

Fleming's discovery of the strain of *Penicillium notatum* which produces penicillin.

In contrast to penicillin, penatin is not extractable from the crude culture by the common organic solvents. Two ways of purification have been found. One is the precipitation of penatin with phosphotungstic acid (penicillin is not precipitated by this agent) which will form an acid-insoluble penatin-phosphotungstate. Because of losses in free penatin encountered in the decomposition of this compound, another method of purification was used. It consists in the adsorption of penatin on kaolin at pH 4, elution of the washed kaolin with pyridin or sodium phosphate at pH 6.3, and precipitation of the penatin by dioxane. The dioxane precipitate is dissolved in water and dried by the lyophilic method. Concentrated solutions of penatin in this state of purification are yellow. In dry preparation, penatin is a yellowish hygroscopic powder, completely soluble in water and stable for months. Penatin is sensitive to the action of alkalis, but more resistant to acids.

A single intravenous injection of 16.5 mg of penatin into a rabbit and a single intramuscular injection of 250 mg of penatin into a guinea pig were made without obvious ill effects. Furthermore, its action is not impeded in 90 per cent. serum. Further experiments on toxicity and antibacterial action *in vivo*, as well as some peculiarities of penatin, will be reported elsewhere.

WALTER KOCHOLATY

SOME CHEMICAL AND PHARMACOLOGICAL OBSERVATIONS ON "LOW NICOTINE" TOBACCO

THROUGH the kindness of Dr. W. D. Valleau, of the Kentucky Agricultural Station, we were supplied with sufficient "low nicotine" Kentucky burley tobacco to make certain chemical and pharmacological observations which appear of interest. The only previous report of similar studies is that of Wenusch and Maier¹ whose observations our present work confirms in general.

On chemical analysis, the leaf web of this tobacco was found to contain 0.13 per cent. nicotine and 0.27 per cent. nornicotine.² Cigarettes weighing one gram each were made of the granulated leaf web and the main stream smoke analyzed (excepting for nornicotine) by the methods described by Bradford, Harlow, Harlan and Hanmer.³ The smoke from each cigarette contained 0.42 mg total volatile bases (calculated as ammonia) including 0.23 mg nicotine and 0.058 mg nornicotine.

¹ Adolph Wenusch and Gerda Maier, *Munchen. Med. Wchnschr.*, 87: 1263, 1940.

² Methods of analysis to be published.

³ J. A. Bradford, E. S. Harlow, W. R. Harlan and H. R. Hanmer, *Ind. and Eng. Chem.*, 29: 45, 1937.

The amount of nicotine is less than 10 per cent. of that found in the smoke of the average standard cigarette. The presence of nornicotine in the tobacco adds an unusual feature to these cigarettes, since this substance is not normally reported present in cigarette tobaccos. Wenusch⁴ has stated that only a small amount of nornicotine is transferred into the smoke from material containing it. The data presented here indicate that the transfer is less than 4 per cent. compared to 29 per cent. for nicotine. The percentage transfer of nicotine from average standard cigarettes is about 22 per cent., the higher value obtained here representing a recognized tendency for low nicotine tobaccos to transfer a higher percentage of their nicotine content into the smoke.³

Solutions were prepared from the smoke of these cigarettes as well as from that of a standard brand of cigarettes by a method previously described⁵ and studied pharmacologically. These solutions were so made that, calculated on the nicotine content of smoke, each ml contained 0.5 mg nicotine. When tested for their toxicity by intraperitoneal injection into white mice, the L. D.₅₀ for the two solutions was identical and in accord with their calculated nicotine content. The failure of nornicotine to materially affect the result is due to its low percentage transfer in the smoke and to its toxicity being only half that of nicotine by the intraperitoneal mode of administration.⁶ When these solutions were injected intravenously into an anesthetized dog arranged for the recording of blood pressure, the blood pressure response was identical to that produced by a control solution of pure nicotine of similar nicotine content. The pressor potency of nornicotine is only one twelfth that of nicotine.⁶

A limited number of studies were made on the effect of smoking these low nicotine cigarettes on the blood pressure and pulse rate in man, using the method of standardized smoking described by Main.⁷ The results of these preliminary tests showed that the smoke from the low nicotine cigarettes on an average produced effects comparable to those observed after the smoking of nicotine-free cigarettes,^{7, 8} both types of cigarettes evoking circulatory responses, markedly less than those effected by ordinary cigarettes.

SUMMARY

Chemical and pharmacological tests have been carried out on a sample of "low nicotine" tobacco. Laboratory tests involving toxicological studies on mice

⁴ Adolph Wenusch, *Pharm. Zentralhalle*, 77: 141, 1936.

⁵ H. B. Haag, *Jour. Lab. and Clin. Med.*, 25: 610, 1940.

⁶ P. S. Larson and H. B. Haag, *J. Pharmacol. and Exper. Therap.*, in press.

⁷ R. J. Main, *Proc. Soc. Exper. Biol. and Med.*, 48: 495, 1941.

⁸ J. H. Weatherby, *Am. Heart Jour.*, 24: 17, 1942.

and blood pressure determinations in the dog showed that the toxic and vasopressor effects of the smoke from this tobacco was proportional to the amount of nicotine present and relatively independent of the normicotine content of the tobacco. On man the smoke from these low nicotine cigarettes produced very much less pronounced effects on blood pressure and pulse rate than that produced by ordinary cigar-

ettes. These results indicate that this type of tobacco is sufficiently low in nicotine to make it practically deserving of much further study.

H. B. HAAG
P. S. LARSON

DEPARTMENT OF PHARMACOLOGY,
MEDICAL COLLEGE OF VIRGINIA,
RICHMOND

SCIENTIFIC APPARATUS AND LABORATORY METHODS

DRY STRIPPED REPLICAS FOR THE ELECTRON MICROSCOPE

THE use of the electron microscope to study the fine structure of the surface of etched metals or other materials has been shown to be a fairly simple process^{1,2} when the direct replica method is used. A still simpler process than those previously described for removing the replica film has now been devised by the writer. It lends itself to a study of practically any flat surface which is free of re-entrant angles or other structure that might prevent the removal of the replica film. The method should be particularly adaptable in permitting the microscopist to make a comparison of identical structures as seen in the optical and electron microscopes.

The replica film of polyvinyl formal dissolved in dioxane is formed on the specimen in the manner previously described. A standard electron microscope, 200 mesh nickelscreen, is then centered above the region to be studied. This positioning process may be carried out with a jig which slips over the objective of the light microscope used for obtaining a photomicrograph. With the screen in place a bit of moist air is applied to the coated surface and immediately a piece of scotch tape is pressed into light contact with the screen and the surrounding film. Holding the scotch tape against the specimen, on one side of the positioned screen, the tape is held rigidly and lifted away from the surface. When this is done the replica leaves the specimen and will be found in contact with the under surface of the screen ready for mounting in the specimen holder of the electron microscope.

The scotch tape is easily removed from the mounting screen by placing the tape holding the screen in contact with a flat surface. The tape is then turned through 180°, a finger placed on the sticky surface and the tape slowly peeled from the surface until the edge of the screen becomes visible. A razor blade or similar thin flat object held against the screen prevents it from becoming bent as the tape is peeled away from it.

The entire process of replica formation, removal

and mounting may be carried out in less than two minutes. Perhaps the most attractive feature of the process described is the fact that the specimen is not injured in any way. For example, from a specimen of 1.1 C Steel (used for developing the technique) at least fifty replicas have been obtained without injuring the surface in any way.

In preparing surfaces of metals for electronographic study it is important that the distance in elevation between high and low spots on the sample does not exceed 400–600 ÅU. To obtain this property and still have a uniform metallographic etch it was found that the standard solutions (such as Nital) should be diluted from four to ten fold, and applied with a swab.

Some evidence has been noted which indicates that conditioning metal surfaces with surface active molecules such as tricresyl phosphate and oleic acid greatly facilitates the removal of replicas. Not more than a monolayer is needed for this purpose. Excess molecules and other contaminations are readily removed by coating the specimen with a film three or four times thicker than that used for making replicas and peeling it off with scotch tape. This thicker film also affords excellent protection to the prepared surface against corroding vapors and moisture.

VINCENT J. SCHAEFER

GENERAL ELECTRIC COMPANY,
SCHENECTADY, N. Y.

BOOKS RECEIVED

- ALBEE, FRED H. *A Surgeon's Fight to Rebuild Men. The Autobiography of Dr. Fred H. Albee.* Pp. 349. E. P. Dutton. \$3.50.
- CLARK, JOHN A., FREDERICK RUSSELL GORTON, FRANCIS W. SEARS and MAJOR FRANCIS C. CROTTY. *Fundamentals of Machines.* Illustrated. Pp. xi + 300. Houghton Mifflin Company. \$1.24.
- COTTON, C. A. *Climatic Accidents in Landscape-Making.* Illustrated. Pp. xx + 343. Whitcombe & Tombs, Ltd. 30 Shillings.
- FRISON, T. H. *Studies of North American Plecoptera.* Illustrated. Pp. 120. Illinois Natural History Survey.
- MARCUS, ABRAHAM and WILLIAM MARCUS. *Elements of Radio.* Illustrated. Pp. xiii + 699. Prentice-Hall. \$4.00.
- SWIETOSLAWSKI, W. *Coke Formation Process and Physico-Chemical Properties of Coals.* Illustrated. Pp. viii + 145. Herald Square Press.
- TEHON, LEO R. *Fieldbook of Native Illinois Shrubs.* Illustrated. Pp. 307. Illinois Natural History Survey.

¹ V. J. Schaefer and D. Harker, *Jour. App. Phys.*, 13: 427, July, 1942.

² V. J. Schaefer, *Phys. Rev.*, 62: 495, 1942.