TABLE II.
Inactivation of Penicillin by Thioglycolic Acid.

Thioglycolic Acid Mg	Penicillin Oxford Units	pH	Destruction %
200	40	6.4	95
50	40	6.6	30-50
12.5	40	6.7	0
3.1	40	6.8	0
0.0	40	6.5	0
200	40	7.6	95
50	40	7.8	88
12.5	40	8.0	50
0.0	40	7.8	0

The reaction was allowed to proceed at 37° for 48 hours.

pounds and cysteine suggests that a free sulfhydryl group is essential for the destruction of the antibiotic activity. If this hypothesis is correct, one might expect thioglycolic acid to be able to inactivate penicillin. The results of a typical inactivation experiment with thioglycolic acid at different pH's are given in Table II.

Thus thioglycolic acid is a far less potent inactivating agent than cysteine. As a matter of fact, we found that simple mercaptans will not inactivate penicillin. These results, therefore, indicate that the inactivation of penicillin by cysteine involves more than the sulfhydryl group.

Summary. The antibiotic activity of penicillin can be easily abolished by cysteine in a slightly alkaline solution. The rate of inactivation is dependent on the amount of cysteine and pH. In an atmosphere of nitrogen, one mg of penicillin can be inactivated by several mg of cysteine at pH 7.8, but to a lesser extent with thioglycolic acid. Inactivation of penicillin does not take place if other amino acids, *e. g.* cystine, methionine, serine, or glycine are used. Hence we believe that the inactivation of penicillin by cysteine is a chemical reaction and that it may involve both sulfhydryl and amino groups of cysteine.

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Seasonal Fluctuations in Susceptibility of Guinea Pigs to Experimental Cavian Poliomyelitis.*

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Following the adaptation of SK poliomyelitis virus from monkey to mouse through intermediate cotton rat passage, further transmission of the murine strain to guinea pigs has been described.[1,2] A fixed strain of cavian virus was finally obtained which has now been carried over more than 70 serial passages. The disease thus produced in guinea pigs is characterized by the occurrence of flaccid paralysis, and the lesions in the central nervous system of paralyzed animals are "remarkably like those of poliomyelitis in monkey and man."[3] Serological identity of the murine and cavian strain was established by cross neutralization tests in mice and in guinea pigs. As far as any relationship between simian and cavian polio-

* Aided by grants from the Dr. Philip Hanson Hiss, Jr., Memorial Fund, the Warner Institute for Therapeutic Research, and anonymous donors.

1 Jungeblut, C. W. and Sanders, M., *J. Am. Med. Assn.*, 1941, **116**, 2136.

2 Jungeblut, C. W., Feiner, R. R. and Sanders, M., *J. Exp. Med.*, 1942, **76**, 31.

3 Wolf, A., *J. Exp. Med.*, 1942, **76**, 53.

TABLE I.
Incidence of Paralysis in Guinea Pigs Following Intracerebral Injection with Cavian or Murine Virus at Different Times of the Year.

Dates of Injection	Cavian Virus (Current Harvest)		Murine Virus					
			Current Harvest		Winter Harvest*		Summer Harvest†	
1/42	7/8	25/26=96%	5/8	11/16=69%				
2	10/10		3/4					
3	8/8		3/4					
4	3/4		3/3					
5	4/4		6/8					
6	4/4	14/14=100%	1/4	11/27=41%	2/4	11/24=46%		
7	4/4		2/8		4/8			
8	4/4		5/11		1/4			
9	2/2		3/4		4/8			
10	2/4		2/4		2/4			
11								
12			2/2	10/13=77%			3/4	14/28=50%
1/43	3/4	6/8=75%	2/3					
2	3/4		3/4				10/23	
3			3/4				1/1	
4								
5	4/7		3/4					
6			3/3	4/10=40%	3/3	4/10=40%		
7	2/4	6/8=75%	0/3		1/4			
8	4/4		1/4		0/3			
9								
10								
11								
12	2/2						8/12=67%	
Totals	66/77=86%		50/85=59%		17/38=45%		22/40=55%	

Numerator = No. of paralyzed guinea pigs; denominator = No. of injected guinea pigs.
* Feb. '42 harvest tested June-Sept. '42; Feb. '43 harvest tested June-Aug. '43.
† June-Aug. '42 harvest tested Dec. '42-Mar. '43; June-July '43 harvest tested Dec. '43.

myelitis virus is concerned, the available evidence rests on the following observations: (1) similarity in the symptomatology and pathology of the two infections, (2) limited but definite pathogenicity of the cavian virus for rhesus monkeys, (3) inability to recover injected cavian virus from the tissues of monkeys previously paralyzed by simian virus, and (4) inactivation *in vitro* of cavian virus by antiserum of a horse hyperimmunized with simian virus. Confirmatory evidence that the simian SK virus can be acclimated from cotton rat to guinea pigs with the production of paralysis, has been adduced by Toomey and Takacs.[4] More recently, another strain of human poliomyelitis virus, *i. e.* MM, has been adapted from man to mouse by intermediary hamster passage.[5] Rodent MM virus was found to be highly pathogenic for guinea pigs, producing symptoms and lesions similar to those induced by rodent SK virus.[6]

In the course of the early experiments with rodent SK virus certain observations were made which suggested the controlling effect of season upon the frequency with which transfer of the murine strain from mice to guinea pigs resulted in the evolution of paralysis. Thus, transfers of murine virus carried out during the winter months of 2 successive years caused a significantly higher incidence of paralysis in the injected guinea pigs than transfers carried out during the intervening summer months. The guinea pigs, however, which had escaped paralysis carried specific neutralizing substances in their serum and proved refractory to repeated reinoculation at later dates. The present communication reports additional observations on the same subject which serve to confirm and amplify these earlier findings.

The SK murine strain was propagated, in serial transmission, by intracerebral passage

[4] Toomey, J. A. and Takacs, W. S., *J. Bact.*, 1942, **43**, 87.

[5] Jungeblut, C. W. and Dalldorf, G., *Am. J. Publ. Health*, 1943, **33**, 169.

[6] Jungeblut, C. W., *J. Exp. Med.*, 1945, **81**, 275.

from mouse to mouse; similarly the SK cavian strain was propagated by serial intracerebral passage from guinea pig to guinea pig. At monthly intervals, intracerebral transfers were made of currently harvested murine virus from mouse to guinea pig. To further analyze the problem, mouse virus was harvested in the winter and, after cold storage in glycerine, was transferred intracerebrally to guinea pigs in the following summer; conversely, mouse virus harvested in the summer, after cold storage in glycerine, was transferred intracerebrally to guinea pigs in the following winter. The amount of virus (murine or cavian) used was 0.1 cc of a 10^{-1} viral brain suspension which represented from 10 to 20 minimal paralytic guinea pig doses; the guinea pigs weighed between 225 and 275 g and were of uniform stock, having been obtained from one dealer throughout this work. The number of animals involved, the dates of transfer, and the incidence of paralysis observed are recorded in Table I.

It is obvious from the data presented in Table I that serial propagation of fixed cavian virus in guinea pigs was not significantly influenced by seasonal fluctuation of any sort, the incidence of paralysis ranging between 75 and 100% through winter and summer periods alike. In contrast herewith, however, the mode of transmission of murine virus from mouse to guinea pig seemed to function more effectively in the winter than in the summer. Thus, current transfers of murine virus during the winter months of 2 years (Jan. '42-March '42; Dec. '42-March '43) paralyzed 69 or 77% respectively, of the injected guinea pigs, whereas current transfers during the summer months of the same 2 years (June '42-Sept. '42; June '43-Sept. '43) paralyzed only 41 or 40%, respectively, of the injected guinea pigs. While the percentages given for each year *per se* do not differ significantly, considered as a whole the data do assume statistical significance. It appears further that winter-harvested mouse virus, when tested in summer-guinea pigs, caused appreciably less paralysis (46 to 40%) than it had produced in winter-guinea pigs (69 to 77%). Again, in this case, we need the evidence of both years to assert statistical significance. It would finally seem that the incidence of paralysis obtained with summer-harvested mouse virus ran consistently higher in winter-guinea pigs (50 to 67%) than in summer-guinea pigs (41 to 40%), even though, in this instance, the number of animals involved is not sufficient to eliminate the element of chance. The possibility that the virus had undergone appreciable deterioration upon storage can be discounted since earlier experience had shown a remarkable stability of glycerinated virus when kept for 1 year in the icebox. We are indebted to Dr. John W. Fertig, professor of Biostatistics at the De-Lamar Institute of Public Health, Columbia University Medical School, for the statistical appraisal of the figures given above.

The above data form a pattern, with a distinct trend, that has now been traced over a period of 3 continuous years. It seems reasonable enough to assume that the periodic drops in the incidence of paralysis observed in guinea pigs following injection with murine virus at different times of the year are caused, preponderantly, by seasonal variation in the receptivity of the host. However, since the disease in mice runs also a somewhat milder course during the summer months,[7] the possibility cannot be dismissed that concomitant changes in the quality of the virus at the source were a contributory factor. The observed phenomenon, in many ways, shows similarity with the well-known seasonal fluctuation in the response of guinea pigs to diphtherial toxin.[8] The precise mechanism of such seasonal variations in the susceptibility of laboratory animals to infectious or toxic agents is still obscure. However, to suspect that the experimental data—even though difficult to interpret at present—bear some valid relationship to the factors that control the epidemiology of the corresponding diseases in man, would be at least a justifiable working hypothesis.

Summary. Serial propagation of cavian poliomyelitis virus from guinea pig to guinea pig was not significantly influenced by seasonal fluctuation of any sort. However, the

[7] Jungeblut, C. W., Sanders, M. and Feiner, R. R., *J. Exp. Med.*, 1942, **75**, 611.

[8] Suedmersen, H. J. and Glenny, A. T., *J. Hyg.*, 1909, **9**, 399.

mode of transmission of murine poliomyelitis virus from mouse to guinea pig functioned more effectively in the winter than in the summer, as judged by the ratio between paralyzed and non-paralyzed animals. The described phenomenon appears to be due, preponderantly, to cyclic variations in the susceptibility of the guinea pig to the paralyzing effect of the virus when passed by intracerebral injection from one host to another.