtained here with the barbiturates used are very similar to those obtained by Welsh and Taub (*14*) when investigating the structure-activity relationships of Ach and quarternary ammonium ions. In this work it was found that quarternary ammonium ions with 3 or 4 alkyl groups other than methyl have an excitatory action on Venus heart and also reduce the effect of added Ach, just as sodium phenobarbital and sodium diethyl barbiturate were found to do in the present investigation. Holton and Ing (*15*) and Welsh and Taub (*14*) also found that at least 2 methyl groups are required for Ach-like activity of quarternary ammonium ions on the Venus heart. A study of the action of a large series of barbiturates on Venus heart might provide valuable information on the structure-activity relationships of these compounds and also serve to elucidate any relationship that may exist between these compounds and Ach.

As mentioned earlier, the finding of Schütz (*4–6*),

that prolonged barbiturate treatment results in a decrease of cholinesterase activity in serum and other tissues of mammalian subjects *in vivo,* has been confirmed in our *in vitro* studies with insect cholinesterase. Our studies on the changes in levels of the Ach-like substance in the insect nervous system during the course of poisoning by the chlorinated hydrocarbon insecticides show significant increases of this substance and also suggest a potentiation of the cholineacetylase system during such poisoning. Taking these facts into consideration, it may be suggested that the efficiency of the barbiturates as anticonvulsants depends, if not entirely, at least mainly, on their blocking of the action of Ach. The fact that withdrawal of barbiturate treatment may often give rise to *de novo* convulsions, may be the result, after metabolism of the barbiturate, of the partial destruction or inhibition of cholinesterase by the active barbiturate at its site or sites of action.

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Manuscript received August 10, 1951.

Electron Microscopy of Human Erythrocytes from Healthy and Sludged Blood[1]

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Microscopic observation of the circulating blood of man, ill with a variety of disease processes, has shown that the red blood cells were struck into aggregates which varied in size and rigidity (*1–3*). In many diseases where tough red cell aggregates were seen *in vivo,* and coatings were presumed to hold these aggregates together, such coatings could not be demonstrated *in vitro* by light- or dark-field microscopy. Electron microscopy was undertaken to obtain better definition of the surface of the agglutinated red cells and thus to be able to demonstrate the morphology of the coatings.

The blood vessels of the human bulbar conjunctiva were obliquely illuminated, and a binocular biobjective microscope was focused on the circulating blood, which was studied at 48–150 diameters magnification (*4*). The results of the observations were recorded on a special chart.

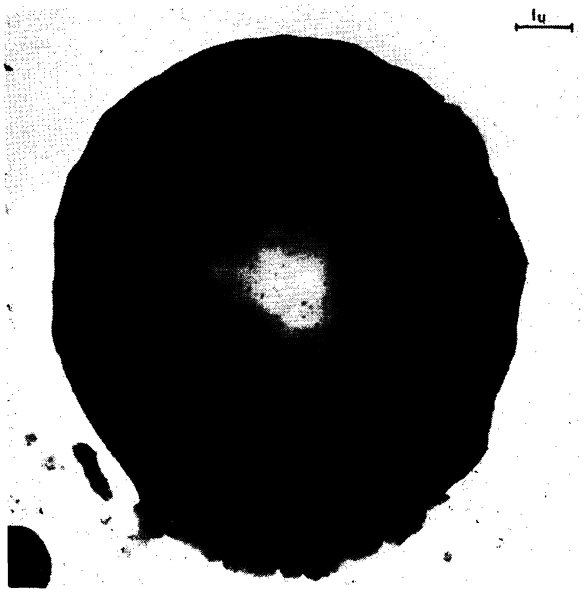

FIG. 1. Electron micrograph of a human red blood cell obtained from a healthy circulation. ×2000. Salt crystals are stuck to the lower border of the cell and can also be seen through center portion.

Two groups of subjects were used. In the first group were those who had a healthy circulation (*2*). This kind of circulation was demonstrated in clinically healthy subjects, as well as in those who were clinically ill but had a disease process which did not produce a sludge, or those who had had a pathologic circulation but had been treated and a normal circulation had been re-established. In the second group of patients were those who were clinically ill and had definite intravascular pathology characterized by the presence of a sludge which significantly reduced flow through arterioles. Such patients often had red cell aggregates with visible coatings.

Each patient's circulation was studied 24 hr before and immediately prior to the withdrawal of the blood for electron microscopy. The blood was obtained from the median cubital vein of the arm and was drawn directly into heparin, with the ratio of blood to heparin varying from 1:1 to 1:6. After withdrawal the blood and heparin were gently agitated in the syringe, and then 0.2–1.0 ml of the mixture was slowly ejected into 8–10 ml of mammalian Ringer's solution.

Electron microscopy of such blood was usually made within a period of 30–60 min after withdrawal. The diluted blood was prepared for electron microscopy by placing a small drop of the mixture upon a Parlodion film, which was placed on a 200-mesh screen. After 2 min the blood droplet was removed by absorption into filter paper, and the film with adhering red blood cells was air-dried and placed on the object holder. The

[1] This investigation was supported in part by a grant from the Life Insurance Fund.
[2] Established Investigator, American Heart Association.