tive, although the specific fluorescence appeared to be stronger in the P_2 and P_3 segments. Distal tubules and collecting ducts were negative. The glomeruli, except for the parietal layer of Bowman's capsule, were negative. There was a marked reduction in fluorescence from the outer stripe to the inner stripe of the outer medulla (not shown). Low-lying cells, showing a faint fluorescence and probably representing thin limbs of the loop of Henle, were seen in the inner stripe, but all other segments of the nephron in the inner stripe and inner medulla were essentially negative. Minipig kidneys (Fig. 2h) resemble the rat kidneys with one exception: occasional brightly staining cells were seen among essentially negative cells in cortical collecting ducts; these cells may represent the "dark" cells of the pig collecting duct.

We found no differences in the distribution or intensity of specific fluorescence between the lungs of phenobarbital- and saline-treated rats (Fig. 2, c and f). Positive fluorescence was seen in bronchi and bronchioles and in an additional cell type in the lung parenchyma. Minipig lungs (Fig. 2i) showed a similar distribution of NADPH-cytochrome *c* (P-450) reductase.

Our results concerning the distribution of NADPH-cytochrome *c* (P-450) reductase in the liver agree with those of Baron *et al.* (*6*). To our knowledge, the immonohistochemical demonstration of NADPH-cytochrome *c* (P-450) reductase in kidney or lung has not been reported previously. Our results agree with morphologic and biochemical evidence suggesting that the proximal tubule is the site of drug metabolism in the kidney (*11*). Boyd (*12*) has shown by autoradiography that a metabolite of the pulmonary toxin, 4-ipomeanol, is localized in pulmonary nonciliated bronchiolar (Clara) cells, and has postulated that the Clara cell is the primary location of P-450-mediated mixed function oxidase enzymes in the lung. Our results are consistent with the presence of the enzyme in the bronchioles, but indicate that additional cell types in the bronchi and bronchioles, and possibly a parenchymal cell type, are positive for NADPH-cytochrome *c* (P-450) reductase. Although the Clara cell may be the predominant cell metabolizing ipomeanol, the more general method of determining the distribution of NADPH-cytochrome *c* (P-450) reductase, which (according to current knowledge) exists in only one form and is presumably present wherever any of the multiple forms of cytochrome P-450 occur, may be a more sensitive way to identify cells containing cytochrome P-450-specific mixed function oxidase enzymes.

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Social Environment as a Factor in Diet-Induced Atherosclerosis

Abstract. *Rabbits on a 2 percent cholesterol diet were individually petted, held, talked to, and played with on a regular basis. Measurements of aortic affinity for a Sudan stain, serum cholesterol levels, heart rate, and blood pressure were made at the end of the experimental period. Compared to control groups, which were given the same diet and normal laboratory animal care, the experimental groups showed more than a 60 percent reduction in the percentage of aortic surface area exhibiting sudanophilic lesions, even though serum cholesterol levels, heart rate, and blood pressure were comparable.*

The apparent relation between stress and cardiovascular disease is based on a variety of evidence implicating physical, emotional, and behavioral factors (*1-6*). Data from animal studies link psychosocial disruption to pathological changes in the cardiovascular system (*7-14*). These studies include several in which states of severe emotional disturbance were produced with negative stressors. To our knowledge, however, there have not been any studies in which the effects of positive factors were investigated.

We designed a series of studies to investigate the influence of social environment on diet-induced atherosclerosis in rabbits. In the experimental groups (groups A, B, and D), the animals experienced social interaction with an experimenter (M.J.L.). In the control groups (groups C and E), the animals received normal laboratory animal care. Group A was studied during late 1977 without a concurrent control, although earlier control studies had been carried out. The results for group A, although initially considered anomalous, ultimately led us to conduct two additional studies. One was carried out in early 1978 and involved experimental group B and control group C. The other was carried out in late 1978 and involved experimental group D and control group E.

It should be noted that the essence of the experimental environment studied here was to establish a one-to-one relationship between each animal and the experimenter. This was achieved through an early morning, half-hour visit during which each animal was handled, stroked, talked to, and played with; an hour-long feeding period during which the animal was also touched and talked to; and a number of 5-minute visits during the day. Through this daily process, the animals quickly learned to recognize the experimenter, and when present, many even sought her personal attention. They were left alone for 10 hours each night.

The animals used in this study were young male New Zealand White rabbits. Upon being received, they were separated and subjected to a 2-week adaptation period during which they and the experimenter became acquainted. All experiments were carried out by the same experimenter with the same protocol.

After the adaptation period, the ani-

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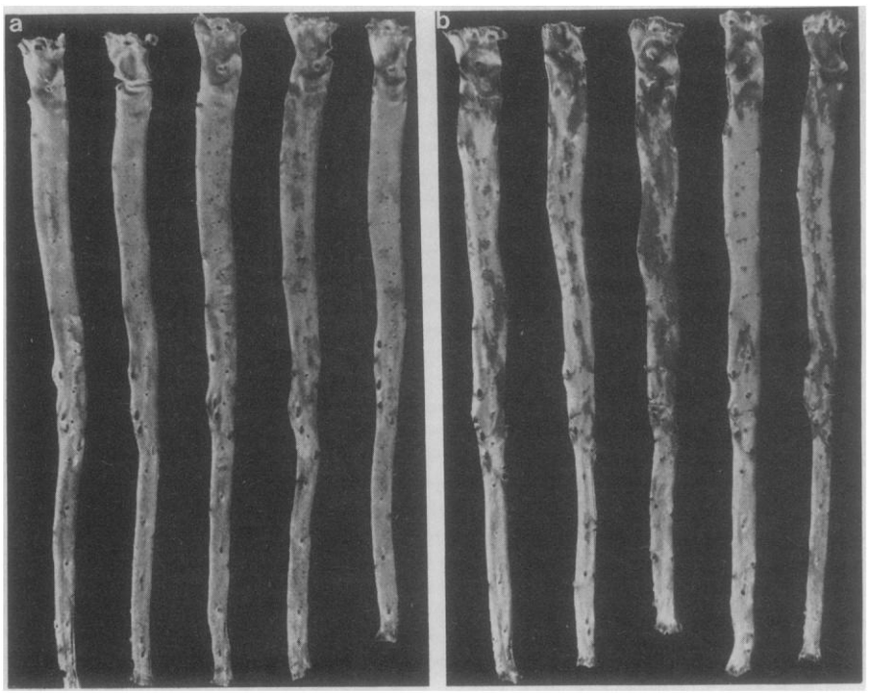

Fig. 1. Photographs of rabbit aortas stained with Sudan IV. (a) Experimental group B; (b) control group C.

mals were fed a regular rabbit diet supplemented with 2 percent cholesterol. The members of groups A, B, and C were killed after 5 weeks, and those of groups D and E were killed after 6 weeks. For blood chemistry analysis, blood samples were withdrawn after a 12-hour fast; total serum cholesterol levels were determined weekly. Indirect blood pressure was determined weekly by using an ear cuff in groups A, D, and E. Immediately before groups D and E were killed, direct blood pressure measurements were made through catheterization of the left carotid artery and the heart rate was determined.

At the end of the experimental period, the aorta was perfused in situ at physiological pressure (95 mm-Hg) with 10 percent isotonic Formalin for 3 hours. The specimens were then stained with Sudan IV and photographed (Fig. 1). Sudanophilia, as evidence by dark regions corresponding to the uptake of Sudan IV, was determined quantitatively with point counting by independent investigators.

Figure 2 shows the percentage of total aortic surface area exhibiting sudanophilia for each of the five groups. Sudanophilia in the experimental groups was more than 60 percent less than in the control groups. The results for group B, compared to those for its control, group C, are significant at $P = .015$ (Student's t-test); those for group D, compared to those for group E, are significant at $P = .034$.

For groups B and C, the mean final

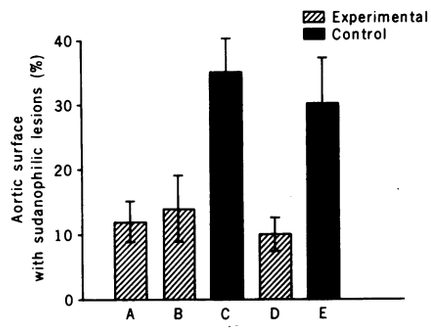

Fig. 2. Average percentage of aortic surface area exhibiting sudanophilia in experimental and control groups; bars show standard error of the mean.

serum cholesterol levels were 1527 ± 125 and 1426 ± 298 mg/dl, and for groups D and E, 1980 ± 419 and 1881 ± 214 mg/dl. For groups D and E, the direct blood pressure levels were 72 ± 9 and 74 ± 4 mm-Hg, and the heart rates were 191 ± 35 and 219 ± 19 beats per minute. None of these differences is statistically significant.

These studies indicate that the establishment of a pet relationship has a dramatic effect on diet-induced aortic atherosclerosis in rabbits. Having, in effect, conducted three separate experiments, we feel that our results are reproducible in spite of the fact that the social interventions we employed are less quantifiable than physical or biochemical interventions. However, the mechanisms behind this demonstrated effect of a particular social environment on atherogenesis remain to be discovered. With regard to this, measurements should be made of the effect of social environment on blood hormonal levels, arterial wall permeability, endothelial regeneration rates, and many other factors.

It is possible that the results obtained by different laboratories for essentially the same experiment are contradictory solely because of a difference in sociopsychological environment. If this is true, then it may also explain anomalous results within a single laboratory. For example, the differences reported here are as great as or greater than those obtained in many studies in which the effect of a particular intervention on atherosclerosis was investigated with the hypercholesterolemic rabbit used as an animal model. If no attention was given to social environment in the experimental protocol, what was the real effect observed? Clearly, more must be learned about the effects of social environment in animal studies of disease. If nothing else, our results suggest that, in specifying the protocol for an animal study, careful consideration should be given to sociopsychological factors.

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