TABLE 1

THE RECOVERY OF ESTROGENIC ACTIVITY FROM THE PERFUSING MEDIUM IN VARIOUS PERFUSIONS OF RAT ORGANS *in vitro* WITH α-ESTRADIOL

Experiment No.	Organ perfused	Perfusion medium	Amount of α-estradiol in perfusate (r.u.)	Perfusion time (hours)	Activity recovered from perfusate (r.u.)* Total	Weak phenolic non-ketonic (estradiol)	Weak phenolic ketonic (estrone)	Strong phenolic (estriol)	Remarks
1	Liver	25 cc defibrinated blood, plus 100 cc Ringer-L.	3200	3	1000	400	400	200	
2	"	20 cc defibrinated blood, plus 100 cc Ringer-Locke	3200	6	570	220	220	132	An aliquot taken at 3 hrs. assayed 1000 r.u.
3	"	25 cc defibrinated blood, plus 100 cc Ringer-Locke	4080	3	1200† 1000‡	...	...	...	¼ of total plus 416 r.u. of α-estradiol assayed 700 r.u. ¼ of total acid hydrolyzed plus 416 r.u. α-estradiol assayed 730 r.u.
4	"	80 cc rabbit serum plus 15 cc Ringer-Locke	1664	6	134† 128‡	...	...	...	
5	"	25 cc defibrinated blood, plus 100 cc Ringer-Locke	300	3	43	...	...	...	
6	"	20 cc defibrinated blood, plus 100 cc Ringer-Locke	208	5	15	...	...	...	
7	"	25 cc defibrinated blood, plus 100 cc Ringer-Locke	0	3	14	...	...	...	
8	Heart	50 cc rabbit serum plus 75 cc Ringer-Locke	300	3	270	270	4	0	

* By our assay method 1 r.u. = 0.125 microgram α-estradiol, 1.0 microgram estrone, 1.0 microgram estriol.
† By routine extraction (see text).
‡ After acid hydrolysis (see text).

estradiol fraction, the small amount (4 r.u.) in the estrone fraction being accountable to fractionation error.

These data controvert the findings of Heller[10] and Zondek[19] that α-estradiol is not converted to other estrogens by liver *in vitro*. It is possible that autolyzed tissue, as represented by liver slices or brei, may release substances (enzymes or oxidants) that are destructive, whereas the intact organ exerts no such effect. Macroscopic examination of the livers of these experiments gave no indication of retrogression or autolysis. Our data do indicate that the liver may normally be concerned in the conversion of α-estradiol to estrone and estriol observed in injection and urinary recovery experiments.[15, 20, 21]

A fractionation of the urine of male rats receiving estrone by injection indicates a similar conversion by the intact animal; furthermore, partially hepatectomized animals show less destruction of the exogenous estrogen and also less conversion than normal animals (Schiller, unpublished data).

It is notable that these data give no indication of estrogen detoxification by the perfused rat liver. Either conjugation occurs at a much slower rate than conversion or the participation of another organ is requisite. Alternatively, the conditions of our experiments result in a breakdown of a detoxifying mechanism in the liver.

Details of these and related experiments will be published elsewhere.

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[20] G. Pincus, *Symposium Quant. Biol.*, 5: 44, 1937.
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INABILITY TO PASS PRIMARY ATYPICAL PNEUMONIA TO HUMAN VOLUNTEERS[1]

THE chief obstacle facing investigation of the etiology of primary atypical pneumonia (pneumonitis) has been the lack of suitable laboratory animals which regularly exhibit pneumonia after intranasal inoculation with throat washings, sputum or lung specimens from sick patients.

Stokes *et al.*[2] and Reimann[3, 4] among others have reported indifferent results after previously obtaining a passage virus. Recently Dingle *et al.*[5] have reported

[1] Published with the approval of the chief of the Bureau of Medicine and Surgery, United States Navy.
[2] J. Stokes, Jr., A. Kenny and D. Shaw, *Trans. Coll. Phys.*, 6: 329, 1939.
[3] H. Reimann and J. Stokes, Jr., *Trans. Asn. Am. Phys.*, 55: 123, 1939.
[4] H. Reimann and W. Haven, *Arch. Int. Med.*, 65: 138, 1940.
[5] J. Dingle *et al.*, *War Med.*, 3: 223, 1943.

that an agent isolated by one of their group could be passed directly to the cotton rat, but not after filtration through a Berkefeld N filter. The relation of this virus to pneumonitis has not yet been established.

Following an outburst in 1942 of continued and sporadic cases of primary atypical pneumonias, occurring in male personnel, several hundred mice, 12 ferrets and 8 hamsters were given intranasal inoculations of pooled and unpooled throat washings and sputa from acute x-ray positive cases. The results obtained were insignificant. Mice would die with some regularity if unfiltered washings were used. Where Berkefeld V filtration of throat washings and ground sputum was used intranasally, occasional mice of a series would die in 7 to 12 days, or longer, but successive serial transfers of the sterile lung suspensions were negative.

In 1942, sputum and nasal washings were collected from twelve patients (subsequently Lygranum negative) exhibiting typical symptoms by roentgenogram of severe primary atypical pneumonia. Their sputum and nasal washings were suspended in veal infusion broth emulsified by grinding and shaking and filtered through Berkefeld V candles. Previous experiments with N filtrates had already been found to be negative for mice and 2 human volunteers.

The veal infusion filtrates were given intranasally to 5 human volunteers in amounts from 20 to 40 cc each. The volunteers remained healthy and did not subsequently exhibit signs of illness. Control mice also remained healthy.

The use of human volunteers is reported. The negative results obtained are consistent with the apparent relatively low degree of infectivity.

The twelve patients mentioned were Naval personnel. The five human volunteers were non-military personnel.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

STERILITY TEST FOR PENICILLIN

WITH the introduction of the use of penicillin in the successful treatment of systemic and wound infections caused by many gram positive and some gram negative bacteria,[1, 2, 3, 4, 5, 6] the necessity of providing a suitable method for neutralizing the antibacterial effects of this highly active substance prior to sterility test becomes apparent. The approach used in developing a satisfactory method for testing penicillin for sterility involved a consideration of some chemical or physical agent which would inactivate the substance completely and yet in itself have no antibacterial effects on possible contaminating organisms in the product.

Among the various agents tested, which appeared to meet the necessary requirements given, were two enzyme preparations, Taka-diastase and particularly Clarase,[7] a more active diastatic enzyme system. These enzymes were found to inactivate the antibacterial effects of penicillin in two hours or less when incubated in the presence of the agent in a water bath at 40° C and tested by the Oxford cup-plate method. Taka-diastase was effective under these conditions at pH 6.0 and 8.0 but not in buffer solution of pH 4.0. Clarase, on the other hand, neutralized penicillin activity completely at all the pH ranges given. Additional studies on the relative inactivating actions of the two preparations indicated that while Taka-diastase was effective in this test in dilutions of 1:200, a similar action was had with Clarase in a final dilution of 1:4,000.[8]

Since it was found that relatively high dilutions of Clarase would neutralize the antibacterial effects of penicillin when tested by the Oxford cup-plate procedure, it appeared worth while to study the effects of the enzyme system against the agent in the presence of a fluid medium. This study was carried out as follows: A 1 per cent. stock solution of Clarase was prepared in buffer solution, of pH 7.0 and sterilized by Berkefeld filtration. Samples of sodium penicillin powder,[9] as received in the sterility control laboratory, were inoculated with dry cultures of *Clostridium tetani, Clostridium septique, Bacillus subtilis, Eber-*

[1] E. P. Abraham, E. Chain, C. M. Fletcher, A. D. Gardner, N. G. Heatley, M. A. Jennings and H. W. Florey, *Lancet*, 2: 177, August 16, 1941.
[2] J. E. Bordley, S. J. Crowe, D. A. Dolowitz and K. L. Pickrell, *Ann. Otol., Rhin. and Laryngol.*, 51: 891, 1942.
[3] M. E. Florey and H. W. Florey, *Lancet*, 1: 387, March 27, 1943.
[4] W. E. Herrell, *Staff Meet. Mayo Clin.*, 18: 65, 1943.
[5] W. E. Herrell, E. N. Cook and L. Thompson, *Jour. Am. Med. Asn.*, 122: 289, 1943.
[6] C. H. Rammelkamp and C. S. Keefer, *Am. Jour. Med. Science*, 205: 342, 1943.
[7] "Standardized" Clarase from the Takamine Laboratories, Clifton, N. J.
[8] Details of this and additional studies on enzymes *vs.* penicillin to be published elsewhere.
[9] 10 mg containing approximately 240 units per mg.