valuable suggestions and to I. Dorrell and D. Klingelhoffer for their technical assistance.

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BLOOD LEVELS AND URINARY EXCRETION IN PEANUT OIL, BEESWAX AND PENICILLIN MIXTURE

CLINICALLY, penicillin has proven to be a highly effective drug, yet its application in non-hospitalized patients is a difficult problem because of its rapid utilization and excretion from the body. This has necessitated repeated injections at frequent intervals; a procedure that more or less disrupts both patients' and doctors' daily schedules. Recently several methods of prolonging the action of this drug have been described. Of these, the most promising is the use of a penicillin-peanut oil and four per cent. beeswax mixture, as described by Romansky and Rittman[1] and confirmed by Zinnamon and Seeberg.[2] The prolongation is achieved by delaying the absorption of penicillin from the area injected.

Recently, an opportunity to use this mixture was afforded this department, and the data obtained pertaining to the excretion of this drug form the basis of this report. The clinical results obtained were good, but will not be considered here, as a more complete report of these findings has been published by the U. S. Public Health Service.

TECHNIQUE

The material used was a mixture of peanut oil and four percent. white beeswax containing 100,000 units of calcium penicillin per cc.[3]

Urine and blood were assayed by the serial dilution "turbidimetric" method of M. H. Dawson and G. L. Hobby.[4] The number of Oxford units were determined by comparing bacterial inhibition of serial dilutions of the fluid to be tested and sets of penicillin solutions of known potency determined by assay against a reference standard of penicillin calcium obtained from the Food and Drug Administration of the Federal Security Agency. The bacteria used were the Oxford strain of hemolytic *Staphylococcus aureus*. This is not the method best suited for the determination of minute amounts of penicillin, as this strain is inhibited by 0.1 units per cc. In our hands, however, it is rapid, practical, and gives easily reproducible results.

The drug was administered intramuscularly in a single dose of 200,000 units. Blood and urine specimens were assayed at the intervals shown in the following table and interval and total excretion values were calculated. A total of 23 patients were treated. The values shown represent the average amounts of penicillin found for each interval. The minimum and maximum amounts are also given.

TABLE 1

PENICILLIN EXCRETION FROM BEESWAX AND PEANUT OIL MIXTURE

Time interval hrs.	Blood levels (Units per cc)			Urine levels (Units per cc)			Urine levels (Total units excreted)		
	Low	High	Average	Low	High	Average	Low	High	Average
2	.225	.45	.343	45.	135.	109.5	3,600	18,225	10,660
4	.225	.45	.235	33.7	135.	83.6	3,375	18,900	8,790
6	0	.23	.152	22.5	135.	75.3	0	17,100	7,672
8	0	.225	.020	22.5	90.	61.4	1,854	14,400	6,884
10				22.5	135.	69.1	2,812	11,250	5,838
12				22.5	135.	54.3	2,250	13,500	5,333
14				22.5	90.	48.4	1,800	13,500	5,323
16				0	67.5	38.1	0	14,087	4,495
18				0	90.	35.1	0	7,414	3,104
20				0	90.	33.4	0	6,000	2,495
22				0	67.5	23.5	0	4,275	2,233
24				0	67.5	20.0	0	5,063	1,786
24 to 36				0	45.	9.5	0	15,750	4,866
36 to 48				0	11.5	1.7	0	5,175	640
Totals . .							15,691	164,639	70,119

In short, our findings confirmed the prolongation of demonstrable blood levels as reported by Romansky and Zinnamon. An additional fact, however, which was noted and considered of more import than has previously been accorded it, was the even more prolonged presence and concentration of penicillin in the urine. The implications of this will be considered later.

DISCUSSION

It will be noted that penicillin could be detected in significant amounts in the blood for an average of over five hours. Since all reports[5, 6, 7] thus far agree that the water or saline solutions of penicillin are excreted in 3 to 4 hours, this is a definite prolonga-

[1] Romansky and Rittman, *Bull. U. S. Army Med. Dept.*, No. 81, p. 43, October, 1944.

[2] Zinnamon and Seeberg, *Venereal Disease Information*, 26: 2, 27, February, 1945.

[3] Prepared and furnished by Squibb in cooperation with the U. S. Public Health Service.

[4] Dawson and Hobby, personal communication.

tion. Actually, the degree of prolongation may be greater as the assay methods used by the above investigators gave values as low as .01 unit per cc, whereas the method in this report had a minimum reading of .1 unit per cc.

TABLE 2

SHOWING DEGREE TO WHICH PENICILLIN IS CONCENTRATED BY KIDNEY

Case	Interval Hours	Blood units/cc	Urine units/cc	Concentration
No. 15	2	.225	135	?
	4	.225	135	600
	6		135	600
No. 17	2	.45	90	
	4	.225	90	200
	6	.225	90	400
	8		37.5	150
No. 20	2	.225	90	
	4	.225	135	600
	6	.225	45	200
	8		37.5	150

During this work it became apparent that penicillin was consistently present in the urine in much greater concentration than in the blood stream and that it may be detected much longer. The rate of excretion dropped rather rapidly in the first 6 to 8 hours and then remained fairly constant for 8 to 16 hours despite the low or absent blood levels. The unit excretion per interval of time was for the most part independent of the urinary volume.

It has been suggested that penicillin is excreted from the tubules of the kidney in addition to filtration through the glomeruli. Concentration values were calculated on the basis of urinary concentration in units per cc divided by the blood level at the end of the previous two-hour period. Sample concentrations appear in Table 2 and varied from 100 to 600 times. As the kidney excretes non-threshold substances creatinine and sulfates in concentrations of 75 to 90 times, respectively, the markedly high values found with penicillin can be explained only by renal tubular excretion. Dawson *et al.*[8,9] have shown that blood serum and whole blood do not inhibit penicillin, therefore the persistence of high concentrations of penicillin in urine long after demonstrable blood levels are absent can be explained by the remarkable power of the kidney to concentrate and excrete it. Another explanation might be storage in the tissues, which would tend to make the blood levels lower and prolong the excretion in the urine.

The total amount of penicillin excreted was relatively constant around 68,000 units, which represents 34 per cent. of the original dose given. This rather low total excretion when compared to values of 50 to 60 per cent. excretion after injection of penicillin in water or saline can be explained by its destruction in the body due to its heat lability or to some other factor in which the time element plays a part. Rammelkamp and Keefer[7] have shown that while the liver excretes penicillin in bile, the total excretion is probably small.

The prolonged presence of penicillin in the urine, however, does suggest the possibility of penicillin being clinically available in the body long after our present concepts have led us to believe. It is true that the levels are minute, but for sensitive organisms and in the presence of leucocytes and antibodies they may prove sufficient.

CONCLUSIONS

(1) Penicillin in combination with peanut oil and beeswax is detectable in the blood stream for longer periods of time following its intramuscular injection than when a water or saline suspension is used.

(2) Penicillin is present in the urine in greater concentration and for much longer intervals than in the blood stream, the concentration being 100 to 600 times. This may prove to be of clinical significance and of value in studying renal physiology.[10]

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IMMUNIZATION AGAINST MALARIA: VACCINATION OF DUCKS WITH KILLED PARASITES INCORPORATED WITH ADJUVANTS[1,2]

IN his recent review of immunity in malaria, Coggeshall[3] came to the conclusion that "the acquisition of immunity following the inoculation of killed malarial organisms is only demonstrable under exceptional conditions." Jacobs[4] published evidence indicating that partial immunity against *P. lophurae* in the duck may be obtained by injecting killed parasites in combination with staphylococcus toxoid. In his experiments the immunized ducks were challenged three days after the fifth injection of vaccine and

[5] Herrel, Nichol, Heilman, *Jour. Am. Med. Asn.*, 125: 15, August 12, 1944.
[6] Cooke and Golding, *Jour. Am. Med. Asn.*, 127: 80, January 13, 1945.
[7] Rammelkamp and Keefer, *Jour. Clin. Invest.*, 22: 425, May, 1943.
[8] Rammelkamp and Bradley, *Proc. Soc. Exp. Biol. and Med.*, 53: 30, May, 1943.
[9] Dawson, Hobby, Meyer and Chaffee, *An. Int. Med.*, November, 1943.

[10] It is desired to acknowledge appreciation to Sergeant Hugh Woosley, who performed the laboratory work herein reported.
[1] Manuscript completed December, 1943.
[2] This study was aided by a grant from the John and Mary R. Markle Foundation.
[3] L. T. Coggeshall, *Medicine*, 22: 87–102, 1943.
[4] H. R. Jacobs, *Am. Jour. Trop. Med.*, 23: 597–606, 1943.