When it was given intravenously first and subcutaneously later the animals did develop arteritis and glomerular changes, but produced circulating antibody in scarcely detectable quantity, late in the course of the experiment.

The following theory is offered as a possible explanation of the reactions here observed. When sodium salicylate is administered subcutaneously sufficiently in advance of the first injection of antigen, it blocks the fixation of antigen in the tissue cells. This precludes any need of antibody in the tissues and there is, therefore, no lymphocytic or monocytic migration to the tissues. In this absence of tissue antigen-antibody union the chain of events leading to production of lesions is never started. The intravenously injected antigen circulates for a prolonged period, and in its passage through the lymph nodes and other reticulo-endothelial structures gives rise to production of antibody. Union of this circulating antibody with its antigen is prevented by reason of the inability of antigen to become fixed to tissue cells. The presence of this circulating antibody thus becomes an indifferent matter to the well being of the host, at least insofar as this type of allergic reaction is concerned. The observations of others concerning the potentiating effect of hyaluronidase in a certain type of allergic reaction[26] and the anti-hyaluronidase activity of sodium salicylate,[20,21] suggest that sodium salicylate blocks antigen fixation in the tissues by inhibiting hyaluronidase activity.

No conclusions can be drawn concerning the effect produced when sodium salicylate is given intravenously prior to the first injection of the antigen and subcutaneously thereafter, since only 2 animals were treated in this manner. However, the identical findings in both suggest a lowering of the protective action against antigen-tissue fixation, and a heightening of the depressant effect on the reticulo-endothelial system. This problem is at present under further investigation.

Conclusions. 1. The subcutaneous administration of large doses of sodium salicylate to rabbits well in advance of the initial contact with horse serum antigen prevents the development of arterial lesions.

2. The arterial lesions fail to develop even though circulating antibody is present in moderate quantity.

3. It is believed that the lesions fail to develop because there is no antigen-antibody reaction, and that this reaction can not take place because salicylate has prevented antigen from uniting with tissue cells.

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Specificity of the Response of Various Assay Organisms to Nicotinic Acid.*

BENJAMIN E. VOLCANI† AND ESMOND E. SNELL.

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

It has recently been shown with *Neurospora* mutants that tryptophan can be converted to nicotinic acid and that kynurenine and hydroxyanthranilic acid are intermediates in the conversion.[1,2] This conversion, which also appears to be carried out by the intestinal flora of animals,[3,4] and possibly by animal tis-

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

† Present address: Daniel Sieff Research Institute, Rehovoth, Palestine.

1 Beadle, G. W., Mitchell, H. K., and Nye, J. F., *Proc. Nat. Acad. Sci.*, 1947, **33**, 155.

2 Mitchell, H. K., and Nye, J. F., *Proc. Nat. Acad. Sci.*, 1948, **34**, 1.

3 Ellinger, P., and Abdel Kader, M. M., *Nature*, 1947, **160**, 675.

sues.‡ is of considerable interest in human nutrition.[5] Since microörganisms are widely used for the determination of nicotinic acid, and since these intermediates occur naturally under some conditions, it was of interest to determine whether the commonly used assay organisms could utilize *l*-kynurenine or hydroxyanthranilic acid for growth in place of nicotinic acid. For the organisms tested, these compounds were uniformly inactive. The data essential for establishing this fact are presented below.

Experimental. Cultures and Methods. The cultures used were *Lactobacillus arabinosus* 17-5, *Leuconostoc mesenteroides* P-60, *Streptococcus faecalis* R, *Proteus vulgaris* and *Torula cremoris* 2512. The niacin assay medium employed with the first three organisms was the complete amino acid assay medium described by Henderson and Snell,[6] with niacin omitted. For *Proteus vulgaris,* the medium of Saunders *et al.*[7] was used. With *Torula cremoris,* the medium and assay conditions described by Williams[8] were employed. In all cases, solutions of *l*-kynurenine and hydroxyanthranilic acid§ were sterilized by filtration, and added aseptically to the basal media after these had been sterilized by autoclaving. The final volume in all assay tubes was 2 cc. After a three-day incubation period, which permitted heavy growth of all organisms except *Proteus vulgaris* in media containing excess niacin, cultures were diluted

TABLE I.

Growth Response of Various Organisms to *l*-Kynurenine and Hydroxyanthranilic Acid in Presence and Absence of Nicotinic Acid.*

Substance added γ per 2 cc	Nicotinic acid			*l*-Kynurenine		Hydroxyanthranilic acid		Hydroxyanthranilic acid plus kynurenine 0.5 γ each	Niacin (.05 γ) plus hydroxyanthranilic acid (0.5 γ) plus kynurenine (0.5 γ)
	0	.05	10	0.1	10	0.1	10		
Organism	% of incident of light transmitted (diluted cultures)								
L. arabinosus	91	70	43	91	97	95	94	95	74
Leuc. mesenteroides	95	74	60	95	96	96	96	98	76
S. faecalis	92	79	82	92	92	92	92	92	80
P. vulgaris	99	92	—	99	99	99	99	99	92
T. cremoris	99	77	53	99	99	97	99	96	76

* Several levels of each substance in addition to those shown were tested. In no case was evidence of any growth-promoting action apparent.

[4] Schweigert, B. S., and Pearson, P. B., *J. Biol. Chem.*, 1946, **163**, 343.

‡ *Cf.* the synthesis of niacin which occurs during incubation of hen (Quarles, E., and Snell, E. E., *J. Nutrition*, 1941, **22**, 483) and turkey (Furman, C., Snell, E. E., and Cravens, W. W., *Poultry Science*, 1947, **26**, 307) eggs, where no microörganisms are present.

[5] Anonymous, *Nutrition Rev.*, 1947, **5**, 247.

[6] Henderson, L. M., and Snell, E. E., *J. Biol. Chem.*, 1948, **172**, 15.

[7] Saunders, F., Dorfman, A., and Koser, S. A., *J. Biol. Chem.*, 1941, **138**, 69.

[8] Williams, W. L., *J. Biol. Chem.*, 1946, **166**, 397.

§ We are indebted to Dr. H. K. Mitchell for samples of *l*-kynurenine and hydroxyanthranilic acid. Synthesis of the latter compound has been described recently (Nye, J. F., and Mitchell, H. K., *J. Am. Chem. Soc.*, in press).

with water to 10 cc and turbidity values determined with the Evelyn photoelectric colorimeter. Results are given in Table I. In no case did kynurenine or hydroxyanthranilic acid, either singly or combined, in the presence or absence of sub-optimal quantities of niacin, enhance growth. Ornithine, glutamine and arginine, which increase nicotinamide production by some strains of *E. coli*, have also been shown to be ineffective in substituting for the niacin requirement of *L. arabinosus*,[3] the organism most commonly used for assay of this vitamin.

Summary. Kynurenine and hydroxyanthranilic acid, which appear to be intermediates in the conversion of tryptophan to nicotinic acid by *Neurospora*, neither replace nicotinic acid nor enhance the growth response to sub-optimal amounts of nicotinic acid for *L. arabinosus, Leuc. mesenteroides, S. faecalis, Proteus vulgaris,* or *Torula cremoris*. These substances do not, therefore, interfere in microbiological assays for niacin which employ these test organisms.

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Paper Partition Chromatographic Analysis and Microbial Growth Factors: The Vitamin B_6 Group.

WALTER A. WINSTEN AND EDWARD EIGEN. (Introduced by A. E. Sobel.)

From Food Research Laboratories, Inc., Long Island City, N.Y.

Microbial growth factors often exist in nature in more than one chemically defined form. In the past, differential assays with several microörganisms have been used to demonstrate this multiplicity.

The present report deals with an alternate procedure for recognizing the presence of different forms of a growth factor. The method involves the combined use of paper partition chromatography and a microbial indicator and represents, as far as the writers are aware, a new methodological principle for the recognition and resolution of different chemical forms of a growth factor.

As will be seen, the method to be described is the counterpart of the technique used in the differention of the several forms of penicillin by Goodall and Levi[1] and independently by Winsten and Spark[2] except that in dealing with growth factors, zones of microbial growth serve to delineate the different members of a complex instead of zones of inhibition, as in the case of the antibiotics. The use of zones of microbial growth to assay vitamins was suggested by the work of Mager and Aschner.[3]

In the present method, the underlying principle involves the separation on a paper strip chromatogram of the different chemical forms of a growth factor. After the separation has been achieved, the positions of the different forms on the paper strip are revealed by the use of a microbial indicator, that is, a microörganism capable of utilizing at least one and usually several forms of the factor for growth. The positions of the various chemical entities on the chromatogram serve to characterize and identify the particular substances present. This is a consequence of the fact that the distance each substance moves is related to its specific partition coefficient for the solvents employed in developing the chromatogram.

In what follows, the application of this method to the resolution and identification of the members of the B_6 group will be described. This particular complex of growth factors was

[1] Goodall, R. R., and Levi, A. A., *Nature*, 1946, **158**, 675.

[2] Winsten, W. A., and Spark, A. H., *Science*, 1947, **106**, 192.

[3] Mager, J., and Aschner, M., *J. Bact.*, 1947, **53**, 283.