

the basic index for conclusions—electrical manifestations, etc., being evaluated and judged as concomitant and secondary phenomena and of significant practical value only insofar as their meaning is revealed by contractile phenomena.

These conclusions are more in line with the older observations and conclusions than with current beliefs and teachings.

## 6750

**Observations on Behavior of the Anthocyan Pigment from Concord Grapes in the Animal Body.**

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The anthocyanins are not uncommon constituents of our diet. They are especially abundant in berries, grapes and grape products. Nevertheless, facts concerning the metabolism of these pigments are meager. In the following experiments we investigated both the alimentary and parenteral fate of the anthocyan from Concord grapes.

The anthocyan necessary for the experiments was prepared as the chloride according to the method described by Anderson.<sup>1</sup>

(A) 50 mg. of the pigment in saline solution, subcutaneously injected into large rats (450 gm.), resulted in the excretion of the dye in the urine. The specimen at the end of the first day was intensely colored, almost black; the next day's urine had a slightly bluish tinge. Proof of the presence of the dye was obtained by adding to the urines a drop of HCl, which changed the color to a deep red. Other tests confirmed the presence of the pigment. Apparently, a considerable portion of the pigment was excreted without undergoing any change.

(B) In order to determine whether or not the compound was absorbed from the intestine, quantities of 100 mg. of the anthocyan chloride were fed to starved rats by stomach tube. The pigment was not excreted in the urine over a period of 4 days, although the addition of 0.2% of pigment directly to the urine gave the mixture a decided pink color. Heating the urine with acid did not increase the pigmentation. At the same time, qualitative examination of the feces showed some pigment to be present.

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<sup>1</sup> Anderson, R. J., *J. Biol. Chem.*, 1924, **61**, 97.

(C) The possibility that the pigment was partially destroyed by intestinal bacteria was investigated. Small amounts of different bacterial media, to which a trace of anthocyan chloride had been added, were inoculated with saline extractions of human feces and incubated. No change in color was apparent after periods of one, 2 or 3 days. Suitable controls, which differed only in that they contained saline extract of feces that had been sterilized, were run.

(D) A dog with a simple permanent gastric fistula of the valve type<sup>2</sup> was anesthetized with amytal. The common bile duct, pancreatic duct and the thoracic duct were cannulated. A milk suspension of 500 mg. of dye was placed in the duodenum by means of a catheter into the gastric fistula and through the pyloric sphincter. "Secretin" was injected intravenously in order to stimulate the flow of pancreatic fluid. Examination of the bile, pancreatic fluid, urine and lymph during the following 2 hours showed that the pigment was not present. Under the conditions of this experiment, there was little, if any, absorption of the pigment through the intestinal wall. Intravenous injection of the anthocyan chloride resulted in its appearance in the bile and urine in 20 minutes and in the lymph in 50 minutes. No pigment was found in the pancreatic fluid, the intraocular fluid, or in the exudations from a cisternal puncture.

(E) The benzene rings in the anthocyan chloride have been suspected as a source of hippuric acid in the normal metabolism of this pigment. Even though absorption of the compound in appreciable quantities was questionable, experiments were planned to determine whether or not there was any measurable increase of conjugated benzoic acid compounds in the urine after ingestion of the pigment. Milk suspensions of 500 mg. of the pigment were administered to rabbits by stomach tube, but the total benzoic acid in the urine, as determined by the method of Kingsbury and Swanson,<sup>3</sup> showed no significant deviation from the normal. The normal excretion of total benzoic acid on a milk diet, determined for each rabbit, averaged about 60 mg. per day. If the fate of ingested anthocyan is the same in the human species as in rabbits, then these pigments probably do not augment the hippuric acid excretion in man.

It is noteworthy that the urine and feces of the rabbits which were fed the anthocyan chloride became highly pigmented. Colorimetric

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<sup>2</sup> Technique for preparing a gastric fistula described by A. J. Carlson: *Control of Hunger in Health and Disease*, Univ. of Chicago Press, 1916, 42.

<sup>3</sup> Kingsbury, F. B., and Swanson, W. W., *J. Biol. Chem.*, 1921, **48**, 13.

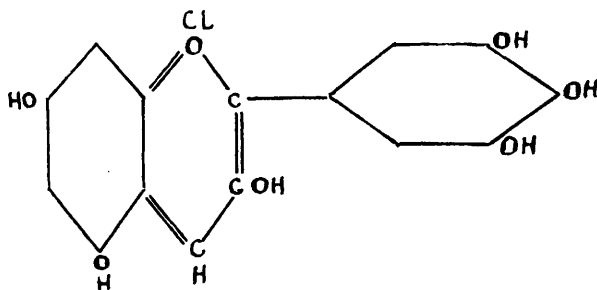


FIG. 1.

Delphinidin chloride. Illustrates nucleus in grape anthocyanins.

examination of these urines showed that from 1 to 2% of the dye was excreted through the kidney. The doses used, however, were greatly in excess of the amounts ordinarily ingested.

*Conclusion.* The above experiments indicate that the anthocyanins are not easily absorbed from the intestine. The small quantity which does pass through to the circulation appears to be excreted by the kidney in its unchanged form.

The author wishes to express his gratitude to Dr. R. J. Anderson, who suggested this problem, and Drs.\* R. W. Jackson and G. R. Cowgill for their valuable advice and assistance.

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### Effect of Ferric Chloride Injections on Spread of Tuberculosis from Site of Subcutaneous Inoculation.\*

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Previous studies have demonstrated that repeated intravenous injections of a 0.25% solution of ferric chloride crystals in tuberculous rabbits are followed by an accumulation of iron in the caseous areas of the lungs.<sup>1, 2</sup> Subsequent studies revealed that concomitant with this accumulation of iron in tuberculous areas there was a definite increase in the survival time of infected rabbits.<sup>3, 4</sup> When control and experimental animals were sacrificed at given intervals

\* Aided by a grant from the Delamar Mobile Research Fund.

<sup>1</sup> Menkin, V., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 1020.

<sup>2</sup> Menkin, V., and Menkin, M. F., *J. Exp. Med.*, 1931, **53**, 919.

<sup>3</sup> Menkin, V., *J. Exp. Med.*, 1932, **55**, 101.

<sup>4</sup> Menkin, V., *Am. J. Med. Sci.*, 1933, **185**, 40.