ever, on diluting the inocula 100 times the eyes remained normal.

Neutralization tests were carried out by injecting a mixture of equal parts of undiluted serum and virus into the rabbit eye. Homologous antiserum from convalescent ferrets afforded complete protection. Normal ferret serum and heterologous influenza antiserum, type A and B, failed to protect the eye.

Tests for the persistence or multiplication of virus in the eye were carried out at intervals ranging from 8 hours to 4 days after inoculation. Aqueous humor or a suspension of tissues from the anterior portion of the eye were injected into 6-11-day-old chick embryos in each test. The results of two experiments showed that the virus disappeared quickly from the aqueous humor. Tissues from the anterior portion of the eye retained small amounts of virus (end points 10^{-2} or 10^{-3}) for three days. On the fourth day the virus was practically absent as only one out of 12 eggs injected with a 10^{-1} dilution of ocular tissues was infected.

Discussion. Inasmuch as there appeared to be a complete lack of multiplication of influenza virus in the eyes of rabbits, the severe corneal damage was attributed to a toxic effect and not to infection. The type specificity of the neutralizing power of antiserum shows that the toxic substance is not a nonspecific irritant in the inoculum but must be either the virus particle itself or some other antigenic substance generated by the virus in the infected ferrets as well as in chick embryos.

The recent reports of Rake and Jones[5] have shown a toxic effect of the viruses of lymphogranuloma venereum, mouse pneumonitis, and meningo-pneumonitis. It would appear that the corneal opacity observed in these experiments and neurologic disturbances observed by the Henles in mice[6] are probably caused by toxic properties of the influenza viruses.

5 Rake, G., and Jones, H. T., *J. Exp. Med.*, 1944, **79**, 463.

6 Henle, G., and Henle, H., *Science*, 1944, **100**, 410.

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Prolongation of the Action of Penicillin After Intramuscular Injection.*

CHARLES D. ARMSTRONG, RICHARD M. HALPERN, AND WINDSOR C. CUTTING.

From the Departments of Medicine and of Pharmacology and Therapeutics, Stanford University School of Medicine, San Francisco, Cal.

Penicillin is so rapidly excreted from the body after parenteral injection that frequent administrations are necessary to maintain it continuously in the blood in measurable amounts. Although intermittent therapy may often be adequate, particularly when this involves spikes of high penicillin concentrations immediately after the injections, it is probable that a long high plateau is still more desirable. To this end, several procedures have been devised to delay the excretion, or retard the absorption, of penicillin after injection.

Rammelkamp and Bradley[1] and Beyer and co-workers,[2] using diodrast or para-aminohippuric acid to interfere with renal function, succeeded in depressing the excretion of penicillin in the urine of men and dogs. So far, this rather unphysiological method has apparently not received adequate clinical trial.

Raiziss[3] found that, when penicillin was injected intramuscularly into rabbits as a suspension in a vegetable oil, the blood remained bacteriostatic considerably longer

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1 Rammelkamp, C. H., and Bradley, S. E., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 30.

2 Beyer, K. H., Woodward, R., Peters, L., Verwey, W. F., and Mattis, P. A., *Science*, 1944, **100**, 107.

3 Raiziss, G. W., *Science*, 1944, **100**, 412.

TABLE I.
Duration of Penicillin in Blood of Dogs After Intramuscular Injection.

No. of trials	Vehicle for penicillin	Average duration measurable penicillin in the blood, in minutes
5	Physiological salt solution in distilled water	30
1	Epinephrine 2 mg in physiological salt solution	0
1	Methyl cellulose	15
1	Human plasma	15
1	Sucrose 12%	15
1	Lactose 12%	15
3	Sesame or peanut oil	25
2	Pitressin 1 cc subcutaneously, penicillin in dextrose 5%	30
2	Sorbitol 4½ to 25%	35
3	Gelatin 8.5%	40
2	Congo red 0.5 to 1% in dextrose 5%	51
1	Gelatin 8.5% with urea 5%	60
4	Lecithin 2 to 7%	67
11	Dextrose 3 to 10%	68
4	Epinephrine 2 mg in dextrose, gelatin 8.5%, or gelatin 8.5% with urea 5%	75

than when it was injected in water, and also, that such suspensions were slow to deteriorate and lose potency. Trumper and Bradley[4] delayed the absorption of penicillin after intramuscular administration by placing an ice-pack over the area of injection to limit the local circulation. Clinical results in gonococcal infections appeared to be satisfactory, but the method was somewhat cumbersome and unpleasant. Finally, Romansky and Rittman[5,6] used suspensions of penicillin in peanut oil containing from 1 to 5% beeswax. While this method may be criticized on the basis of the residue of oil and wax which will be left at the site of injection, the foreign accumulation apparently is not harmful.

In a survey of the problem, various agents were tried by us, in dogs and in man, in an attempt to obtain a more satisfactory prolongation of the residence of penicillin in the body.

Methods. Various solutions or suspensions of sodium or calcium penicillin were prepared, given intramuscularly to dogs or humans, and blood samples taken at appropriate times thereafter. Estimations of penicillin in the blood were made by means of the method of Wolohan and Cutting.[7]

Results in Dogs. Penicillin was given intramuscularly to 11 dogs on 48 occasions, in amounts of 1000 to 4000 units. The dogs weighed from 7.7 to 18 kg, but 7 weighed about 10 kg. The variations in penicillin dosage, and in the weights of the dogs appeared to have little effect on the conclusions reached, perhaps because several different agents were tried in each dog. Likewise, no differences were noticed on 3 tests in which calcium penicillin was substituted for sodium penicillin. The total volume of solution injected was 1 or 2 cc. The lowest concentration of penicillin measured was 0.01 unit per cc.

The results as seen in Table I, showed that water, vegetable oils, methyl cellulose, human plasma, sucrose, lactose, sorbitol, gelatin, and pitressin did not substantially prolong the stay of penicillin in the body. On the other hand, epinephrine, congo red or lecithin in dextrose, gelatin with urea, and dextrose significantly prolonged the time over which penicillin could be demonstrated in the blood. None of these latter agents, with the possible exception of epinephrine, was more effective than dextrose alone. There was no essential difference in the height of the penicillin level in the blood 15 minutes after injection with the different vehicles.

[4] Trumper, M., and Hutter, A. M., *Science*, 1944, **100**, 432.

[5] Romansky, M. J., and Rittman, G. E., *Science*, 1944, **100**, 196.

[6] Romansky, M. J., and Rittman, G. E., *Bull. U. S. Army Med. Dept.*, No. 81, 1944, 43.

[7] Wolohan, M., and Cutting, W., *J. Lab. Clin. Med.*, in press.

TABLE II.
Duration of Penicillin in Blood of Humans After Intramuscular Injection.

No. of trials	Dose of penicillin in units	Vehicle for penicillin	Average duration of measurable penicillin in the blood, in hours
11	80,000	Physiological salt solution	2.5
11	"	5% dextrose	3.5
4	"	5% dextrose plus 0.25 to 0.5 mg neosynephrine hydrochloride	4.0
1	40,000	Physiological salt solution	3.0
1	"	5% dextrose	4.0
1	25,000	5% dextrose	2.0
1	"	5% dextrose plus 0.25 mg neosynephrine hydrochloride	4.0
1	20,000	Physiological salt solution	1.0
2	"	5% dextrose	1.1
2	"	5% dextrose plus 0.25 mg neosynephrine hydrochloride	1.0
2	15,000	Physiological salt solution	1.25
2	"	5% dextrose	1.5

Results in Man. On the basis of the trials in dogs, penicillin was administered to patients and normal humans in dextrose alone, and in a combination of dextrose with a vasoconstrictor. Thirty-nine sets of observations were made on 12 patients. The concentration of penicillin was 5000 units per cc.

In nearly all cases, as shown in Table II, dextrose as a vehicle extended the period over which penicillin could be measured in the blood. With the smaller doses this extension could hardly be called important, but with large doses it is great enough to be therapeutically valuable. The addition of neosynephrine to the solution prolonged the active penicillin concentration still further, but probably not enough to justify the slight hazard involved. One of the patients had a symptomless rise in blood pressure from normal to a systolic pressure of 180 mm of mercury.

Discussion. Penicillin was administered to dogs and humans intramuscularly in a variety of vehicles in an attempt to prolong its sojourn in the body. Only dextrose, of the agents tried, appeared to furnish a satisfactory and harmless extension of its action. No explanation is offered for this effect, but it appears to be substantial enough to be of clinical value when large doses of penicillin are used. Unlike the low blood concentrations produced by mixtures of penicillin with peanut oil and beeswax, a high concentration of penicillin is present in the blood immediately after injection, and no foreign substance remains in the tissues. However, it may be that a long-continued low concentration is more effective therapeutically than separated spikes of higher concentration, as suggested by the work of Romansky and Rittman.[5,6] When such mixtures are not chosen, and at present their use is limited, there seems no doubt that the penicillin should be dissolved in dextrose, and not in physiological salt solution.

Conclusion. Penicillin is demonstrable in the blood after intramuscular injection significantly longer when the vehicle is 5% dextrose instead of the usual physiological salt solution.

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