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.

added, rarely exhibit this property at the higher wave-lengths of visible violet light. Therefore, the method attains considerable specificity if "tagged" derivatives are prepared which fluoresce in light of approximately 440 millimicrons wave-length. We have applied these principles successfully to the determination of nicotinic acid and p-aminobenzoic acid, using the König reaction.

In 1904, König[2] and Zincke[3] described reactions in which the very stable pyridine molecule, by combining with CNBr, 2,4-dinitrophenyl chloride, or similarly reactive reagents, formed relatively unstable pyridinium compounds which yielded highly colored crystalline derivatives of glutaconic dialdehyde on further reaction with aromatic amines. These reactions have since been adapted to minute quantities of nicotinic acid, and they form the basis of all present methods for the colorimetric determination of the vitamin.

König found that many of the derivatives possessed desirable properties as dyes, and he noted that the dyed fabrics had a fluorescent sheen in bright light. Although the reaction was subsequently studied in great detail by König,[4] Zincke[5] and others,[6] no mention of the fluorescent properties[7] of the dyes was made, as far as we are aware, except in the first communication by König.

In a study of the conditions for the maximum production of fluorescent derivatives, it was found that the intermediate N^1-cyanbromo nicotinic acid fluoresced markedly in ultraviolet light. Many of the amines which were tested and the guanidine derivatives[8,9] which are produced by the reaction of the excess of CNBr and amine in the solution gave varying intensities of fluorescence in ultraviolet light. However, these substances gave negligible fluorescence when a primary violet filter, having a maximum transmission at approximately 440 millimicrons (Corning filters 511 and 038), and a secondary orange-colored filter (Corning filter 351) were used. This filter system is commonly used in the determination of riboflavin. Under the latter conditions, many of the glutaconic dialdehyde derivatives in aqueous solution gave a greenish-yellow fluorescence similar to that of riboflavin.

The structure of the amine used in the reaction and a final high acidity of HCl are very important. Relatively little or no response in violet light was seen when the reaction was carried out with various amines as in the procedure of Melnick and Field.[10] Perhaps for this reason the phenomenon has been overlooked. However, when HCl was added, either with the aromatic amine or after the latter had reacted with the intermediate pyridinium compound, then a marked fluorescence was obtained with certain of the amines. The maximum effect was obtained at acidities above 0.5 *N* HCl. Polar radicals, especially when substituted in the para-position in the aniline molecule, conferred fluorescent properties on the dialdehyde derivative. The radicals, from the least to the most effective, may be placed in the following approximate order: H, CH_3, OCH_3, Cl, NH_2, OH, SO_3H, COOH, $COCH_3$. Thus p-aminobenzoic acid and p-aminoacetophenone, particularly the latter, yielded highly fluorescing derivatives.

The following procedure is suggested for the determination of nicotinic acid in suitably prepared extracts. Transfer 5.0 cc volumes of extract, containing from 1 to 6 micrograms of nicotinic acid, to two cork-stoppered colorimeter tubes, A and B. Warm the tubes and contents 5 to 10 minutes in a water bath which is maintained at 50 ± 2° C. To tube A add *from a burette* 2.0 cc of 0.5 *M CNBr* in 5 per cent. KH_2PO_4. To tube B, the "amine blank," add 2.0 cc of 5 per cent. solution of KH_2PO_4. Return both tubes immediately to the water bath and incubate for a period of exactly 10 minutes. Cool the tubes 5 to 10 minutes in a water bath at approximately 25° C. Now add 3.0 cc of a freshly prepared 5 per cent. solution of p-aminoacetophenone in 2 *N* HCl. Protect the tubes from strong light. Depending upon the temperature of the room and the reagents, the maximum fluorescence will be attained in 30 to 45 minutes; it remains constant for 15 minutes, and then declines slowly. Readings should, therefore, be made 30 and 45 minutes after addition of the amine reagent.

Some types of sample may contain cyanogen bromide-reacting substances. For such samples it is necessary to prepare two additional tubes, C and D. To tube C, the "CNBr blank," add 2.0 cc of CNBr reagent *from the burette,* and 3.0 cc of 2 *N* HCl. To tube D, the "color blank," add 2.0 cc of 5 per cent. KH_2PO_4 solution and 3.0 cc of 2 *N* HCl. The conditions of incubation are the same as for tubes A and B. The corrected reading then is: A − (B + C − D).

Because of the strong absorption of the violet light by the yellow glutaconic dialdehyde derivatives, the relation between fluorescence and concentration of the vitamin is no longer linear when more than 6 micrograms are assayed. With such small quantities,

[2] W. König, *Jour. prakt. Chem.,* 70: 1, 1904.
[3] T. Zincke, *Annalen d. Chemie,* 330: 361, 1904.
[4] W. König and R. Bayer, *Jour. prakt. Chem.,* 83: 325, 1911.
[5] T. Zincke and coworkers, *Annalen d. Chemie,* 333: 296, 1904; 338: 107, 1905; 339: 193, 1905; 341: 365, 1905; 353: 380, 1907.
[6] F. Reitzenstein and W. Bruening, *Jour. prakt. Chem.,* 83: 97, 1911.
[7] Zincke observed marked dichroism of solutions of the crystalline derivatives.
[8] A. W. Hoffman, *Annalen d. Chemie,* 67: 129, 1848.
[9] W. König, *Jour. prakt. Chem.,* 69: 1, 1904.
[10] D. Melnick and H. Field, Jr., *Jour. Biol. Chem.,* 134: 1, 1940.

a sensitive instrument, such as the Coleman photofluorometer, Model 12, must be used.

By means of an apparatus which was developed three years ago by Mr. George S. Liebeck, of the American Telephone and Telegraph Company, and which has been in constant use since, from 0.1 to 6 microgram quantities in 5 cc of extract can be determined. This range is made possible by very high stable electronic amplification and a variable shunt which increases or decreases the sensitivity of the microammeter with which the readings are obtained. At one-half of the maximum sensitivity of the instrument, 0.1 to 1.0 micrograms in 5 cc of standard solution, at increments of 0.1 micrograms, gave the following corrected (blank, 1.6) galvanometer deflections: 2.9, 5.9, 8.2, 10.1, 13.1, 17.3, 19.2, 22.7, 25.4, 29.5. One galvanometer deflection, therefore, indicated the presence of 0.0068 microgram of nicotinic acid per cc of extract.

The procedure has given results with wheat flours, cornmeal and animal and green plant tissues which generally check closely with those obtained by the microbiological method. The results by the latter method were obtained on acid digests. In a few instances, for example, with soybean meal, the fluorimetric method has given definitely higher results than the microbiological or colorimetric methods. This effect is not due to other fluorescent substances such as riboflavin, or amine-, or CNBr-reactive materials. It appears to be due to pyridine-like substances which react with *both* CNBr and the amine. Whether or not these substances belong in the category of physiologically active pyridine compounds studied by Elvehjem and coworkers[11] is now being determined in this laboratory.

The procedure and its application to biological materials will be described in detail in a later publication.

Summary: In a study of the König reaction, it was found that nicotinic acid, on reacting with CNBr and certain substituted aromatic amines, yields glutaconic dialdehyde derivatives which fluoresce with a greenish-yellow light in visible violet light of about 440 millimicrons wave-length. A procedure was described which is applicable to 0.1 to 6 micrograms in 5 cc of solution or extract. It is suggested that the principle of preparing fluorescing "tagged" derivatives be applied to other vitamins of the B-complex.

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[11] W. A. Krehl, C. A. Elvehjem and F. M. Strong, *Jour. Biol. Chem.*, 156: 13, 1944.

THE INHIBITION OF POLLEN PRODUCTION IN RAGWEED BY THE USE OF CHEMICAL SPRAYS[1]

DURING the course of a pollen survey, made for the Michigan Department of Health in the summers of 1940, 1941, 1942, and 1944, it became apparent that, while data in regard to the number of ragweed pollen grains in the air at any time are valuable to physicians working in the field of pollen allergy, there is a much more important problem in those regions where ragweed grows freely. The more fundamental problem is not the accumulation of numerical data concerning pollen incidence, but rather the development of some method of control for this menace to the health and well-being of many people.

A program designed to eradicate ragweed from large areas frequently faces some opposition because of the possibility that successful eradication of these plants may tend to increase soil erosion in certain regions and because the weeds have some value as cover and food for wild-life.

The ideal solution of this pollen problem would be the development of a method by which large areas may be treated with some agent which will prevent flower formation, or at least pollen production, and not destroy the vegetative portion of the offending plants. The chief obstacles to the use of chemicals for this purpose are the cost of materials and the possible danger to cultivated crops and livestock. Observations on weed control in vegetable crops by the use of selective herbicides, however, have shown that certain chemicals are available which are not a hazard to animal life and may be useful in this connection.

In the period of August 20 to September 16, 1944, a number of test sprayings were made on areas having a heavy growth of ragweed. Ragweed on these plots began pollinating the last week of August and spray treatments were begun as soon as pollen release was evident. All sprays were applied at 100 pounds pressure and at the rate of 100 gallons per acre.

RESULTS

G–412[2] (di-nitro-secondary-butyl-phenol) in kerosene gave a complete kill of ragweed within a period of six hours. The vegetative portion of the plant, as well as the flower spikes, turned brown, and pollen release was stopped. Water solutions of this material killed more slowly, and frequently killing was not complete.

G–410[2] (penta-chlor-phenol) gave a 75 per cent. kill in twelve hours, but some stems remained alive and continued growth until frost. Pollen was not again produced by these plants.

[1] Journal Article No. 755 (n.s.) from the Michigan Agricultural Experiment Station.

[2] Supplied by the Dow Chemical Company.