

(2) erythema and induration were uniformly present; (3) delayed reactivity could be transferred by cells; and (4) a typical histologic picture could be demonstrated by skin biopsies.

Summary. Histoplasmins from two strains of *Histoplasma capsulatum* have been fractionated by salting-out and electrophoretic procedures. Fractions obtained in this manner have been assayed for ability to induce a delayed type of hypersensitivity in sensitized guinea pigs. Carbohydrate-rich fractions appear to be more effective in this regard, and one histoplasmin fraction has been found which exhibits no precipitin activity but is highly effective in the skin test. The skin test reaction with histoplasmin is also demonstrated to be an example of a delayed hypersensitivity reaction.

1. Cross, F. W., Howell, A., Jr., Public Health Rep., 1948, v63, 179.

2. Palmer, C. E., *ibid.*, 1946, v61, 475.

3. Salvin, S. B., Smith, R. F., J. Infect. Dis., 1959, v105, 45.

4. Heiner, D. C., Pediatrics, 1958, v22, 616.

5. Markowitz, H., Proc. Soc. Exp. Biol. and Med., 1964, v115, 697.

6. Kaufman, L., Blumer, S., J. Bact., 1966, v91, 1434.

7. Knight, R. A., Marcus, S., Am. Rev. Resp. Dis., 1958, v77, 983.

8. Müller-Eberhard, H. J., Scand. J. Clin. Lab. Invest., 1960, v12, 33.

9. Kabat, E. A., Experimental Immunochemistry, Ed. 2, Charles C Thomas, Springfield, Ill., 1961, 905 pp.

10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, Rose, J., J. Biol. Chem., 1951, v193, 265.

11. Ornstein, L., Ann. N. Y. Acad. Sci., 1964, v121, 321.

12. Davis, B. J., *ibid.*, 1964, v121, 404.

13. Crowle, A. J., Immunodiffusion, Academic Press, Inc., New York, 1961, 333 pp.

14. Gell, P. G. H., Benacerraf, B., Adv. Immunol., 1961, v1, 319.

15. Chase, M. W., Proc. Soc. Exp. Biol. and Med., 1945, v59, 134.

16. Jankovic, B. D., Waksman, B. H., J. Immunol., 1962, v89, 598.

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Influence of Mother's Milk on Incidence of Spontaneous Aortic Lesions in Weanling Rabbits.* (31912)

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A recent survey of aortas from approximately 1100 untreated rabbits disclosed a relatively high incidence of spontaneous, non-lipid medial lesions(1). These lesions have a variable gross, but fairly uniform microscopic appearance(2). They are always confined to the inner one-half to one-third of the media. Fragmentation and disruption of the elastic lamina with accumulation of acid mucopolysaccharide is seen, frequently accompanied by calcification of the fragmented portions of the elastic fibers. The morphologic characteristics of these spontaneous medial lesions and of the diet-induced atheroma are dissimilar at all stages of their development

(3). The early atheromatous plaque is characterized by accumulation of lipid-filled foam cells in the intima and of lipid droplets in the subjacent media. The development of atheroma, however, is influenced by the presence of the spontaneous lesions. There is an increased tendency for lipid deposition at the raised borders of nodular medial lesions projecting from the luminal surface and in areas of acid mucopolysaccharide accumulation within the medial lesions. Atheroma formation is absent at sites of medial calcification. On the basis of our survey(1) and studies which cover rabbits from birth to maturity (2,4), it seems that these medial lesions are laid down by the sixth week, and in most cases are irreversible, but do not necessarily

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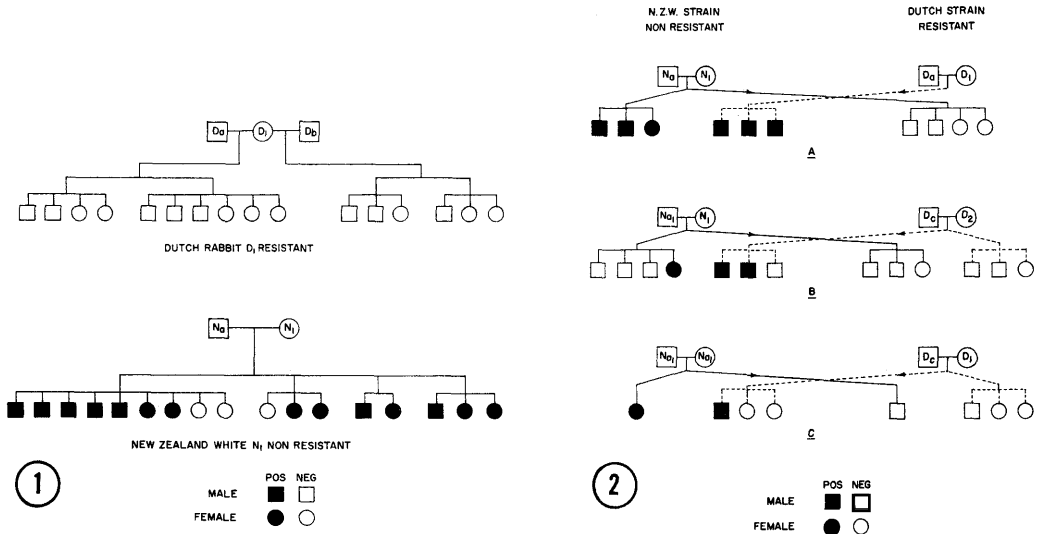


Fig. 1. Incidence of aortic lesions in offspring of resistant and non-resistant does.
 Fig. 2. Incidence of aortic lesions in weanling rabbits which were nursed by resistant and non-resistant does. Position of offspring in the figure indicates source of milk.

increase with age or shorten the life span of affected rabbits. The effect of these lesions remains unknown.

While lesions were found in all of the 11 breeds included in our survey, there were significant differences between breeds with respect to the frequency of occurrence. Furthermore, when appreciable numbers of animals of a particular breed were procured from more than one source, significant differences between sources were found as well. These findings suggested a familial factor that could be either genetic or environmental, such as a factor related to the nursing process. A careful breeding program had led to the establishment of families that are either "resistant" or "non-resistant" to the lesions in question, based on incidence at 8 weeks of age. The following study was designed to test the possibility of a non-genetic factor.

Methods. Two of the eleven breeds used in the survey were chosen for the present study. One of the breeds (New Zealand White) has a relatively high incidence of lesions, the other (Dutch) a relatively low incidence. In one New Zealand White family (non-resistant) we have found 32 rabbits with lesions, out of 61 examined. In a resistant family of Dutch rabbits, we found no lesions in any of the 16 aortas examined. All rabbits

were maintained in an outdoor rabbitry. They received a stock commercial diet with an antibiotic additive and water *ad libitum*.

Baby rabbits were exchanged between mothers on the day of their birth. Aortas were removed when the babies were 8 weeks of age. The aortas were opened longitudinally and examined under a hand lens. The appearance of a single lesion was sufficient to categorize an aorta as being involved.

Results and discussion. Fig. 1 gives some background on the breeding record of 2 does, one from each strain. The Dutch doe (D_1) had 16 offspring in 4 litters, none of which had any lesions. The New Zealand White doe (N_1) had 17 babies in 4 litters, of which 14 had lesions. Although not shown here it was noted that the incidence of lesions was also high when N_1 was bred with other bucks. It seemed possible therefore that a factor responsible for the production of these lesions was transmitted by this doe. Each of these does gave birth to a litter on the same day and we immediately exchanged babies between them. The results are shown in Fig. 2A. The non-resistant N_1 had 7 babies in her litter. Four of these, 2 male and 2 female, were placed with the resistant doe D_1 , the other 3 remained with N_1 . D_1 had only 3 babies and all were given to N_1 . Lesions were

found in all of the rabbits nursed by N_1 , but none were found in any of the rabbits nursed by D_1 .

Due to a number of circumstances an exact repetition of the above experiment was not possible. Different, but related, bucks had to be used in both strains. While both original does were used, this time their babies were exchanged with the offspring of 2 other does (Fig. 2B and C). No lesions were found in rabbits nursed by the resistant, but unrelated does, D_1 and D_2 . Lesions were present in the animals nursed by the non-resistant does, N_1 and her daughter N_{a1} .

Summary and conclusion. Newborn rabbits were exchanged between does previously shown to be members of families that were either "resistant" or "non-resistant" with re-

spect to incidence of spontaneous, non-lipid, medial lesions. Lesions were found at 8 weeks of age only in the rabbits nursed by does of the non-resistant family. These data suggest that a factor is present in rabbit milk which either enhances the formation of the non-lipid, medial aortic lesion found in weanling rabbits of non-resistant strains or which, in resistant rabbits, protects against an ubiquitous lesion-inducing agent.

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1. Gaman, E. M., Feigenbaum, A. S., Schenk, E. A., J. Atherosclerosis Res., 1967, v7, 131.
 2. Schenk, E. A., Gaman, E. M., Feigenbaum, A. S., Circulation Res., 1966, v19, 80.
 3. ———, *ibid.*, 1966, v19, 89.
 4. Kesten, H. D., Arch. Pathol., 1935, v20, 1.

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Changing Activity of Erythrocyte Glucose-6-Phosphate Dehydrogenase and Tolerances to Glucose and Tolbutamide in Growing Sheep.* (31913)

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A metabolic syndrome—low erythrocyte glucose-6-phosphate dehydrogenase (G6PD) activity coupled with diabetic type tolerances to glucose and tolbutamide—first reported in man(1) has now been found to develop in growing sheep. Previous workers have demonstrated that blood glucose concentration and glucose tolerance decrease progressively in young sheep(2-4), and that erythrocyte G6PD activity is low in adult sheep(5,6). We now report that erythrocyte G6PD activity and tolbutamide tolerance of young lambs resemble those of normal man, and gradually change during 100 days of extrauterine life to values considered pathologic in man. This enlarges the group of metabolic conditions in ruminants which may serve as models for medical problems in man(7).

Methods. Standard clinico-chemical methods were used for the estimation of G6PD

activity(8), biuret protein(9), glucose concentration(10), intravenous glucose tolerance (2-4) and tolbutamide tolerance(11). Packed cell volumes were read after centrifugation at 1500 g for 25 minutes. Erythrocyte glucose concentration was calculated from whole blood and plasma concentrations without correction for trapped plasma in the packed cell volume. Glucokinase activity (V of hexokinase using glucose as substrate) was assayed by NADPH generation(12) in 50% saturated ammonium sulfate precipitates of the hemolysates prepared for G6PD assay(8). The same activity was found with 1 or 100 mM glucose in the cuvette. We studied 6 adult sheep (crossbred females) and 4 growing lambs. They were fed poor quality grass hay *ad libitum* and 0.5 kg of grain concentrate daily per adult. The lambs had free access to the feed or to suckle.

Results. Erythrocyte G6PD activity was 95-190 U/100 ml red cells in the lambs when

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