containing approximately 5,000 units of penicillin/ml (4.1% content of solids including phosphate buffer) was added in 0.5-ml volume/flask.

TABLE 1

INHIBITION OF GLYCOLYSIS OF MOUSE TISSUES BY IMPURE PENICILLIN

No. of experiment	Liver				Muscle				Sarcoma			
	Aerobic		Anaerobic		Aerobic		Anaerobic		Aerobic		Anaerobic	
	C*	IP*	C	IP	C	IP	C	IP	C	IP	C	IP
1	58	30	64	37	68	37	75	39	59	41	29	16
2	61	32	69	39	71	40	88	46	60	45	29	16
3	57	29	64	31	66	30	79	40	56	43	26	14
4	59	31	66	35	65	33	86	44	57	46	29	14
5	63	30	70	40	69	41	82	45				
Average	60	30	66.7	36	68	36	82	43	58	44	28	15
Difference	50%		46%		47%		48%		24%		47%	

* C = control; IP = impure penicillin added.

Results are given in Table 1. These show that both aerobic and anaerobic glycolysis of liver and muscle homogenates was equally inhibited, suggesting that the site of action of the impure penicillin is in the anaerobic part of the glycolytic cycle. The sarcoma gave a higher aerobic than anerobic glycolysis, which is typical for malignant tissues. The impure penicillin caused less inhibition of the aerobic glycolysis of the sarcoma, but inhibited its anaerobic glycolysis to the same degree as in normal tissues.

Crystalline penicillin G (15,000 units/ml) had no effect on glycolysis, indicating that the inhibition of glycolysis is due to some other factor present in the impure preparation. It cannot yet be stated whether or not the glycolysis-inhibiting factor is identical with the factor responsible for the protection of mice against endotoxins.

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IN THE LABORATORY

Benzene-Ether Extracted Rabies Vaccine

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In 1946–47 DeBoer and Cox (*1*) described a method for the preparation of complement-fixing antigens from brain by benzene (C_6H_6) extraction. Espana and Hammon (*2*) confirmed the validity of the principle and slightly modified the method of extraction. It occurred to us that the procedure might be adapted to the removal of lipid from brain tissue, as an initial step in the purification of rabies vaccine. Previously we had attempted by various common procedures to separate rabies virus from tissue elements. In some instances it seemed that the lipids of the brain may have interfered with sharp separation. In view of the results obtained by DeBoer and Cox, we attempted the preparation of vaccines by extracting lipids with various solvents.

Earlier experiences with the exposure of rabies virus to ether revealed that the virus is quite labile to ether when wet. Consequently, the first step was drying of the homogenized, infected, brain tissue from a frozen state under vacuum. Various lipid solvents were then added to the dry tissue, the volumes in each case being about twice the wet volume of the original tissue suspension. After a period of about 2 hrs in the cold room, the solvent was removed either by centrifugation and decanting or by filtration through a sintered glass filter of "C" porosity. In either case the sediment (or residue) was resuspended in the solvent and immediately recentrifuged or refiltered. Following the extration with benzene or other solvent, ether [$(C_2H_5)_2O$] extraction was done twice in a similar way but using ether at – 50° C. The tissue was then freed of the small amount of residual ether by placing the container in a jar and exhausting by water suction. Rehydration was accomplished simply by shaking with water and churning in a Waring blendor. The tissue suspensions were stored

in the refrigerator at +5° C, and samples were removed at intervals for the vaccination of mice, utilizing the technique of Habel and Wright (*4*).

TABLE 1

POTENCIES OF ANTIGENS EXTRACTED WITH VARIOUS SOLVENTS

Solvent used	Viability at 10^{-3} dil.	Antigen LD_{50} protection
Acetone	–	15,000
Benzene	+	290,000
Carbon tetrachloride	–	100
Dichlorethylene	–	3,000

The results of the first test of antigens extracted with various solvents are presented in Table 1.

The virus used in this experiment was not inactivated before extraction, and it was found after extraction that benzene had failed to kill all of the rabies virus. No viable virus was demonstrated at 10^{-3} dilution following extraction with the other solvents. Since benzene yielded the most potent antigen and was found to be least toxic to the virus, further experiments were undertaken using this solvent for extraction. It was determined that extraction as outlined above effected a marked reduction of the lipid content of the dry vaccine. The effect of cold benzene and ether on the titers of virus in two different lots is presented in Table 2.

TABLE 2

THE EFFECT OF DRYING AND OF BENZENE-ETHER EXTRACTION ON THE TITERS OF RABIES VIRUS

	Original infective titer	Infective titer after drying	Infective titer after extraction
1)	10,000,000	1,000,000	1,000
2)	501,000	13,000	400

Since extractions with benzene and ether in the cold did not completely inactivate rabies virus, subsequent extractions were done on mouse brain suspensions previously inactivated by ultraviolet light (*3*, *5*). In each case another sample of the same irradiated lot, dried but not extracted, was used as a control. In two experiments the dried, or dried and extracted, tissue was treated with phenol to kill the virus. The relative potencies of extracted and unextracted vaccines are compared in Table 3.

TABLE 3

COMPARISON OF THE POTENCIES OF EXTRACTED AND UNEXTRACTED, NONVIABLE VACCINES

Experiment No.	LD_{50} protection by control vaccines (unextracted)	LD_{50} protection by extracted vaccines
1*	0	36,000
2	10,000	274,000
3	10,000	274,000
4	568,000	568,000
5	4,000	400
6†	700	9,000
7†	100	9,000

* The source material was an outdated vaccine.
† Phenol killed.

It is evident that the antigenicity of extracted vaccines is as good as, or better than, that of unextracted material of the same lot in most instances. No explanation is offered for the observation, but the experiments listed, and others to be reported later, consistently suggest significant difference in favor of the extracted vaccines. In our hands, experimental phenolized vaccines have tended to be of lower antigenicity than corresponding irradiated vaccines.

It has thus been found that extraction of rabies vaccine with cold benzene followed by cold ether will remove much of the lipid (41.5% of the total dry weight) of vaccine. The process causes a marked reduction in the infective titer of rabies virus in brain tissue, but does not kill all of the virus under the conditions described. Rabies vaccines killed with ultraviolet light and extracted do not lose antigenicity, but in most instances are actually improved as antigens. As a result of our experiments we have concluded that benzene and ether-extracted vaccines may offer some advantage over unextracted vaccine for the prevention of rabies. However, the greatest advantage of lipid extraction is the elimination of one of the major nonspecific components of a very crude vaccine.

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A Constant-Temperature Micromanipulation Chamber

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Although the constant-temperature micromanipulation chamber to be described here was developed in connection with certain studies in progress on mammalian tissue, it, unlike the special constant-temperature apparatus described by Peterfi (*3*), has a wide range of application not only in this general field of investigation but also in other fields not employing the micromanipulative technique. This instrument was designed specifically for use with the Chambers micromanipulator (*1*), but its basic

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