TABLE III.
Effect of 2,3-dichloro-naphthoquinone on Growth and Survival of Weanling Mice Fed a Highly Purified Ration Free of Vitamin K.

Amt in diet, %	No. of animals	Deaths	Avg change in wt, g per wk
0	8	0	+5.0
0.1	4	0	+3.3
0.2	4	0	+1.4
0.5	14	7	—1.0

growth was diminished detectably when 0.1% of the diet consisted of the quinone, and when this was increased to 0.5%, half the animals died within 3 weeks (Table III). The mice which died did not, however, show signs of hemorrhage macroscopically as do mice fed 3,3'-methylenebis-(4-hydroxycoumarin). Furthermore, the prothrombin time of the diluted plasma of mice fed 0.5% of the quinone was not significantly greater than that of the controls or of normal mice. Finally, simultaneous administration of 2-methyl-naphthoquinone or of vitamin K_1 did not erase the toxic manifestations of 2,3-dichloro-naphthoquinone.

Since vitamin K has not been demonstrated in yeast, it may be that dichloro-naphthoquinone and the vitamin are just 2 foreign substances contending non-specifically in the yeast cells, which fall prey to either agent or to both, or to neither, depending on relative and absolute concentrations. However, vitamin K is known to exist in some microörganisms, and to be an essential for growth of the Johne's bacillus.[11] The concentration of the vitamin in yeast may be so small as to be undetectable by present methods, and yet sufficient to supply each cell with many molecules.

From the examples now at hand it would seem that the replacement of methyl groups attached to benzenoid nuclei in metabolites with Cl atoms can lead to inhibitory structural analogs which compete with these metabolites. More examples would be desirable to determine whether it is a type of change which is generally applicable.

Summary. 2,3-dichloro-1,4-naphthoquinone, an antifungal agent now in practical use, has been recognized as an analog of vitamin K. This substance has been found to be exceedingly toxic to yeasts and moderately harmful to the growth of bacteria. Its effect on yeast was reversed competitively by vitamins K over a limited range of concentration. While the effect on the growth of bacteria was not influenced by vitamin K in the form of 2-methyl-naphthoquinone, it was probable that this was due to the toxicity of the vitamin for these species.

11 Woolley, D. W., and McCarter, J. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 357.

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The Porphyrin of Harder's Gland.

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The fact that choline poisoning stimulates the flow of brick red tears, designated as chromodacryorrhea,[1] has been reported and confirmed; however, the nature of the pigment, protoporphyrin[2] or blood,[3] is in dispute. The presence of a porphyrin in Harder's

1 Tashiro, S., and Stix, H., *Biol. Bull.*, 1935, **64**, 327.

2 Barnard, R. D., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 254.

3 Hodge, H. C., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 26.

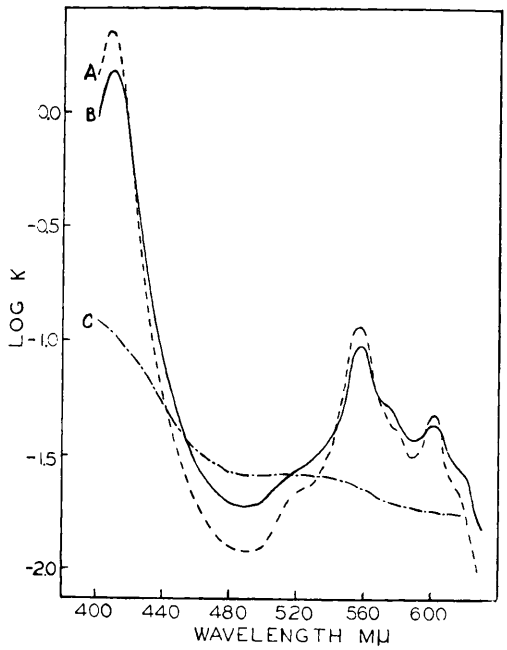

FIG. 1.
Spectrophotometric curves for the red pigment of "bloody" tears (A), extract of Harder's gland (B), and extract of rat blood (C).

gland in the rat and its excretion from that gland has been described.[4] On the basis of these observations, our attention was directed to the role of Harder's gland in chromodacryorrhea produced by choline poisoning. In the present study, young adult rats of both sexes were given intraperitoneal injections of choline chloride, about 200 mg in aqueous solutions. Convulsions and secretion of deep red tears were followed by death in each of about 20 rats.

Microscopic examination of the red tears revealed a field full of spherical, colorless, clear droplets among which were fragments of yellow-brown pigment. A source of ultraviolet light, a Corning ultraviolet filter and a glass optical system provided illumination in the near-ultraviolet. This light when directed through the condenser upon the microscopic field caused the pigment particles to fluoresce a brilliant fuchsia, whereas the spherical droplets remained dark.

Absorption spectra. A sample was prepared for spectrophotometric analysis as follows: The red tears from several animals were stirred with 2 cc of water and centrifuged at high speed. The turbid, nearly colorless supernatant liquid was decanted, leaving a dark red solid at the bottom of the tube. The red solid was washed by trituration and centrifuging with two 1-cc portions of distilled water. A small portion of the washed pigment was dissolved in 0.1 *N* hydrochloric acid. Centrifugation threw down a thin film of colorless material, leaving a clear, slightly pink solution that showed intense red fluorescence under the ultraviolet lamp. Determination of its absorption spectrum, using the Beckman quartz spectrophotometer, gave curve A in Fig. 1.

$$K = \text{Log} \frac{I_o}{I}$$

Comparison of the absorption curve of our red pigment with data published by Hans Fischer[5] (Table I) showed that our pigment is a protoporphyrin. This finding confirmed the observation of Barnard.[2]

TABLE I.
Comparison of Absorption Characteristics of Protoporphyrin and Pigment.

		Protoporphyrin (5) (mμ)	Pigment from red tears (mμ)
I	Band width	607.6-597.3	606-596
	Band maximum	602.4	601
II	Band width	586.5-577.9	586-576
	Band max.	582.2	580
III	Band width	565.3-549.2	564-549
	Band max.	557.2	557

To determine whether hemoglobin might also be in the red tears, Dr. Alexander Dounce performed a pyridine hemochromogen test and examined a second sample of the tears for methemoglobin. These examinations were made with a hand spectroscope and in each case, bearing in mind the limits of the hand spectroscope, the absence of the characteristic bands showed that no appreciable amount of blood was present in the red tears.

Gland pigment. Two pairs of fresh Harderian glands were prepared for the spectro-

[4] Derrien, E., and Turchini, J., *C. R. de la Soc. de biol.*, 1924, **91**, 637.

[5] Fischer, H., Abderhalden, *Handbuch der biologischen Arbeitsmethoden*, 1936, **1**, 169.

photometer by washing with physiological saline to remove superficial blood. The glands were dried on filter paper and ground in a mortar with a pinch of sand and a little quick-setting plaster of paris. The fine, dry powder was ground with 2 portions of ether to remove fat. It was then ground with 2 cc of 0.1 N hydrochloric acid. The acid solution was centrifuged and the absorption spectrum of the clear, pink supernatant solution was determined, giving curve B in Fig. 1, which is very much like the curve of the pigment from the tears. This solution also showed intense red fluorescence under the ultraviolet lamp.

A few drops of fresh, normal rat blood in water was put into a mortar and treated in the same manner as the gland had been. The extract thus obtained had an absorption spectrum which is shown by curve C in Fig. 1. The porphyrin which was extracted from the red tears and from Harder's gland cannot be extracted from blood.

Unilateral extirpation. Under ether anesthesia, Harder's gland was removed from the right ocular orbit in 11 rats. After 2 weeks, the right eyes of these rats seemed normal except for their sunken appearance. Toxic doses of choline were injected intraperitoneally, and in every animal red tears welled up into the left eye but not in the right eye. In at least 6 of the animals a large pool of colorless tears could be detected in the right eye, indicating that in these animals, at least, lachrymal ducts and glands remained intact. The pigment in the red tears induced by choline poisoning undoubtedly has its origin in the glands of Harder.

Gland stimulation. Some interesting observations were made with reference to the stimulation of Harder's gland. If the common carotid artery and the external and internal jugular veins were ligated on one side of the rat, then the chromodacryorrhea following intraperitoneal choline injection was just perceptible on the ligated side but profuse on the normal side. Injection of choline into the common carotid produced highly pigmented tears, of a chocolate rather than brick red color. Careful observation revealed that the tears first appeared on the same side as the injection. When such an injection is made in a rat not more than a few minutes dead, the red tears appear only on the same side as the carotid which is injected. Choline injected topically behind the eyeball and into the orbit also produces chromodacryorrhea. These observations indicated that the choline affected the Harder's gland directly rather than through some central mechanism. Electrical stimulation of the gland with an inductorium produced red tears from that eye, but intracranial stimulation of the fifth and seventh nerves had no effect.

Histological observations. Under light ether anesthesia the right Harderian gland of a rat was electrically stimulated to produce red tears, and then both glands were removed and fixed in 10% formalin. Frozen sections were prepared and the uncleared, unstained sections were put on slides (in either water or glycerine) and gelatin-sealed under coverslips. Microscopic examination showed that many of the collecting ducts were filled with yellow-brown pigment. The number of filled ducts was much greater in the control gland than in the stimulated gland. With near-ultraviolet illumination, all of the tissue fluoresced a brilliant fuchsia. The control sections seemed to fluoresce somewhat more than the sections of stimulated gland but, since some sections of the latter equaled the former in brilliance, no definite difference can be reported. The pigment showed no fluorescence. At first this was attributed to its thickness and opacity; but even when illuminated from above, these areas remained dark. These observations were made of the sections as they appeared immediately after their preparation.

After the sections had been prepared for about 3 weeks they were again examined with the fluorescence microscope. The brilliance of the fluorescence in areas where there were no pigment-filled tubules had faded markedly. The cells surrounding the pigment fluoresced as much as, or more than, they had at first; and some of the thinner (as judged by their appearance in white light) pigment areas showed a deep ruby-red fluorescence. Apparently the pigment in the tubules has undergone some change while standing.

Summary. A pigment from Harder's gland

has been identified as a protoporphyrin. It is this pigment and not blood that appears in choline-induced chromodacryorrhea.

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Serological Differentiation of Primary Atypical Pneumonia from Virus Pneumonia of the Psittacosis Group.*

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Despite the widespread occurrence of agents of the psittacosis-lymphogranuloma group in birds and mammals, there is now definite evidence that few cases of virus pneumonia are caused by psittacosis or related agents, while many atypical pneumonias may be caused by a new filterable virus recently transmitted to chick embryos, cotton rats, and hamsters.[1] Eaton and Corey,[2] using complement fixation tests with the virus of meningopneumonitis propagated in allantoic fluid and the virus of lymphogranuloma venereum grown in yolk sacs of chick embryos, found that among 70 patients with pneumonitis of unknown etiology, 10 to 15% showed an increase in complement-fixing antibodies for the psittacosis group. Isolation of a psittacosis-like virus was successful in only 10 of over 250 specimens of sputum or lung inoculated intranasally into mice or cotton rats.[2,3] In a series of 45 cases Smadel,[4] using agar slant tissue cultures of psittacosis virus as antigen, demonstrated a significant rise in complement-fixing antibodies in 10 cases, or about 22%. The virus was isolated from 2 of these patients. Workers in Francis' laboratory,[5] using an antigen from mouse lung infected with meningopneumonitis virus and refined by differential centrifugation, obtained a significant increase in titer (fourfold or greater) in only 1 case among 57 cases of atypical pneumonia. In the same laboratory, psittacosis-like viruses were isolated from the sputum or throat washings of 5 of 20 patients by inoculation of Java rice birds and subsequent intranasal passage in mice or by passage in mice alone. The relation of these findings to the etiology of atypical pneumonia was not clear, since none of the 5 patients had any increase in complement-fixation titer for meningopneumonitis associated with their illness. In a recent article Dingle[6] states that in the army the diagnosis of psittacosis or ornithosis was not once established in a series of more than 500 patients with atypical pneumonia and other respiratory infections. The method of testing was not stated.

During an epidemic of 19 cases of pneumonitis in the bayou region of Louisiana, a virus similar to the "psittacine group" was isolated from the throat washings of 3 of 4 patients studied and from the lungs at autopsy of 2 of these patients.[7] This outbreak seems to have been quite different in its general characteristics from primary atypical pneumonia. In other publications since 1940 psitttacosis-like viruses were identified as the

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6 Dingle, J. H., *Bull. N. Y. Acad. Med.*, 1945, **21**, 235.

7 Olson, B. J., and Larson, C. L., *Pub. Health Rep.*, 1944, **59**, 1373.