from smoking; 3 definitely missed the nicotine but became adapted to the change in one to two weeks; 9 definitely missed the nicotine and continued to do so throughout the period (approximately 1 month). The symptoms experienced by the latter 2 groups for the most part took the form of varying degrees of heightened irritableness, decreased ability to concentrate on mental tasks, feeling of inner hunger or emptiness, hypoesthesia (1 case), in short, virtually the same symptoms experienced by many individuals on stopping smoking. Some of the individuals in the last group "just could not take it" and admitted to interspersing a few cigarettes of ordinary nicotine content during their period on low nicotine cigarettes.

CONCLUSIONS

It would seem clear from these results that with many individuals nicotine becomes a major factor in their cigarette habit. Equally certain, with many individuals nicotine is not a factor in their cigarette habit. Even in those individuals in whom nicotine has become a major factor we feel that a cigarette containing no nicotine would be grudgingly accepted as better than no cigarette at all.

There is seemingly no correlation between the number of cigarettes smoked daily and the degree to which nicotine becomes a factor. Indicative of this is the heaviest smoker in the series, a man who for many years has smoked 3 packages daily. This individual made the switch to low nicotine cigarettes without the slightest difficulty.

Groupings on the basis of subject age or duration of the habit showed no correlation with the degree to which nicotine was missed. However, the number of subjects involved was too small to arrive at any definite conclusions in these respects.

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PENICILLIN IN EXPERIMENTAL SPOTTED FEVER

UNTIL relatively recently the treatment of spotted fever (Rocky Mountain spotted fever) has been purely supportive and symptomatic. Published reports on chemotherapeutic treatment are scant. In 1938 Baker reported that the intravenous administration of neoarsphenamine in metaphen solution was beneficial in relieving symptoms and, in a later publication, that all treated cases had survived.[1, 2] The number of cases was not stated. No doubt the sulfonamides have been given in many instances not reported in the literature. In infected guinea pigs the use of sulfapyridine and prontosil resulted in no improvement,[3] and Topping concluded that there was evidence of their being harmful and that they should not be used. We have confirmed and extended these observations, studying the effect of sulfamerizine, sulfadiazine, sulfapyridine and sulfathiazole.[4] In our hands, the latter two drugs were very definitely contraindicated, deaths occurring in the treated animals sooner than in the controls. The first two drugs were without effect. Steinhaus and Parker,[5] in addition to testing some of the sulfonamides, treated guinea pigs with atabrine and tyrothricin and concluded that none of the substances used was of any value. Serotherapy (rabbit immune globulin) is coming into wider use,[6] but it will take some years to evaluate the benefit of this form of treatment in human spotted fever where the mortality with supportive treatment alone is only about 20 per cent. Its striking effects in guinea pigs, however, even when very small quantities are employed,[4] tend to make one exceedingly enthusiastic about its use in the human disease.

In view of the extraordinary effectiveness of penicillin in many other infectious diseases, it seemed pertinent to study its effects on experimental spotted fever. Its use in man has been reported in one case diagnosed as spotted fever; the patient recovered.[7]

Male guinea pigs weighing from 450 to 600 grams were infected by the intraperitoneal route with 1 cc of a 10 per cent. suspension of spleens obtained from guinea pigs infected with the Bitter Root strain of spotted fever. Temperatures were taken twice daily to determine exactly the time of onset of fever. In the first experiment, groups of 4 guinea pigs were selected for treatment 24, 48, 72 and 96 hours after the temperatures rose above 103.5° F. All guinea pigs, except the 96-hour group, received intramuscularly 200 Oxford units of penicillin contained in a volume of 0.2 cc every 4 hours for 36 hours, or a total of 1,800 units per animal. The disease in the guinea pigs selected for treatment 96 hours after the onset of fever had progressed so far that it was deemed advisable to give them larger doses. Five hundred units at 4-hour intervals were administered for 36 hours, or a total of 4,500 units per guinea pig. No beneficial effect could be observed in any of the treated animals.

A second experiment was undertaken to see if the larger dose of penicillin given earlier in the disease

[1] G. E. Baker, *Rocky Mt. Med. Jour.*, 35: 36, 1938.
[2] *Idem*, *Ann. Intern. Med.*, 17: 247, 1942.
[3] N. H. Topping, *U. S. Pub. Health Rep.*, 54: 1163, 1939.
[4] Unpublished experiments.
[5] E. A. Steinhaus and R. R. Parker, *U. S. Pub. Health Rep.*, 58: 351, 1943.
[6] N. H. Topping, *U. S. Pub. Health Rep.*, 58: 757, 1943.
[7] P. K. Edmunds, *Rocky Mt. Med. Jour.*, 41: 910, 1944.

and over a longer period of time would produce better results. Six guinea pigs approximately 500 grams in weight were given 500 units of penicillin intramuscularly every 4 hours, the initial injection being given 48 hours after the first elevation of temperature. Treatment was continued for 4 days, a total of 12,000 units being administered per guinea pig (the equivalent of about 2 million units for an adult weighing 160 pounds). Six other animals received one injection of 1 cc of spotted fever rabbit immune globulin intraperitoneally 48 hours after the onset of fever. Sixteen untreated guinea pigs served as controls. The results are summarized in Table 1.

TABLE 1

No. of guinea pigs	Treatment	Result
6	500 units penicillin every 4 hours for 4 days. Total 12,000 units per animal	6 died
6	1 cc spotted fever rabbit immune globulin	6 recovered
16	None—Controls	8 died 8 recovered

It will be seen that all the animals receiving the penicillin succumbed to the disease. The treatment had no beneficial effect on any of the signs and symptoms characteristic of this strain of spotted fever, *i.e.*, loss of appetite, loss of weight, sustained high temperature, scrotal involvement, etc. Smears of spleen and lung at autopsy showed rickettsiae to be as numerous as in the untreated controls. In the globulin-treated animals, on the other hand, the progress of the disease was arrested. The scrotal swelling subsided in about 2 days, whereas in most of the controls and in the guinea pigs treated with penicillin the lesion progressed to petechial hemorrhages, adhesions of the tunica vaginalis and finally necrosis.

Studies of plasma levels showed that the dosage and time schedule employed in the second experiment should have been adequate for treatment. Three male guinea pigs weighing 510 to 525 grams were given 500 units of penicillin into the muscles of the leg. Plasma was obtained 30 minutes and 4 hours after the injection, and the drug levels determined by the Rammelkamp method,[8, 9] These are recorded in Table 2.

TABLE 2

Guinea pig no.	Units of penicillin per cc of plasma	
	30 minutes after drug	4 hours after drug
1	0.25	0.03
2	0.12	0.02
3	0.19	0.03

It will be noted that absorption of penicillin in the guinea pig was very rapid following intramuscular injection and that even after a lapse of 4 hours detectable quantities of the drug were still present.

SUMMARY

Guinea pigs infected with a virulent strain of spotted fever received large doses of penicillin intramuscularly every 4 hours. The injections were begun 48 hours after the onset of fever. Controls included untreated animals and others that received one dose of spotted fever rabbit immune globulin. The penicillin had no effect on the classic symptoms of this disease and all the treated animals died. (The toxicity of penicillin for guinea pigs probably was a contributing factor.)[10, 11] Eight out of 16 controls died; all guinea pigs receiving globulin survived. Penicillin plasma determinations led to the belief that the treatment was adequate to bring about recovery had the agent been of any value.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF "TAGGED" DERIVATIVES IN THE FLUORIMETRIC ASSAY OF VITAMINS[1]

THIS preliminary note is the first of a series of papers dealing with the application of fluorimetry to the assay of vitamins of the B-complex. At present, such methods are used only in the case of riboflavin and thiamin; in the first instance, the fluorescence of the vitamin itself, and in the second instance, that of an oxidation product, thiochrome, is measured. In this communication, we present a new principle in fluorimetric vitamin assay methods, the use of "tagged" derivatives. By this means, the presence of a relatively light-insensitive compound may be determined quantitatively through the preparation of a highly fluorescent derivative. Extracts of biological materials, if properly prepared, contain relatively few fluorescent substances, and most of the latter give emissions in ultraviolet light. Those which fluoresce in ultraviolet light, including the reagents which are

[1] This study was aided by the Clara A. Abbott Fund of Northwestern University, Chicago.

[8] We are indebted to R. McC. Woodward, of the Department of Bacteriology, Medical Research Division, for the penicillin assays.

[9] C. H. Rammelkamp, *Proc. Soc. Exp. Biol. and Med.*, 51: 95, 1942.

[10] D. M. Hamre, G. Rake, C. M. McKee and H. B. MacPhillany, *Am. Jour. Med. Sci.*, 206: 642, 1943.

[11] H. Pinkerton, Personal communication.