ronidase is still present in the cultures when tested on rabbit skin, and the resulting spreading of any virus left in the inoculum should lead to enhanced lesions.

In all the cases where total inactivation of the virus was obtained the effect was noticeable from the first or second passage by progressive reduction of titer and of size of the lesions till complete suppression. It is important to point out that we have occasionally obtained unpredictable results with some of the steamed preparations of hyaluronic acid. These preparations inactivated the virus in some experiments but failed to do so in others, the same happening when hyaluronidase was added. An effort is being made to elucidate the cause of the phenomenon, keeping in mind the possibility that steaming might affect the hyaluronic acid in such a way as to permit the development of resistant variants; in one instance, at least, we suspect this to be the case.

When passing the cultures by cells, we obtained only complete inactivation of the neuro vaccine with autoclaved preparations from umbilical cord in 2 out of 3 cases. All the other preparations, with or without hyaluronidase, were ineffective, in the course of perhaps 20 passages, except for a decrease in titer of 1–2 tenfold dilutions below the control.[6]

The protection thus afforded the virus by the cell seems to indicate that the effect of the polysaccharide preparation is directly on the virus and not on the cells, resulting in interference with the process of virus multiplication. To investigate this point further the hyaluronic acid preparation was added to the media from 30 min to 24 hr before or after being seeded with the virus, with no differences in the results. Still another test was to inject intradermally in the rabbit the washed cells of cultures the supernatant fluid of which, undiluted, had become inactive through the effect of the hyaluronic acid preparation: vaccinial lesions were obtained of about the same size and severity as those induced by the cells of control cultures.

Several of the conditions in our experiments and phenomena observed therein duplicate what takes place during infection of the animal. First of all, the concentration of hyaluronic acid, at about 1:1000, was not above what can be roughly assumed is present in the ground substance of several tissues, even disregarding a probable increase of the polysaccharide during inflammation. Further, it is well known that the hyaluronic acid of this ground substance is depolymerized and hydrolyzed by hyaluronidase as well as by other means, and it is this specific enzyme that, attacking its substrate in our cultures, brought to a maximum the inactivating effect of hyaluronic acid on vaccinia. Finally, the generally recognized fact in animal infection of the protection against injurious agents afforded to viruses by cells was also clearly observed in our tests regarding the effects of the hyaluronic acid preparations.

This investigation should obviously be expanded before what is above suggested becomes a conclusion, but if the results obtained so far do in any way duplicate what happens in the organism during infection it could be assumed that hyaluronic acid, and conceivably other polysaccharides, of the ground substance, especially after its degradation during infection, act to inactivate vaccinia and other infectious agents.[7] If this were so the ground substance besides being a *mechanical* barrier (*1*) would also be a *sterilizing* barrier to the progress of infection.

[6] The cell passages were carried out by transfer of most of the sediment obtained by light centrifugation of the cultures, so that in the successive passages cells progressively accumulated in amounts high above those generally employed in virus cultures. Incidentally, this method of cell passage has resulted in the practically indefinite maintenance of ordinary cultures, which, if passed by transfer of the supernatant fluid, would soon have been lost.

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[7] Alice E. Moore, of the Sloan-Kettering Institute, kindly agreed to test some of our preparations of hyaluronic acid on the virus of Russian encephalitis. Her experiments, although preliminary, confirmed with that virus the results we obtained on vaccinia.

Manuscript received August 3, 1951.

Reversal of the Diabetogenic Action of Alloxan by Sulfhydryl Compounds

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Large doses of sulfhydryl compounds, given prior to intravenous diabetogenic doses of alloxan, prevented diabetes in rats (*1, 2*). Prevention failed to occur, however, when the sulfhydryl compounds were given 5 min after the alloxan. Thus the action of alloxan could not be reversed.

The fundamental action of alloxan is completed within a few minutes after it reaches the blood, the necrosis of the β-cells of the islets of Langerhans being the ultimate result of this initial action (*3*). Attempts to reverse the action of alloxan should therefore be made after the completion of the action but before the onset of β-cell degeneration. The action of alloxan carried to its final stage of cell necrosis is not likely to be reversed. In rats changes in the β-cells only become evident microscopically 24 hr after intraperitoneal injection of alloxan as contrasted to their early appearance (5 min to 1 hr after injection) when alloxan is given intravenously (*4*). This suggested reinvestigation of the problem after intraperitoneal or subcutaneous administration of alloxan. The subcutaneous route was preferred, as it produced diabetes more consistently. A 175 mg/kg dose of alloxan produced diabetes in 100% of young rats

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previously fasted for 24 hr. This dose was used throughout the experiments. BAL and cysteine were the sulfhydryl compounds used.

Young rats, each weighing about 100 g and previously fasted for 24 hr, were subcutaneously injected with 175 mg/kg dose of alloxan. Solution of BAL in arachis oil (Boots) was then given to the rats intramuscularly at varying intervals. The presence of sugar in the urine for 2 consecutive days and a blood sugar value over 160 mg% for a similar period of time were considered to indicate a diabetic condition. The results are given in Table 1.

TABLE 1

EFFECT OF INTRAMUSCULAR INJECTION OF BAL ON PREVENTION OF DIABETES PRODUCED BY SUBCUTANEOUSLY INJECTED ALLOXAN IN 175 MG/KG DOSE*

No. rats used	Interval between administration of: Alloxan and first dose of BAL (75 mg/kg)	First and second doses of BAL (50 mg/kg)	Second and third doses of BAL (50 mg/kg)	Diabetic rats (%)
5	10 min	Not injected	Not injected	100
6	10 "	2–3 hr	" "	30
6	10 "	1 hr	1 hr	0
5	15 "	Not injected	Not injected	100
8	15 "	2–3 hr	" "	75
6	15 "	1 hr	1 hr	0
3	30 "	2–3 hr	Not injected	100
6	30 "	1 hr	1 hr	50
10	30 "	½ "	1 "	20
3	60 "	1 "	1 "	100
6	60 "	½ "	1 "	100

* BAL was given after the alloxan.

In the course of the above studies the effect of the administration of BAL before the alloxan was also studied. A 75 mg/kg dose of BAL when given 15 min before alloxan offered complete protection from diabetes. The same dose of BAL when given 10 min after alloxan could not, however, prevent diabetes (Table 1). Thus the fundamental action of the subcutaneous dose of alloxan was completed within this period of time. Repeated administration of BAL, however, offered considerable protection even when the treatment with BAL was started as late as 30 min after alloxan. Pancreas of rats in which diabetes had been completely prevented by BAL showed normal islet tissue. Thus the prevention of diabetes was essentially due to a reversal of the fundamental action of alloxan.

In the above-described experiments maximum protection was obtained with a 175 mg/kg total dose of BAL given in divided doses—a first dose of 75 mg/kg, followed by a second dose of 50 mg/kg ½ hr later and a third similar dose after another hour. These experiments were repeated with cysteine-HCl, which

TABLE 2

EFFECT OF REPEATED ADMINISTRATION OF CYSTEINE-HCl ON PREVENTION OF DIABETES PRODUCED BY SUBCUTANEOUSLY INJECTED ALLOXAN IN 175 MG/KG DOSE*

No. rats used	Interval between the administration of: Alloxan and first dose of cysteine-HCl (200 mg/kg)	First and second doses of cysteine-HCl (125 mg/kg)	Second and third doses of cysteine-HCl (125 mg/kg)	Diabetic rats (%)
10	30 min	30 min	1 hr	30
3	60 "	30 "	1 "	100

* Cysteine-HCl was given subcutaneously after the alloxan.

was given subcutaneously in aqueous solution without prior neutralization. A total of 450 mg/kg dose of cysteine-HCl approximately equivalent in its SH content to a 175 mg/kg dose of BAL was administered in proportionately divided doses and at the same rate as BAL. The first dose of cysteine was given only 30 and 60 min after alloxan. Earlier administration of cysteine was considered unnecessary. The results given in Table 2 show that repeated administration of cysteine also offered protection from diabetes and that the degree of protection was practically the same as with the corresponding dose of BAL.

The effect of the total 175 mg/kg dose of BAL given in a single injection after alloxan could not be studied because of the lethal effect of such a single dose on rats. The corresponding 450 mg/kg dose of cysteine-HCl was found to offer no protection when given in a single injection as early as 10 min after alloxan. This, together with the general sequence of the results obtained, shows that maintenance of a high concentration of the sulfhydryl compounds in the blood for a fairly long period of time was essential for the reversal of the action of alloxan.

Inhibition of essential sulfhydryl enzymes of the β-cells has been suggested as the mechanism of the diabetogenic action of alloxan (*1*). The reversal of the action of alloxan by BAL and cysteine reported here adds considerable strength to this view. The mechanism of the inhibition of the enzymes, however, seems to consist of oxidation of the essential SH groups to the —S—S— stage and not of formation of addition compounds between alloxan and the enzymes, as has been suggested by Lazarow and his co-workers (*2, 5*). Had the latter hypothesis been true, cysteine, which does not form an addition compound with alloxan (*5*), would be incapable of reversing its action. Both BAL and cysteine could not prevent diabetes when given 1 hr after alloxan. The inhibition of the enzymes was probably carried to an irreversible stage after this period of time. This may be due either to a further oxidation of the SH groups beyond the —S—S— stage, or to the onset of subsidiary irre-

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.