parenchyma cells; the next two had necrosis plus edema of the gland, with infiltration of polynuclear leucocytes and hemorrhages inside the septa; the fourth dog showed only pancreatic hemorrhages, without much necrosis or infiltration. Accompanying this acute pancreatic disease, all 4 dogs had fat necrosis of the adipose tissue around the pancreas and in the mesentery and omentum. These fat necroses had the typical aspect of white specks which is so characteristic of the fat necrosis that accompanies acute pancreatitis in the human. Three of the 5 dogs showed recent hemorrhages in the mucosa of the stomach or small intestine.

One other dog was fed 10,000 units of vitamin A and 5 mg of vitamin B_1/day in addition to the bacon diet. These vitamin supplements were unable to modify the course or the ultimate outcome of the experiment. This dog, which died after 7 months, had a fatty liver and acute necrosis with hemorrhages in the pancreas, fat necrosis in the omentum, and uric acid kidney stones, just as did the animals that had not received any supplement.

The last dog received 25 gm of raw liver daily for 13 months. At the end of this time it was still in perfect condition. The liver supplement was then replaced by 25 gm of raw pancreas daily. After 5 months on this supplement the animal died in a cachectic condition. Postmortem examination showed fatty degeneration of the liver. The pancreas showed hemorrhages and areas of inflammation. There were fat necroses in the omentum, and both kidneys contained uric acid stones. It appeared, therefore, as if 25 gm of raw liver had protected the animal, whereas 25 gm of raw pancreas had been unable to do so.

The severe fatty infiltration of the liver was obviously due to the combination of a high fat content of the diet and a lack of lipotropic factors. The finding of kidney stones consisting of uric acid is remarkable. The normal end-product of purine metabolism in dogs is not uric acid but allantoin, and only small amounts of uric acid normally appear in the dogs' blood and urine. Mann and his associates (*1*) have shown that the transformation of uric acid into allantoin in the dog takes place in the liver. In the liverless dog uric acid accumulates in the blood and is excreted into the urine. The same was found when the liver was damaged by chloroform or phosphorus intoxication. It seems likely, therefore, that the severe fatty infiltration of the liver had impaired its function in our animals and had acted as chemical hepatectomy. Moreover, many of the dogs had a low urinary output with a high concentration of solids and a very high urinary acidity. All these factors may have contributed to the precipitation of uric acid and the formation of stones in the urinary tract.

The hemorrhagic pancreatic necrosis in these animals had apparently occurred as a terminal event. Its explanation is uncertain. From the microscopical aspect one got the impression that the inflammation and hemorrhage had spread along the septa. In some of the sections the lumen of the pancreatic ducts appeared blocked by a material that could not be identified. It seems possible that obstruction of the ducts by this material has been the primary event after which necrosis, hemorrhage, and inflammation of the gland followed. Whatever the pathogenesis of the acute pancreatic necrosis may have been, it is interesting to note the association of acute pancreatic disease with fatty degeneration of the liver in these dogs. It is well known from human pathology that acute pancreatic necrosis occurs as a complication in patients with disease of the gall bladder and bile ducts, in alcoholics, and sometimes in diabetic coma. Fatty degeneration of the liver is a common feature in all these conditions. These experiments suggest that the presence of a fatty liver may play a part in the pathogenesis of acute pancreatic necrosis.

In a further communication the effect of the diet on blood and urine chemistry and blood morphology will be presented.

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Photochemical Oxidation of Nicotine in the Presence of Methylene Blue

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The photochemical oxidation of nicotine in the presence of methylene blue produces a compound which has not been completely characterized. During the course of this investigation, it was learned that a similar, if not identical, product had been isolated by W. G. Frankenburg (*1*) from the alkaloids of fermented tobacco. It was therefore deemed advisable to disclose our findings simultaneously.

If an aqueous solution of *l*-nicotine is irradiated in the presence of oxygen and a small amount of methylene blue, a rapid oxidation of the nicotine takes place which, in the dark, comes to a standstill. The effective wave length (around 6,700 A) coincides with the maximum absorption of the dye, and thus the "light-excited" dye can serve as a hydrogen acceptor, as shown by its rapid bleaching. The leuco methylene blue formed is reoxidized and serves continuously as a hydrogen acceptor. The oxygen required for the reoxidation of the reduced dye or, in terms of total effect, for the oxidation of nicotine thus serves as a means to follow the rate of the reaction.

An adaptation of the manometric technique of Warburg and Negelein (*3*) was used. White light of high intensity was passed horizontally through the glass wall of the water bath and reflected on the bottom of the vessel by a mirror placed at 45° under the respirometers. In general, 20 mg of the compound to be studied was dissolved in 1.5 cc of water and placed in the main chamber,

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

and 0.5 cc of water solution of methylene blue containing 0.1 mg was placed in the side arm. After the vessel was filled with oxygen and the dye dumped into the main chamber, the oxygen uptake was measured in the usual fashion at the desired water-bath temperature. A rapid oxygen uptake took place which stopped sharply after about 4 hrs at 40°, when an equimolar amount of oxygen had been taken up. The optimum pH for the oxygen uptake was about 9. There was no CO_2 evolution.

Irradiation experiments under anaerobic condition in Thunberg tubes, to prevent reoxidation of the leuco dye, showed that the bleaching time is practically not affected at temperatures of 30°, 40°, 50°, 60°, and 70°, thus indicating a true photochemical reaction in this phase. The reaction appeared to be reversible, although it proceeded slowly, as in the thionine-iron system (*2, 4, 5*). The aerobic photo-oxidation of nicotine, however, is strongly accelerated by increasing temperature, which would indicate the participation of a dark reaction in addition to the previously observed light-sensitive reaction.

The N—CH_3 group of the pyrrolidine ring seems to be important in the reaction, since under similar conditions *d,l*-nornicotine[2] acts sluggishly. Pyridine, β-picoline, β-vinylpyridine, nicotinic acid, and nicotinamide are unreactive. N—CH_3-pyrrolidine, however, which is a part of the nicotine molecule, is photo-oxidized in the presence of the dye at the same rate and to the same extent as nicotine itself. These experiments indicate that the chemical changes due to the irradiation are confined to the N—CH_3-pyrrolidine ring.

The product (obtained by larger-scale experiments) is a yellow viscous oil, not volatile in steam, practically insoluble in ether, but soluble in chloroform, in polar solvents, and in water. It is optically active ($\alpha_D^{20} = -35.2°$), indicating that the asymmetric carbon atom is not involved in the reaction. During the irradiation, the pH of the reaction mixture dropped from 10.4 to 6.8, suggesting that the strongly basic pyrrolidine nitrogen is involved in the reaction by forming, for example, an amine oxide. The elementary analysis of the ether-insoluble irradiation product of nicotine was: Calculated for $C_{10}H_{14}N_2O_2$: C, 61.85%; H, 7.21%; N, 14.43%; Found: C, 62.69%; H, 7.21%; N, 14.08%. Upon reduction with Zn and acetic acid in alcoholic solution, it was to a large part reconverted into nicotine (85–100%). Qualitative tests for aldehyde, keto, peroxide, and alcoholic groups were negative. The compound is not identical with oxy-nicotine. Further work is in progress to identify the product, but in the meantime, the following over-all equations are proposed for the reaction:

$$\text{(I)}\quad C_{10}H_{14}N_2 + M\text{—}B^* \xrightarrow{\text{light}} C_{10}H_{12}N_2 + M\text{—}B(H_2)\dagger$$

$$\text{(II)}\quad M\text{—}B(H_2) + O_2 \longrightarrow M\text{—}B + H_2O_2$$

$$\text{(III)}\quad C_{10}H_{12}N_2 + H_2O_2 \longrightarrow C_{10}H_{14}N_2O_2$$

* Methylene blue

† Leuco methylene blue

Reaction I would represent the light reactions; II and III, the dark ones. An attempt to demonstrate the intermediate formation of H_2O_2, as required by reaction II, was not successful. Preliminary work indicated that the failure might be due to the high reactivity of certain tertiary amines with nascent H_2O_2.

[2] Kindly supplied by P. G. Haines, of this laboratory.

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Transformation Products of Nicotine in Fermented Tobacco

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One of the chemical changes concurring with the fermentation of cigar leaf tobacco[1] is a considerable decrease of the nicotine contained in the leaf tissues. Table 1 shows the average nicotine contents (30 samples each) of four crops of Pennsylvania Seedleaf tobacco, before and after fermentation. For the individual samples, the nicotine decreases range from 6 to 94% of the amount present before fermentation. A definite parallelism exists between the degree to which each individual sample has been fermented and the extent of its nicotine decrease. To the author's knowledge, no systematic studies have been published concerning the fate of the disappearing nicotine, except that occasionally volatilization of the alkaloid has been assumed without experimental proof.

TABLE 1

Decrease of Nicotine Contents of Pennsylvania Cigar Tobacco for Various Crops

(Averages are based on analyses of 30 samples for each crop.)

Crop Year	Avg. nicotine contents (% of dry weights)*		Avg. decrease of nicotine due to fermentation (%)	Decrease of nicotine for individual samples	
	Before fermentation (BF)	After fermentation (AF)		Minimum (%)	Maximum (%)
1936	3.08	1.51	51	6	94
1938	2.82	1.76	38	7	72
1939	2.26	0.98	57	18	89
1941	3.85	2.04	47	27	83

* All values based on dry weights before fermentation.

In this laboratory, analytical studies have been conducted for several years on the nitrogenous components and particularly on the alkaloids and related substances in the leaf tissues of Pennsylvania tobacco. Specifically, we investigated the changes of these compounds resulting from fermentation.

[1] The fermentation of Pennsylvania tobacco has been described briefly by the writer (*Arch. Biochem.*, 1947, **14**, 157–181).