Hemolytic Disease in Newborn Dogs Following Isoimmunization of the Dam by Transfusions[1]

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Four different isohemagglutinins have been demonstrated in sera of dogs following transfusions with dog erythrocytes containing antigenic factors lacking in their own cells. The factors thus far identified serologically may conveniently be designated as "canine A, B, C, and D" and the corresponding antibodies as "canine anti-A, -B, -C, and -D." In our experience to date, A antibodies have been most easily produced in high titer, and in many respects their behavior *in vitro* is similar to that of Rh antibodies. Possible relationships between antigenic factors in dog red blood cells and factors present in erythrocytes of other species are under investigation. Pending completion of these studies, it is convenient to refer to dog cells containing the canine A factor as "Do-positive" and to cells lacking the canine A factor as "Do-negative."

Observations on hemolytic reactions following transfusion of Do-positive cells into immunized Do-negative recipient dogs and of anti-Do plasma into Do-positive recipients are described in separate reports (*2, 4, 5*). The object of this paper is to record the production of hemolytic disease in four Do-positive puppies born to a Do-negative bitch (mostly pointer) that had been immunized by transfusions of Do-positive cells and then mated with a Do-positive male (mostly German shepherd). Four Do-negative puppies in the same litter showed no evidence of hemolytic disease.

Erythrocytes of the four affected whelps were agglutinated by high dilutions of anti-dog-serum rabbit serum (positive direct Coombs' test), and were also agglutinated when suspended in undiluted normal dog serum, presumably because the cells were coated with Do-antibody. Unsuccessful attempts were made to separate the antibody from the puppies' cells by heating the cells at 48° to 56° C. Plasma drawn from the four unaffected Do-negative whelps agglutinated cells of the sire in a titer equal to that of the maternal serum at the time of delivery. Isoantibodies in the blood of the four affected Do-positive puppies, on the other hand, were apparently completely bound by cells, since plasma from these dogs did not agglutinate the sire's erythrocytes.

The maternal isoantibody titer fell from 1: 64 (against the sire's cells suspended in autologous serum) at the time of mating to 1: 4 at the time of delivery. The dam was not transfused during pregnancy, but was given 17 ml of the sire's fresh citrated whole blood intravenously immediately after delivery. Breast milk from the dam agglutinated the sire's red corpuscles in titers considerably lower than those found in the maternal serum during the postpartum period of rising antibody titer. Absorption of isoantibody from the milk by the puppies could not be demonstrated. It is significant that Do-antibodies could no longer be detected in plasma from the unaffected Do-negative puppies on the 9th day, and Coombs' tests on cells of Do-positive affected puppies became negative on the 12th day, despite the fact that all whelps suckled breast milk containing antibody for 24 days.

Erythrocytes from the four affected puppies showed increased susceptibility to lysis in hypotonic solutions of sodium chloride, but spheroidicity of these cells could not be detected in smears or wet preparations. Nucleated red blood cells and reticulocytes were more numerous in most of the smears prepared from blood of the affected puppies than in the Do-negative puppies, but in some instances the differences were not striking.

Only one Do-positive whelp showed icterus of skin and mucous membranes and was found to have distinctly increased concentration of bilirubin in the plasma. This puppy was moderately anemic. Another Do-positive puppy became severely anemic without developing jaundice. Neither icterus nor anemia of significance was found in the other two Do-positive puppies. The severely anemic whelp and a normal litter mate were sacrificed for histologic studies on the third day. The affected animal showed splenomegaly and more marked erythropoiesis in the liver and spleen. The erythroid elements in the bone marrow, liver, and spleen of the affected puppy were also more immature than those in the marrow of the normal litter mate. There was no evidence of kernicterus. The three remaining Do-positive puppies gained weight more slowly than their normal litter mates, but ultimately made complete recovery.

The observations cited are considered sufficient to establish the diagnosis of hemolytic disease of varying severity in the four puppies with erythrocytes containing a factor present in the red cells of their sire but lacking in the red cells of their mother. The four litter mates with erythrocytes lacking this factor were apparently normal. A separate paper (*3*) will describe more completely the observations on this litter and on litters born of dams immunized against other canine factors.

Abelson (*1*) has observed what appeared to be naturally occurring hemolytic disease in newborn dachshunds. It is believed, however, that the present report records the first unequivocal serologic and hematologic evidence of this disease in puppies born of a mother previously immunized by transfusions. These experiences suggest that hemolytic disease in dogs may be produced with some degree of frequency and that studies on this species may aid in clarifying certain aspects of erythroblastosis fetalis as it occurs in human beings. Opportunities for

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controlled observations on large litters sired by heterozygous males seem particularly attractive.

In the litter described, the pups had suckled for about two hours before the first blood samples were drawn. Do-positive pups in three subsequent litters were similarly affected, but examinations of the blood before and after suckling revealed that most, if not all, of the Do-antibody was acquired from the dams' milk.

References

1. ABELSON, N. Paper read before Interurban Club, Philadelphia, April 5, 1946.
2. ERVIN, D. M., CHRISTIAN, R. M., DAVIS, R. W., YUILE, C. L., and YOUNG, L. E. *Blood.* In press.
3. ERVIN, D. M., *et al. Blood.* In press.
4. YOUNG, L. E., ERVIN, D. M., and YUILE, C. L. (To be published.)
5. YUILE, C. L., *et al.* (To be published.)

The Antithyroid Factor of Yellow Turnip[1]

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It has been well established that goiter may be induced in laboratory animals by diets of certain vegetables such as cabbage, turnip, or rape. A recent study (*2*) of the antithyroid effect of various foods in man disclosed marked activity in the yellow turnip or rutabaga, *Brassica napobrassica.* This antithyroid principle has now been isolated in crystalline form and its structure determined.

The purification of the goitrogen was controlled by antithyroid assay of crude preparations in the rat, and later by measurement of the ultraviolet absorption spectrum. The active substance was released from ground rutabaga root by extraction with cold water, and concentrated by appropriate distribution between ether and alkaline buffers. The concentrates so prepared could be crystallized directly from ether with the aid of seed crystals, which were originally obtained from material that had been further purified by distillation in high vacuum and chromatographic adsorption on alumina. The active principle was isolated in a yield of 0.2 g/kg of root as colorless crystals of formula C_5H_7ONS, mp 50°, $[\alpha]_D^{31} - 71°$ (2% methanol solution). The same substance was obtained from the root of white turnip, and in larger quantities (1–8 g/kg) from the seeds of rutabaga, white turnip, cabbage, kale, and rape. Its antithyroid activity in man approximately equals that of 6-*n*-propylthiouracil.

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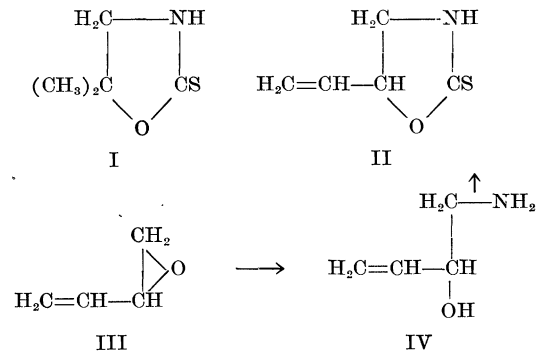
Since no pure chemical degradation product could be obtained from the goitrogen, its structure was deduced largely from physical evidence. The compound in aqueous solution was found to be a weak acid (pK_a 10.5) and to have an intense ultraviolet absorption maximum at 240 mμ and log ε 4.24, which was shifted by alkali to 232 mμ, log ε 4.06. In these properties it closely resembled 5,5-dimethyl-2-thioöxazolidone (formula I), a natural product (*3*) of similar composition. Furthermore, the infrared absorption spectrum of the unknown in chloroform solution exhibited a system of bands at 2.9 μ, 3.15 μ, 6.6 μ (inflected at 6.5 μ), and 8.6 μ, which was found to be highly characteristic of 2-thioöxazolidones, and contained two bands at 10.17 μ and 10.85 μ, indicating the presence of a vinyl group (*1*). Coupled with the absence of terminal methyl groups, these facts required that the rutabaga goitrogen have the structure of a vinylthioöxazolidone. The observation that treatment of the substance with boiling 4N HCl destroyed optical activity without liberating ammonia strongly suggested the attachment of the oxygen rather than the nitrogen atom to the allylic center of asymmetry. Therefore, the antithyroid factor of rutabaga was considered to be L-5-vinyl-2-thioöxazolidone (formula II).



The assigned formula was confirmed by synthesis. Reaction of butadiene 1,2-monoxide (formula III) (*4*) with ammonia produced a mixture from which a pure aminoalcohol, in all probability 1-amino-3-buten-2-ol (formula IV), was separated as its acid oxalate, mp 131°. Conversion of this aminoalcohol to the dithiocarbamate with carbon disulfide and alkali, followed by cyclization with lead nitrate, furnished DL-5-vinyl-2-thioöxazolidone, mp 63°, which had the same infrared spectrum as the rutabaga factor. L-1-Amino-3-buten-2-ol, obtained by resolution of the racemic aminoalcohol with D-α-bromocamphor-π-sulfuric acid, was similarly transformed into synthetic L-5-vinyl-2-thioöxazolidone, identical with the natural product.

References

1. BARNES, R. B., *et al. Anal. Chem.,* 1948, **20**, 402.
2. GREER, M. A. and ASTWOOD, E. B. *Endocrinology,* 1948, **43**, 105.
3. HOPKINS, C. Y. *Canad. J. Res.,* 1938, **16B**, 341.
4. KADESCH, R. G. *J. Amer. chem. Soc.,* 1946, **68**, 41.