probably not of significance. In this respect it should be mentioned that the crystalline sample of penicillin dihydro F used in these studies may have contained 10-20% of "K-type" penicillins. Preliminary experiments on certain mixtures in serum of 2 penicillins which are bound to greatly different degrees suggest that the observed activity is solely that of the penicillin which is bound to the lesser degree. For example, pooled serum containing mixtures of penicillins G and K over a wide range of ratios of concentration, when assayed by the technic employed in these studies has consistently shown an apparent activity equal to that of the penicillin G present. Thus the values obtained for serum concentration after administration of penicillin dihydro F may actually represent what would be obtained after approximately 80% of the dose were given in the form of pure material. This probably would not differ significantly from the results which have been presented.

Summary. The absorption and excretion of penicillins X, G, dihydro F and K in humans have been studied in an effort to evaluate the influence of the marked differences in the degrees of binding of these substances by serum protein.

The duration of the serum concentrations afforded by all 4 penicillins after intramuscular injection of 300,000 units to humans were uniform within the limits of the method of assay. During the period when all of the penicillins were present in measurable concentrations there was no difference among the 4 except for the lower peak of penicillin K 15 minutes after injection. Evidence was presented that previous findings of higher and more prolonged serum concentrations of penicillin X, and lower, rapidly disappearing concentrations of penicillin K were artifacts created by differences in the antagonistic action of the serum on penicillin during the actual bioassay procedure. Thus differences in therapeutic effectiveness of the individual penicillins cannot be explained on the basis of differences in height and duration of serum concentrations after equal doses.

Although the total excretion of penicillin K was low, the rate of excretion and the renal clearance suggest that the mechanism of removal of penicillin K by the kidney differs from that observed with the other penicillins.

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Antidotal Properties of Crude *Penicillium notatum* Filtrate.

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Recent work of Ramon, *et al.* shows that there is present in filtrates of crude *Penicillium notatum* an agent which detoxifies various toxins.[1-3] This property they have called "antidotal." According to these workers this action is enzymatic and they conclude that preparation of highly purified penicillin results in a loss of this factor which, from a therapeutic point of view, may be unwise. No *in vivo* therapy data were given. The following is a summary of *in vitro* and *in vivo* experiments determining the antidotal efficacy of crude penicillium broth.

Materials. Toxin—crude tetanus which assayed about 100,000 LD/ml.

P. notatum filtrate—a crude commercial filtrate from which the penicillin had been extracted.

Mice—white, inbred strain, either sex, 18-21 g.

Broth—1% peptone.

Experimental. To make the experiment

1 Ramon, G., Richou, R., and Ramon, P., *C. R. acad. sci.*, 1946, **222**, 621.

2 Ramon, G., Richou, R., and Ramon, P., *La Presse Medicale*, 1946, Oct. 2.

3 Ramon, G., Richou, R., and Ramon, P., *Rev. Immunol.*, 1944-45, **9**, 161.

TABLE I.

Test	Tube 1	2	3	4	5
Material					
Tetanus toxin (3 ml)	1:100,000 dil.	1:25,000	1:5,000	1:1,000	0
Original filtrate or dialyzed filtrate (ml) or dialysate	3	3	3	3	3
Broth	0	0	0	0	3
Toxin control					
Toxin (3 ml)	1:100,000	1:25,000	1:5,000	1:1,000	
Broth (ml)	3	3	3	3	

inclusive 3 materials were obtained; (a) original crude penicillium filtrate, (b) dialyzed filtrate, (c) dialysate. The dialysis was carried out at 1°C. (b) was obtained by dialyzing against running distilled water for 96 hours. The dialysate (c) was obtained by immersing in a cylinder of distilled water, a cellophane tube containing filtrate, and allowing it to stand for 48 hours. A total of 300 ml filtrate was used for (c).

The above materials were utilized in *in vitro* and *in vivo* experiments in order to determine the efficacy of the antidotal factor. If the active material is dialyzable then the agent is of relatively small molecular size.

Total solid determinations based on corrected volumes for dialysis, gave the following results: (a) original filtrate—34 mg per ml, (c) dialysate—15 mg per ml.

In vitro neutralization test, the scheme in Table I was used.

Three sets were made for each test and controls. All toxin dilutions were made with 1% peptone broth. One set each of each test and control was kept at 2°C, 20°C, and 37°C for 16 hours. Three mice per dilution were then injected intra-abdominally with 1 ml volume each. The results are shown in Table II.

Table II shows that the toxin is completely inactivated by the agent which is dialyzable. The fact that it will dialyze indicates that it is not a protein and therefore the action is not enzymatic.

There appears to be a lethal factor present in the dialyzed filtrate which is not present in the undialyzed filtrate.

Effect of blood on antidotal activity. Two ml of dialysate was added to 2 ml of sterile sheep's blood. Two ml of tetanus toxin containing 6 L.D. per ml was added and the contents mixed. Two sets were made, one being placed at 2°C and one at 37°C for 2 hours. Five mice were injected, subcutaneously to the right of the tail, with each mixture using 1 ml volume per mouse. Control mice received the same mixture without toxin. All the test mice died within 3 days with typical tetanus symptoms. All control mice survived.

The above experiment was repeated except that the incubation period at 2°C and 37°C was for 6 hours. All of the test mice died within 3 days corroborating the above results.

Acute toxicity in mice of antidotal material. The mouse toxicity of the original clear filtrate was determined since it was desirable to utilize optimal nontoxic doses of filtrate and dialysate in the *in vivo* therapeutic experiments. When the intra-abdominal route was used the LD_{50} of the original filtrate was found to be about 177 mg per 18-21 g mouse. The M.L.D. of the dialysate was >76 mg.

In vivo therapeutic test. Five mice were each injected intra-abdominally with 4 L.D. of tetanus toxin. Simultaneously, these mice received 0.5 ml of crude undialyzed filtrate subcutaneously. Two more subcutaneous injections of the filtrate at 2-hour intervals were given. Since each ml contained 34 mg a total of 51 mg was given. All 5 mice died within 2 days. Control mice which received only the filtrate survived.

Another group of 15 mice was injected subcutaneously with 2 L.D. tetanus toxin just to the right of the tail. This was followed

TABLE II.

Material	Incubation temp. °C	Toxin dilution, × 1000	Day of death	
Undialyzed*	2	1:100		
		1: 25		
		1: 5		
		1: 1		
		No toxin		
	20	1:100		
		1: 25		
		1: 5		
		1: 1		
		No toxin		
	37	1:100		
		1: 25		
		1: 5		
		1: 1		
		No toxin	2	
Dialyzed*	2	1:100	333	46
		1: 25	222	223
		1: 5	111	111
		1: 1	111	111
		No toxin	222	
	20	1:100	334	44
		1: 25	222	222
		1: 5	111	111
		1: 1	211	111
		No toxin	223	
	37	1:100	334	12
		1: 25	222	145
		1: 5	111	222
		1: 1	211	111
		No toxin	223	
Dialysate	2	1:100		
		1: 25		
		1: 5		
		1: 1		345
		No toxin		
	20	1:100		
		1: 25		
		1: 5		
		1: 1		
		No toxin		
	37	1:100		
		1: 25		
		1: 5		
		1: 1		
		No toxin		
Toxin controls*	2	1:100	36	443
		1: 25	222	222
		1: 5	211	111
		1: 1	111	111
	20	1:100	344	444
		1: 25	222	24
		1: 5	111	111
		1: 1	111	111
	37	1:100	446	446
		1: 25	222	222
		1: 5	222	111
		1: 1	—	111

* Results for undialyzed, dialyzed, and toxin controls are based on 2 tests or total of 6 mice per dilution.

immediately with 1 ml dialysate intra-abdominally. One ml of dialysate was given intra-abdominally every hour for 13 hours with a 13-hour interval between the 6th and 7th injections. A total of about 220 mg per mouse was given. All of the mice developed typical tetanus symptoms in about 24 hours and all of the mice died in 2 or 3 days. Five control mice which received only the dialysate showed no ill effects.

Test for destruction of antigenicity of toxin. Since the *in vivo* experiments showed the *P. notatum* broth to be without therapeutic activity it was not known whether there was *in vivo* destruction of the agent. However, the "antidotal" factor might prove to be a valuable material for preparation of toxoids. To test this possibility the 36 mice which had received undialyzed filtrate mixed with toxin and survived were challenged by intra-abdominal injection of 4 L.D. of tetanus toxin 21 days following the original test. All 36 mice died within 2 days. These results were corroborated in a similar experiment in which 66 surviving mice were challenged after 21 days with 4 L.D. of tetanus toxin. All the mice died within 3 days. Thus it would seem that the agent in the filtrate not only detoxifies toxin but also destroys antigenicity. This evidence is admittedly indirect but since the tetanus toxin used in these experiments represents an aliquot from a large batch of toxin used for making satisfactory commercial toxoid it is felt that the results are valid.

Determination of antidotal activity in amorphous penicillin. Results similar to those obtained with crude filtrate against tetanus toxin were observed with amorphous penicillin calcium having a potency of 1000 units per mg. *In vitro* neutralization occurred but no *in vivo* therapeutic activity was demonstrated.

Antidotal activity versus the endotoxin of H. pertussis. Four concentrations of *P. notatum* filtrate in a volume of 3 ml were made; undiluted, 1:4, 1:16 and 1:64. The diluent was peptone broth. Three ml of pertussis endotoxin* containing 50 LD_{50} per ml were added to each concentration of filtrate. The contents of the tubes were mixed and kept at 2°C for 6 hours. For each group 5 mice were then injected intra-abdominally with 1 ml volume. There were no deaths in the groups where undiluted or 1:4 filtrate was used. In the 1:16 and 1:64 dilutions all of the mice died.

In vivo experiments identical to those described previously for tetanus toxin (intra-abdominal route) were carried out using 4 L.D. of pertussis endotoxin. All of the mice died.

The *in vitro* and *in vivo* results obtained with pertussis endotoxin were the same as those observed with tetanus toxin. It would appear that the *in vitro* antidotal activity is not specific.

Discussion. Ramon, Richou, and Ramon did not include any *in vivo* results in their communications. However, the *in vitro* results reported seemed to warrant the foregoing experiments. Both the *in vitro* and *in vivo* data contained in this report are very striking in the definite results attained. The fact that blood neutralizes the antidotal activity is in itself, sufficient evidence to predicate failure of *in vivo* therapy.

With the *in vitro* incubation period of 16 hours used in the present experiments there seems to be no substantial difference whether the incubation temperature is 2°C, 20°C, or 37°C.

Summary. 1. The *in vitro* results on the antidotal factor reported by Ramon, *et al.* are essentially corroborated.

2. This antidotal agent is dialyzable.

3. The antidotal activity is inactivated in the presence of blood.

4. No *in vivo* therapeutic activity against tetanus toxin or pertussis endotoxin could be demonstrated.

Both the crude penicillium filtrate and the amorphous penicillin calcium were kindly supplied by the Penicillin Division of Wyeth, Inc. We are indebted to the Biological Division of Wyeth, Inc. for the crude tetanus toxin.

* For the method of preparation see Smolens, J., and Flavell, E. H., *J. Immunol.*, in press.