ried out successfully, entails no blood loss. During the period of temporary portal vein obstruction, the intestines become markedly cyanotic, but normal color is regained immediately upon completion of the anastomosis and release of the temporary ligatures. The operation requires about 30 minutes. The major portion of this time is spent in preparing the renal vein and glass sleeve for insertion into the portal vein. The actual period of obstruction of the portal vein need not exceed 5 minutes.

If the hepatic artery be left patent, the rat will survive for an indefinite period with a complete portal-caval shunt (direct Eck fistula). These animals appear to be perfectly normal. Animals sacrificed 5 days after establishment of the portal-caval shunt show patent functioning anastomoses without evidence of portal obstruction. Secondary or delayed ligation of the hepatic artery may then be carried out at a later date.

Discussion. The survival time in a series of 12 unfasted adult rats averaged 11½ hours (range: 5-17 hours). The technic of nonsuture anastomosis of the portal vein to the inferior vena cava via the left renal vein obviates the use of a cannula directly in the bloodstream, and further does not require heparinization of the animal. The animals recover readily from the anaesthetic and seem normal for about two-thirds of the survival time, when they become progressively less active. Death usually occurred after a series of convulsive attacks. The animals were injected hourly after operation with glucose in saline solution by the subcutaneous route. At autopsy, the portal-renal anastomoses were patent. The liver was pale yellow in color and practically bloodless on incision. It is thought that the functionally hepatectomized rat or the rat with a portal-caval shunt is a useful preparation for experimental use.

Summary. A method is described whereby the portal vein is directly anastomosed to the left renal vein in the rat by a nonsuture method of blood vessel anastomosis. The complete direct Eck fistula produced in a one-stage operation coupled with ligation of the hepatic artery results in the preparation of a functionally hepatectomized animal. The survival time in a series of 12 rats thus prepared averaged 11½ hours.

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Viruses Inactivated by Mustard (*Bis*(β-chloroethyl) Sulfide) as Vaccines.

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For some years we have been using the rapidly advancing knowledge of protein chemistry in an attempt to find an agent other than formaldehyde that would inactivate viruses without destroying their antigenic nature. Formaldehyde reacts primarily with amino groups of protein but it also attacks other radicals. While some formalinized virus preparations are good immunizing agents many are not satisfactory, perhaps because the virus is not present in sufficient quantity. Reagents known to react with amino, tyrosine phenol, or SH groups of protein and several detergents have been used with disappointing results. Either the virus was not completely inactivated or else it failed to immunize animals to the active virus. It has been shown that mustard ($Cl—CH_2—CH_2)_2—S$ reacts with proteins[1] and it is our purpose here to report the tests we have made on the effect of this chemical on a number of viruses.

Eastern equine encephalomyelitis virus, fixed rabies virus, and hog cholera virus have been treated with chemically pure mustard.

[1] Berenblum, I., and Wormall, A., *Biochem. J.*, 1939, **33**, 75.

TABLE I.
Immunity Produced by Eastern Equine Encephalomyelitis Virus 2 Times Saturated with Mustard.

Immunizing injection 0.5 ml into right pad. Dilution used	Time after preparation and results			
	24 hr	2 wks	3 mo.	6 mo.
10^{-1}	0/4*	0/3	0/4	0/4
10^{-2}	0/2	0/3	0/4	0/4
10^{-3}	—	—	1/4	2/4
Controls	2/2	3/3	4/4	3/4

* Numerator—No. of animals that died.
Denominator—No. of animals inoculated.

For most of our experiments virus-containing suspensions were twice saturated, but in some instances they were half saturated by mixing an equal quantity of freshly saturated and centrifuged cold salt solution with the virus material. Care was taken to keep the reaction of the materials between pH 7 and 8 and at 25°C. Contrary to our previous results with other chemical reagents, we have had no difficulty in completely inactivating these viruses and all of the inactive preparations have had some immunizing ability.

Supernatants from suspensions of chick embryos, dead following inoculation with eastern equine encephalomyelitis virus, twice saturated with mustard are completely inactive and appear to be as good immunizing agents as any formalinized preparations. A typical experiment, given in Table I, shows that as little as 0.5×10^{-2} ml immunized guinea pigs regularly up to 6 months after its preparation. The material was injected into the right pad and the test for immunity was made by injecting suspensions of brain of infected guinea pigs into the left pad.

Fixed rabies virus in the form of suspensions of brain from infected guinea pigs, mice, and a dog has been treated and tested for its immunizing ability. Inactivation has been regularly obtained. In Table II are the results of one experiment in which brain from an infected dog was treated with different amounts of mustard and compared with vaccines prepared by adding 1% phenol and 1% chloroform. Guinea pigs were used for the experimental animals and received 2 intraperitoneal injections of 1% brain at an interval of 7 days. Two weeks after the last injection their immunity was tested by intramuscular injection of fixed rabies virus in guinea pig brain. The half saturated suspension gave complete immunity, while the once saturated material and the phenol or chloroform inactivated virus gave questionable protection.

TABLE II.
Immunity Produced by Fixed Rabies Virus.

Inactivation of vaccine	Results of intramuscular injection of active virus. Dilution	
	1/10	1/50
1 X saturated with mustard	4/4	1/4
1/2 saturated with mustard	0/12	0/11
Phenol treated	4/4	2/4
Chloroform treated	3/4	3/4
Controls	4/4	3/4

Hog cholera virus both in the form of sera from infected animals bled on the 4th day of fever and of organ suspensions has been used after two saturations with mustard. The test for immunity was made 3 weeks after the last injection by housing the animals with an infected pig. All of 9 control pigs died and showed typical lesions of hog cholera. Of 23 vaccinated pigs, 2 died from causes other than hog cholera, 3 died from acute hog cholera, and the remaining 18 showed some degree of immunity. All had an increased temperature; in one animal it lasted 1 day, in most of the others it lasted from 3 to 5 days, while in a few it persisted longer than this, but all survived the infection.

While there is much work yet to be done the results obtained show that mustard will inactivate the viruses tested and that the inactivated materials can be successfully used as vaccines.