are released into the medium during a 3- to 6-hour period of incubation with ^{131}I of intact thyroid lobes from severely iodine-deficient rats or of isolated beef thyroid follicular cells. MIT and DIT are found in the medium primarily in peptide-bound form, but T_4 and T_3 are apparently found only as free amino acids. T_4 enters the medium more readily than T_3.

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Effect of an Amyloid Inducing Regimen on Phagocytosis of Carbon Particles.* (30268)

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While the pathogenesis of amyloidosis is uncertain, much speculation has centered on its relationship to the producton of humoral antibody, and several workers in the past have suggested that in fact amyloid deposits are the result of the interaction of antigen and antibody. Light microscopic immunofluorescent studies seemed to offer indirect support to this hypothesis(1,2). Other studies, however, including those utilizing the electron microscopically distinct component of amyloid, the fibril, suggest that this unique element at least is not undenatured gamma globulin(3-7).

The relationship between amyloidosis and phagocytosis, the other major host defense mechanism has until now been little studied, and is the subject of the present report.

Materials and methods. 1) A modified version of the reticuloendothelial system (R.E.S.) evaluation technique described by Benacerraf, McCluskey, and Patras(8) was used. A commercial preparation of carbon ('Pelikan,' Gunther, Wagner, Hanover) was suspended in a 6% gelatin solution such that each milliliter of solution contained 120 mg of carbon. White female New Zealand rabbits were given injections of this carbon gelatin solution in the dose of 4 mg of carbon/100 g of body weight by ear vein. The rate of carbon clearance was established by taking measured samples of arterial blood (0.2 ml) from the opposite ear every 2 minutes after injection for 10-12 minutes (*i.e.*, 5-6 points). Each sample was dissolved in 2 ml of 0.1% sodium carbonate. The carbon concentration of each sample was determined spectrophotometrically on a Coleman Junior Spectrophotometer at wavelength 675.

2) Phagocytic indices were determined according to the formulae described by Biozzi, Benacerraf, and Halpern(9). It has been shown that the clearance from the blood of intravenously injected colloidal solutions, such as the carbon gelatin solution described above, follows an exponential equation:

$$\frac{\log C_1 - \log C_2}{T_2 - T_1} = K$$

where C is the serum concentration of the colloid at time T. K therefore measures the activity of the reticuloendothelial system (R.E.S.) for a given dose of colloid and serves as the index of phagocytosis.

3) The phagocytic indices (K values) of 3 separate groups of 7-8 animals were determined at 3-4-week intervals over the course of several months. The *first group* consisted of young rabbits which were simply allowed to mature and gain weight. These served as

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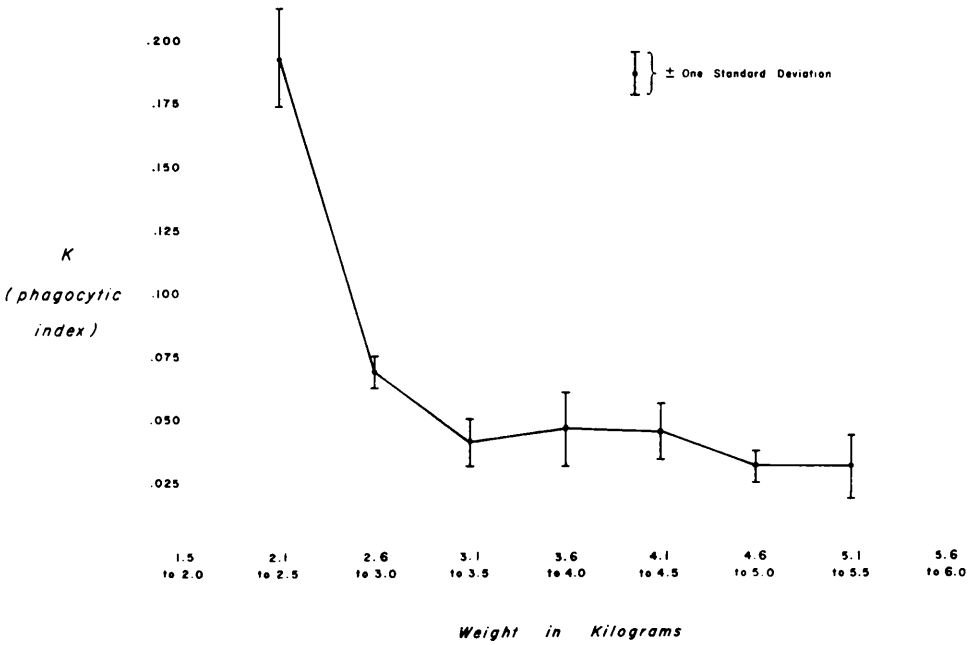

FIG. 1. Relationship of rabbit weight to phagocytic index (K). After early markedly pronounced phagocytic activity, a levelling off occurs once an animal achieves a weight of 3.0 kg or more.

weight and growth curve controls. The *second and third groups* composed of mature animals (weighing 3.8 kg or more) were given twice weekly injections of 5 ml of a 12% casein suspension, as previously described by Cohen, Calkins, and Levene(10). The casein suspension was freshly prepared each week from commercially available sodium caseinate and injected subcutaneously. K values were again obtained at 3-4-week intervals. One group of animals (Group II) was maintained on the casein regimen 15 weeks before sacrifice, the other (Group III) 26 weeks. At time of sacrifice, tissue samples taken from liver, spleen and kidney were fixed in formalin and stained with hematoxylin and eosin, crystal violet and Congo red dyes. Slides were examined with the light and polarizing microscopes.

Results. Group I animals. Eight rabbits weighing between 2 and 2.5 kg were evaluated for R.E.S. activity initially and then at several week intervals while on a normal diet until a weight of 5 to 5.5 kg was achieved. Their K values are shown in Fig. 1. It is apparent from the graph that phagocytic activity of the immature rabbit was quite marked but that once an animal achieved a weight of over 3 kg its R.E.S. activity stabilized and was little, if at all, effected by further increases in weight. Whether this change in phagocytic index is a reflection of maturation or weight gain cannot be determined from these data. The K values of this group of animals at mature weight (3.5+ kg) were regarded as the control values for Groups II and III.

Group II animals. These rabbits, a group of 8, all weighed over 3.8 kg at the start of the experiment and their initial phagocytic indices were comparable to those of the control group of similar body weight. However, once they were started on the amyloid inducing regimen, their K values began to rise (Table I). This rise in phagocytic activity was accompanied by a moderate weight loss.

TABLE I. Group II Rabbits.

Time in wk	Avg wt in kg (range)	K ± standard deviation
0	4.6 (3.8-5.6)	.044 ± .013
1-4	4.1 (3.5-5.4)	.061 ± .023
9-12	3.9 (3.5-4.5)	.145 ± .025
13-16	4.1 (3.5-4.6)	.097 ± .020

TABLE II. Group III Rabbits.

Time in wk	Avg wt in kg (range)	K ± standard deviation
0	4.6 (4.4-5.1)	.037 ± .006
13-16	4.1 (3.6-4.5)	.066 ± .016
21-24	3.6 (2.9-4.3)	.112 ± .025
25-28	3.9 (3.8-4.0)	.045 ± .001

At no time did the weight of any animal fall below the critical level of 3.4 kg. The change in phagocytosis was far greater than could be accounted for by the weight loss alone. K values continued to rise and reached a peak at 10-12 weeks of casein injections after which they began to fall. The decline in phagocytosis was accompanied by a modest weight gain but again the change in weight was insignificant compared to the altered phagocytic activity. These animals were sacrificed after 15 weeks on the casein regimen, when K values were still considerably above those of the control group, even though they were declining. None of the tissues taken at autopsy showed evidence of amyloid.

Group III animals. Each rabbit in this group of 6 weighed over 4 kg at the start of the experiment. They showed a rise in phagocytic activity comparable to that of the animals in Group II after being put on the casein regimen (Table II). Again, this was accompanied by a small weight loss. These animals, however, did not reach peak K values until after 20 weeks, *i.e.*, several weeks later than the Group II animals. Apart from the difference in the time elapsed before peak K values were attained, graphs derived from the data contained in Tables I and II were similar in appearance, that is, both showed a peaking and then a decline of phagocytic activity. Group III animals, however, were continued on casein until their K values had returned to normal or near normal levels. At autopsy all animals showed clearcut evidence of amyloid in liver, spleen or kidney. One animal in this group succumbed after 20 weeks on casein before a fall off in phagocytic activity had been noted. This animal showed only slight deposition of amyloid in its tissues.

Discussion. These results may be interpreted as being indicative of 2 stages in the induction of amyloidosis. In the first stage, phagocytosis is enhanced (as compared to the findings in the non-casein treated animals) and amyloid deposits are not seen. In the second stage, phagocytic activity returns to normal, and amyloid is found in the tissues. The enhanced phagocytic activity seen in the first stage may be either a nonspecific response to injected protein, or a reaction to whatever process will eventually result in amyloid deposition. It could also be an essential phase in the pathogenesis of amyloid. In either case, the cells responsible for amyloid formation are not necessarily those involved in active phagocytosis. Moore *et al*(11) and Schoenberg *et al*(12) have shown that proliferating R.E. cells, usually thought to account for enhanced phagocytosis when an animal is appropriately stimulated, may actually not be phagocytically active. Such cells, under appropriate circumstances, might be the amyloid producers. On the other hand, Smetana(13) has shown that reticuloendothelial blockade delays the appearance of amyloid, and the reticuloendothelial activity is absent in areas of amyloid deposition. This would suggest that cells originally having phagocytic activity are responsible for amyloid formation, and that as they commence to form amyloid phagocytic activity is lost. Although the current study does not solve this dilemma it does indicate at the minimum that 2 sequential, possibly interrelated events, *i.e.*, R.E.S. hyperplasia and amyloid formation, take place under these experimental conditions (*i.e.*, serial casein injections).

In the past, with a few notable exceptions, little emphasis has been placed on the role of the reticuloendothelial system in the pathogenesis of amyloidosis. In addition to Smetana's study, detailed histological studies by Teilum(14) and by Christensen and Rask-Nielsen(15) demonstrated the close relationship of amyloid staining material to the R.E.S. On an ultrastructural level, Cohen and Calkins(16) observed early amyloid deposits occurring in relation to glomerular endothelial cells. Cohen, Gross, and Shirahama have described the synthesis of amyloid by spleen explants in culture(17).

Thus, there is considerable evidence that the reticuloendothelial system plays an im-

portant, if not decisive role in the genesis of amyloid deposits. The results of our study, utilizing indices of reticuloendothelial activity other than morphological, tend to support this theory.

Summary. 1) A group of 8 rabbits was shown to have significantly higher rates of phagocytosis when young and small than when fully matured. However, after the animals achieved a weight of 3.5 kg, further gains in weight did not significantly decrease phagocytic activity. 2) Two groups of 8 and 6 rabbits respectively were given casein injections as used previously to induce amyloidosis. Both groups showed a pattern of accelerating and then decelerating phagocytic activity over many weeks. One group was sacrificed while phagocytic indices were still quite high. The animals in this group showed no evidence of amyloidosis. The second group of animals, sacrificed only after phagocytic indices had returned to control levels, showed definite amyloid deposition. 3) The possible relationships between amyloidosis and the R.E.S. were discussed.

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Effect of Sensitization to BSA on Adjuvant Disease in Normal and Neonatally Thymectomized Rats.* (30269)

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It has been noted that rats are readily sensitized to tuberculin by a single footpad injection of tubercle bacilli suspended in mineral oil(1). This sensitization is reduced or suppressed entirely when a competing antigen, bovine serum albumin (BSA), is included in the adjuvant mixture employed for sensitization. Adjuvant disease, a complex of polyarthritis, ocular, and mucocutaneous lesions occurring in rats sensitized with tubercle bacillus-oil mixtures(2,3,4), was also found to be inhibited when the mixtures contained BSA(5). The suppressive effect of BSA on these forms of immune response appeared related to the development of sensitivity to the BSA itself, and was not noted when amounts insufficient to sensitize were employed. It was supposed that this suppressive effect depended on antigenic competition analogous to that described in a number of systems involving humoral antibody(6,7).

Comparative data have been obtained on the incidence and severity of adjuvant disease

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