THE ROLE OF DIET AND THE RETICULOENDOTHELIAL SYSTEM IN THE RESPONSE OF RATS TO *SALMONELLA TYPHIMURIUM* INFECTION*

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DURING the course of investigations into the role of dietary factors in the resistance of rats to infection with *Salmonella typhimurium*, we observed a much higher mortality rate in groups fed diets deficient in copper or in protein than in groups fed control or vitamin B_{12}-deficient diets. When we repeated the experiment we observed the same results. By investigating this phenomenon with larger numbers of animals we acquired interesting information concerning the interrelationships of the reticuloendothelial system (RES) and deficiencies of copper, vitamin B_{12}, or protein on resistance to infection with *Salm. typhimurium*.

MATERIALS AND METHODS

Male rats of the Charles River Casearean-Derived strain (Charles River Breeding Laboratories, North Wilmington, Massachusetts, U.S.A.) were used for the study. Those to be depleted of copper or vitamin B_{12} were fed their respective diets (Gallagher, Judah and Rees, 1956; Woodard and Newberne, 1967) from weaning until they reached a weight of about 250 g.; this required a period of approximately 3 months. Control rats were fed a standard 20 per cent casein diet throughout the experimental period. Those to be depleted of protein were fed the standard diet until 2 weeks before infection; they were then placed on a protein-free diet (McGuire, Young, Payne and Newberne, 1968). In order to obtain information on relative mortality due to the various dietary treatments, groups of 20 rats each were conditioned on their respective diets, infected, and left on experiment for 30 days. The most obvious change observed in animals dying or killed during the 30-day experiment was enlargement of the spleen and liver; this change was most pronounced in animals fed control or vitamin B_{12}-deficient diets. Copper-deficient rats exhibited splenomegaly only if they survived for a week or longer following infection. Since none of the rats in the 30-day study died until 5 days after infection (Table I), additional groups of 10 animals each were conditioned, infected, and killed 5 days after infection for more definitive studies.

Preliminary observations had indicated that the RES of copper-deficient and, to a lesser extent, protein-deficient rats failed to respond in a normal manner to the infection. For this reason the RES of some groups were blocked with Thorotrast (Testagar Inc., Detroit, Michigan, U.S.A.) (Good and Thomas, 1952). Each rat was given 0·3 ml. containing 77 mg. of a stabilized colloidal suspension of thorium dioxide by tail vein inoculation at the time of infection and again 48 hr. later.

The rats were infected by i.p. injection of $3{\cdot}5 \times 10^7$ organisms of a lyophilized culture of a strain of *Salm. typhimurium* (Young, Chen and Newberne, 1968). Identification and typing of the original isolation, of the standardized culture, and of cultures isolated from infected animals in these studies were confirmed by the Communicable Disease Center,

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United States Public Health Service, Atlanta, Georgia. An antigen was prepared from the culture for determination of agglutination titres (Shaffer and Goldin, 1962) and standard microbiological techniques (Shaffer, Goldin and McQuay, 1962) were used for isolation studies on the blood and organs. Total serum protein was determined by ultraviolet spectrophotometry (Waddell, 1956); serum protein fractions were separated by Gelman techniques and quantitated on a modified Beckman analytrol (Newberne, 1966). Routine histological methods were used to prepare tissues for microscopic assessment (Armed Forces Institute of Pathology, 1960).

RESULTS

Data on mortality and survival time for the groups on the 30-day study are presented in Table I. RES blockade increased mortality and decreased average

TABLE I.—*Effect of Deficiency and RES Blockade on Survival Time of the Rat after Infection with* Salmonella typhimurium

Treatment	Average time to death (days)	Range of time to death (days)	Number of survivors at 30 days*
Control infected	25	7–29	16
Control infected + RES blockade	12	4–15	8
Vitamin B_{12}-deficient infected	27	12–29	17
Vitamin B_{12}-deficient infected + RES blockade	16	5–20	12
Copper-deficient infected	7	5–10	4
Copper-deficient infected + RES blockade	8	5–9	3
Protein-deficient infected	7	5–10	5
Protein-deficient infected + RES blockade	9	7–12	4

* There were 20 rats in each group.

survival time in the control and vitamin B_{12}-deficient groups. However, while the response to infection alone was most severe in copper- and protein-deficient rats, RES blockade in these 2 groups had no further effect on mortality or survival time. At necropsy spleens and livers of infected rats were enlarged in control and vitamin B_{12}-deficient groups. In protein-deficient groups the response was variable and less pronounced while in copper-deficient groups splenic enlargement was not usually observed in animals succumbing during the first week after infection. When rats in these groups survived for longer periods of time an increase in spleen size was observed; however, it appeared to develop slowly and was not of the magnitude observed in the other groups.

In the short-term, 5-day study neither deficiency nor RES blockade had an appreciable effect on enlargement of the liver due to infection in any of the groups (Table II). However, control and vitamin B_{12}-deficient animals had a slightly greater increase in liver size than did those deficient in copper or protein.

A major difference among the groups, observed in both the 5- and 30-day studies, was the failure of the spleens of copper-deficient rats to enlarge in response to infection. There were no remarkable changes in the spleens of rats in this group, either in relative weight or in histologic alteration, as a result of infection. There was a marked enlargement of the spleens of control and vitamin B_{12}-deficient infected rats and a moderate enlargement of the spleens of protein-deficient infected animals during the 5-day period. RES blockade resulted in a further increase in spleen size in infected control and vitamin B_{12}-deficient rats but had no effect on copper- or protein-deficient animals. Thus, of the 4 treatments

TABLE II.—*Effect of Deficiency, Infection, and RES Blockade on Liver and Spleen Weights 5 Days Postinfection*

Treatment*	Average body weight (g.)	Liver per cent of body weight†	Spleen per cent of body weight†
Control	270	3·2±0·2	0·21±0·01
Control infected	260	5·0±0·4	0·92±0·11
Control infected + RES blockade	280	4·0±0·7	1·04±0·14
Vitamin B_{12}-deficient	264	3·1±0·4	0·19±0·02
Vitamin B_{12}-deficient infected	274	5·8±0·6	0·70±0·09
Vitamin B_{12}-deficient infected + RES blockade	252	5·1±0·2	0·82±0·05
Copper-deficient	268	3·0±0·1	0·17±0·06
Copper-deficient infected	274	4·0±0·1	0·22±0·10
Copper-deficient infected +RES blockade	267	4·0±0·3	0·19±0·08
Protein-deficient	236	2·8±0·2	0·18±0·05
Protein-deficient infected	228	4·4±0·2	0·52±0·04
Protein-deficient infected +RES blockade	235	4·2±0·4	0·48±0·05

* There were 10 rats in every group except the copper-deficient infected group, which had 8.
† Each value is given as the mean ± S.E.

only copper deficiency was associated with failure of splenic response during the 5-day period following infection.

Data on the effects of the deficiencies, infection, and RES blockade on serum protein are presented in Table III. Samples were taken when the animals were killed 5 days after infection. Both copper- and protein-deficient rats tended to

TABLE III.—*Effect of Deficiency, Infection, and RES Blockade on Serum Protein in the Rat 5 Days Postinfection**

Treatment	Pre-exposure					Post-exposure				
	Total protein (g./100 ml.)	Albumin	Globulins α	Globulins β	Globulins γ	Total protein (g./100 ml.)	Albumin	Globulins α	Globulins β	Globulins γ
Control	7·3	56	13	15	14	6·7	54	15	17	13
	6·8–8·0					6·3–7·4				
Control infected	7·0	52	18	14	16	7·2	32	30	24	20
	6·6–7·8					6·8–7·4				
Control infected + RES blockade	7·0	52	16	16	16	7·2	27	31	32	12
	6·5–7·4					6·7–7·4				
Vitamin B_{12}-deficient	7·0	50	17	18	15	6·8	54	18	12	16
	6·4–7·6					6·5–7·4				
Vitamin B_{12}-deficient infected	6·7	50	17	17	16	6·7	30	32	27	20
	6·0–7·4					6·6–7·2				
Vitamin B_{12}-deficient infected + RES blockade	6·8	48	18	18	14	7·0	32	32	25	10
	6·5–7·2					6·7–7·5				
Copper-deficient	6·2	52	16	18	14	6·2	48	16	20	15
	5·7–6·8					5·5–6·7				
Copper-deficient infected	6·5	57	13	13	13	6·4	25	28	22	14
	6·3–7·0					6·2–6·9				
Copper-deficient infected + RES blockade	7·0	50	18	17	14	6·5	38	23	28	11
	6·6–7·5					6·2–7·0				
Protein-deficient	6·0	50	17	18	15	6·2	48	19	20	13
	5·6–6·4					5·7–6·5				
Protein-deficient infected	5·6	46	18	19	16	6·2	38	20	23	16
	5·0–6·2					5·6–6·5				
Protein-deficient infected +RES blockade	5·8	48	17	18	15	5·8	36	23	27	14
	5·4–6·0					5·5–6·2				

* There were 10 rats in each group.

have lower total serum protein levels than did either control or vitamin B_{12}-deficient animals. While infection *per se* did not appear to affect total serum protein levels in any of the groups, RES blockade may have caused a slight decrease in the serum total protein level in protein-deficient infected rats. Infection caused a sharp decrease in serum albumin; this fraction was decreased to about half its preinfection level in infected control and vitamin B_{12}-deficient groups but was less affected in copper- and protein-deficient groups. After RES blockade a further decrease was observed only in the control group. In control and vitamin B_{12}-deficient rats γ-globulin was increased only after infection; this was prevented by RES blockade. Animals deficient in copper or protein failed to respond to infection by increasing γ-globulin and RES blockade had no further effect.

In other studies (McGuire *et al.*, 1968) we have observed more widespread lesions in control, adequately fed, infected rats than in protein-deficient ones. Therefore, in this series of experiments we attempted to isolate Salmonella from the various tissues and organs of infected rats. Although not very reliable as an indicator of the severity of infection, the serum agglutination titre provides some indication of the response of the animal to bacterial infection. Isolation data and serum agglutination titres from the various groups are given in Table IV.

TABLE IV.—*Effect of Deficiency and RES Blockade on the Serum Agglutination Titre and on Recovery of Organisms from Salmonella-Infected Rats 2 and 5 Days Postinfection**

	Heart blood		Liver		Spleen		Kidney		Agglutination titre†	
Treatment	2	5	2	5	2	5	2	5	2	5
Control infected	9/10	3/10	1/10	10/10	8/10	10/10	0/10	7/10	2+	4+
Control infected +RES blockade	7/10	8/10	2/10	10/10	2/10	6/10	1/10	5/10	1+	2+
Vitamin B_{12}-deficient infected	6/9	8/10	8/8	7/10	3/9	10/10	0/9	6/10	1+	3+
Vitamin B_{12}-deficient infected +RES blockade	5/8	6/9	2/8	9/9	3/8	8/9	2/8	5/9	0	1+
Copper-deficient infected	6/7	8/8	6/7	8/8	2/7	3/8	0/7	0/8	0	1+
Copper-deficient infected +RES blockade	5/9	6/9	0/9	6/9	0/9	4/9	0/9	0/9	0	1+
Protein-deficient infected	6/6	6/6	1/6	5/6	3/6	6/6	0/6	2/6	0	2+
Protein-deficient infected +RES blockade	7/8	8/9	2/8	6/9	1/8	3/9	0/8	1/9	0	0

* Number of positive cases over total sampled.
† Arbitrarily graded from 0 to 4+ depending upon the intensity of reaction.

Heart blood yielded cultures in all groups on both 2 and 5 days postinfection. Most groups yielded liver and spleen cultures of Salmonella on day 2 postinfection; all groups were positive on day 5. Control, vitamin B_{12}-deficient, and protein-deficient groups were positive for Salmonella in kidney tissues only after 5 days while the copper-deficient groups yielded no cultures on either day. In general, animals other than those in control infected groups seemed to require a longer period of time to sequester or concentrate sufficient organisms to permit isolation.

Histopathology

The characteristic enlargement of the liver and spleen in response to *Salm. typhimurium* infection in the rat is largely a result of the proliferation of cells of the RES, although there is some contribution from congestion and from the bone marrow in the form of granulocytes. The livers of both control and vitamin B_{12}-deficient infected rats in this study had widespread focal necrosis; Figs. 1 and 2 illustrate the characteristic lesion observed in these livers. Lesions typical of those seen in livers of copper- and protein-deficient animals are shown in Figs. 3 and 4. Ocular micrometry measurements of involved areas from 100 high-power fields of liver from each of the 4 groups indicated that 53 per cent of the total area of the liver section of control and 48 per cent of vitamin B_{12}-deficient rats were involved with focal necrosis and reactive cells 5 days after infection. This was in contrast to an average of 26 per cent for copper-deficient and 32 per cent for protein-deficient animals. RES blockade reduced the percentage involvement in all groups to a degree approximating the decrease in relative liver weight.

The cell population of necrotic areas was comprised of large mononuclear

EXPLANATION OF PLATES

FIG. 1.—Liver lesion typical of those observed in control and vitamin B_{12}-deficient rats 5 days postinfection. Lesions were circumscribed, numerous, and involved about 53 per cent of the total area of 100 sections measured by ocular micrometry. H. and E. ×270.

FIG. 2.—Higher magnification (from the section shown in Fig. 1) of a focal area of necrosis and cellular response. Large mononuclear cells predominate. H. and E. ×530.

FIG. 3.—Liver lesions characteristic of both copper- and protein-deficient rats. Focal necrotic areas are small and few in number. H. and E. ×270.

FIG. 4.—Higher magnification of an area of focal necrosis typical of those present in the livers of copper- and protein-deficient rats. Polymorphonuclear cells are numerous and large mononuclears reduced in numbers compared to control and vitamin B_{12}-deficient animals. H. and E. ×530.

FIG. 5.—Section of spleen from a control infected rat. Large mononuclear-type cells predominate. H. and E. ×530.

FIG. 6.—Section of spleen from a protein-deficient infected rat. Fewer mononuclear cells are present in the RES and those present are in focal aggregates. H. and E. ×530.

FIG. 7.—Section of spleen from a copper-deficient infected rat. Note the sparse focal area of RE cell proliferation. H. and E. ×530.

FIG. 8.—Section from the lung of a control infected rat. A focus of large mononuclear cells with an admixture of neutrophils occupies space previously occupied by alveolar spaces. H. and E. ×530.

FIG. 9.—Section of lung from a protein-deficient infected rat. Focal areas such as this were fewer and smaller in size in protein-deficient animals than in infected controls. H. and E. ×530.

FIG. 10.—Lesion observed in the kidneys of most infected animals from all groups. Interstitial nephritis was comprised mainly of neutrophils and lymphocytes. H. and E. ×530.

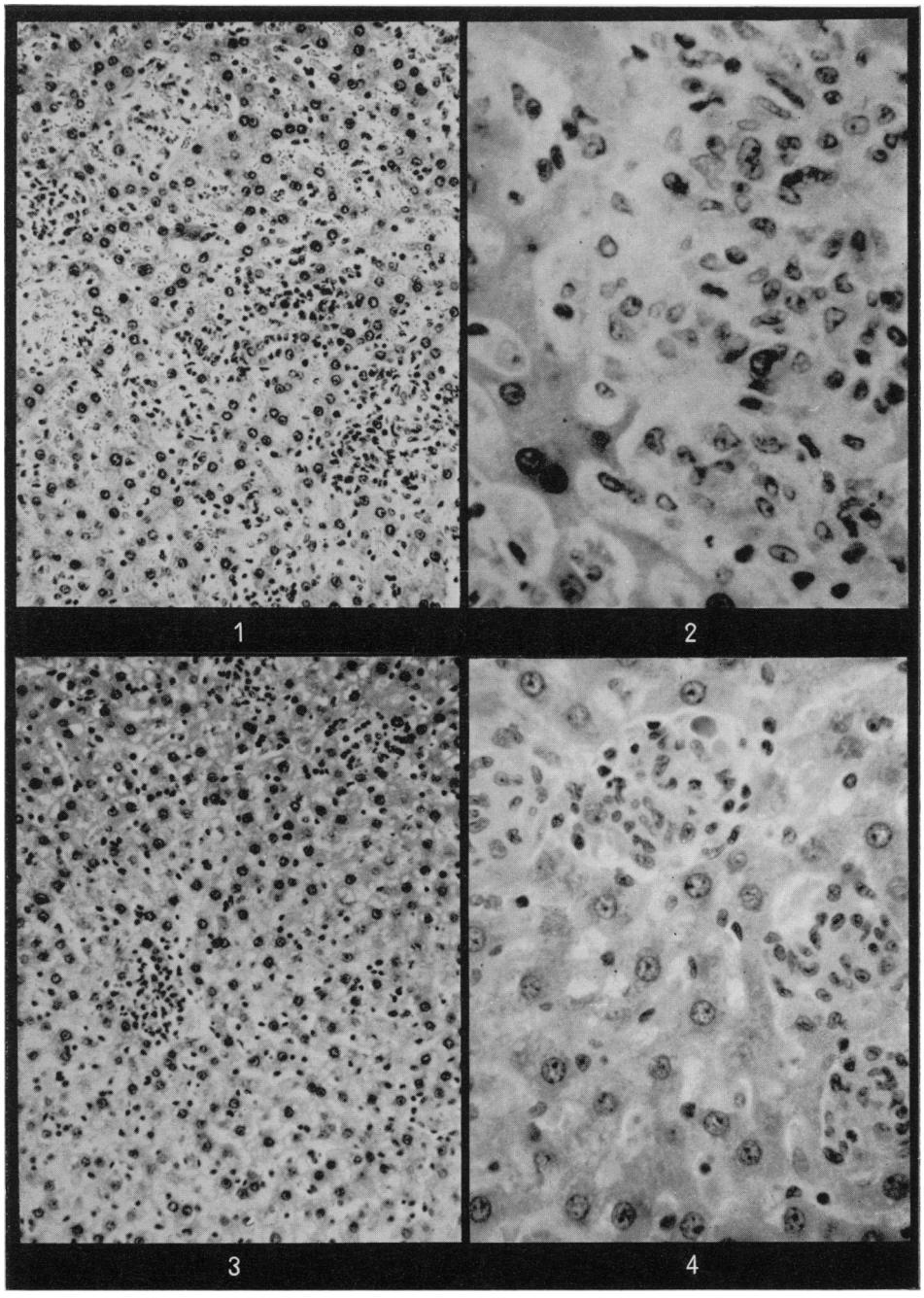
FIG. 11.—Pyelitis and pyelonephritis were observed in many copper- and protein-deficient infected rats. Haemorrhage was a prominent component in some of the copper-deficient animals. H. and E. ×65.

FIG. 12.—Section from the heart of a control infected rat illustrating the widespread focal and diffuse myocarditis characteristic of that observed in control and vitamin B_{12}-deficient rats. H. and E. ×65.

FIG. 13.—High magnification of section from the area of heart shown in Fig. 12. There is an admixture of large mononuclear cells, myocytes, and a sprinkling of neutrophils. H. and E. ×530.

FIG. 14.—Section of heart from a copper-deficient infected rat. Only a few focal areas of myocarditis were observed in these and in protein-deficient rats. H. and E. ×65.

FIG. 15.—Higher magnification of an area of heart illustrating focal myocarditis in a copper-deficient rat. Although a few mononuclear cells and myocytes are present, neutrophils make up the larger proportion of reactive cells. H. and E. ×530.



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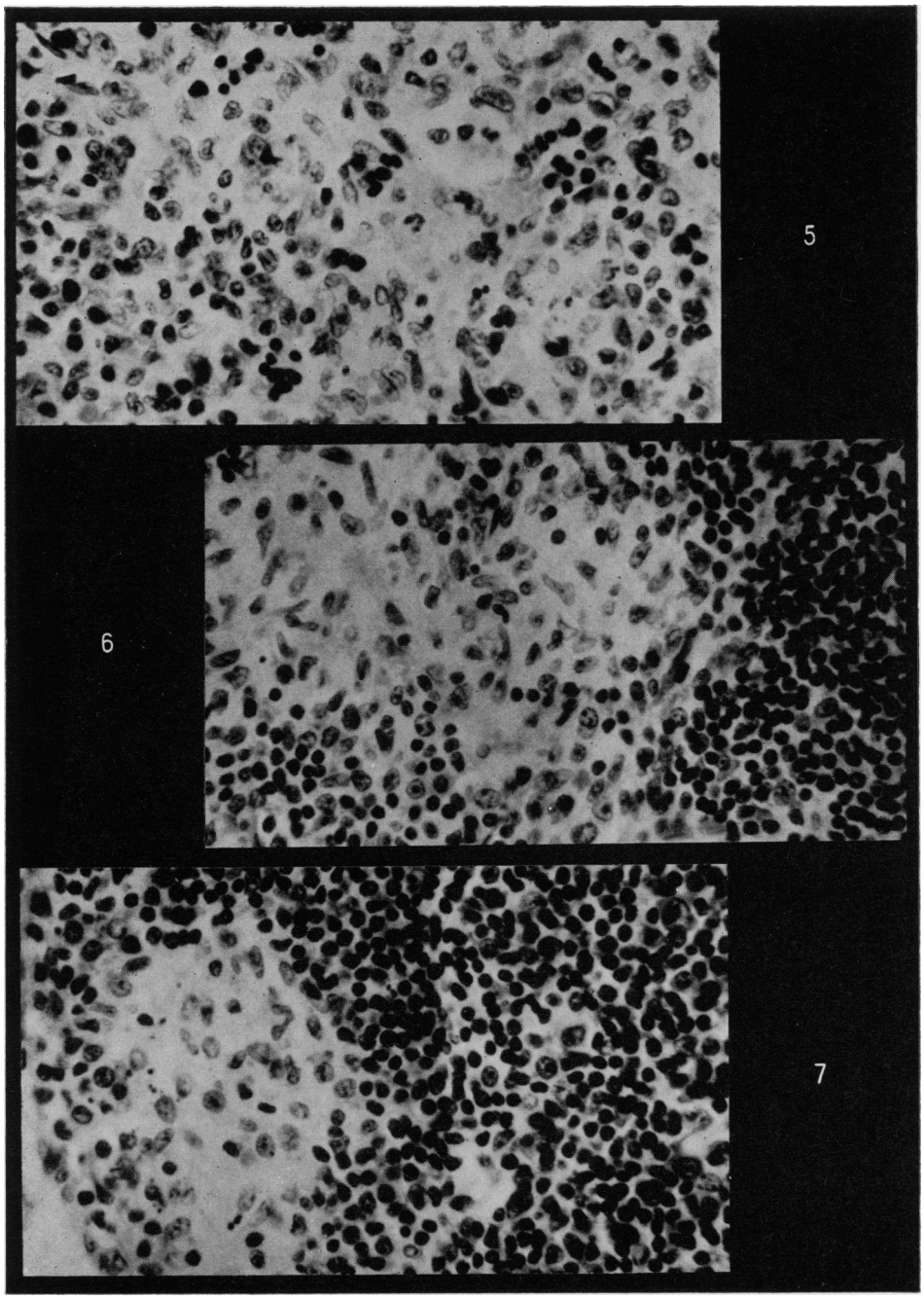

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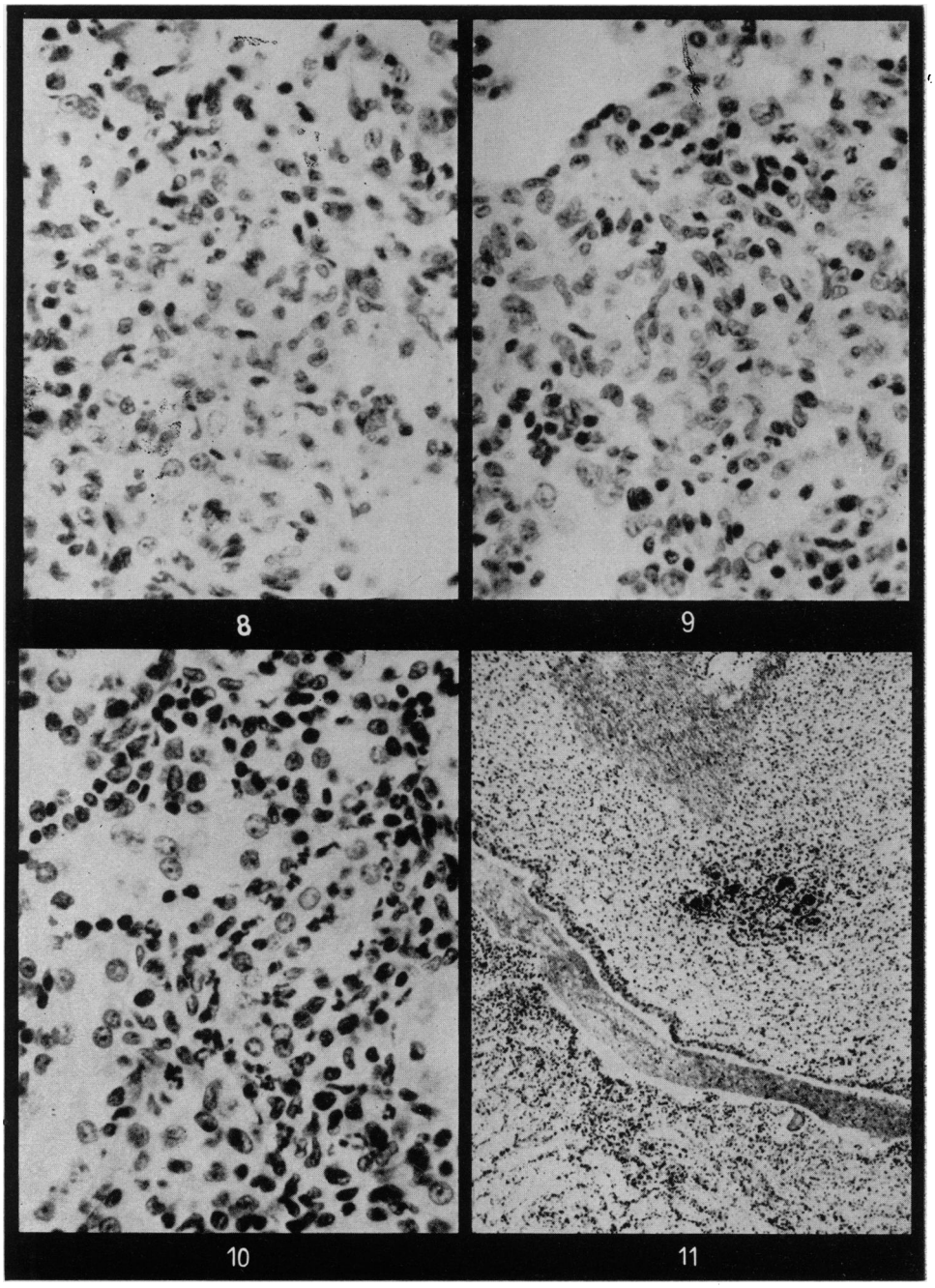

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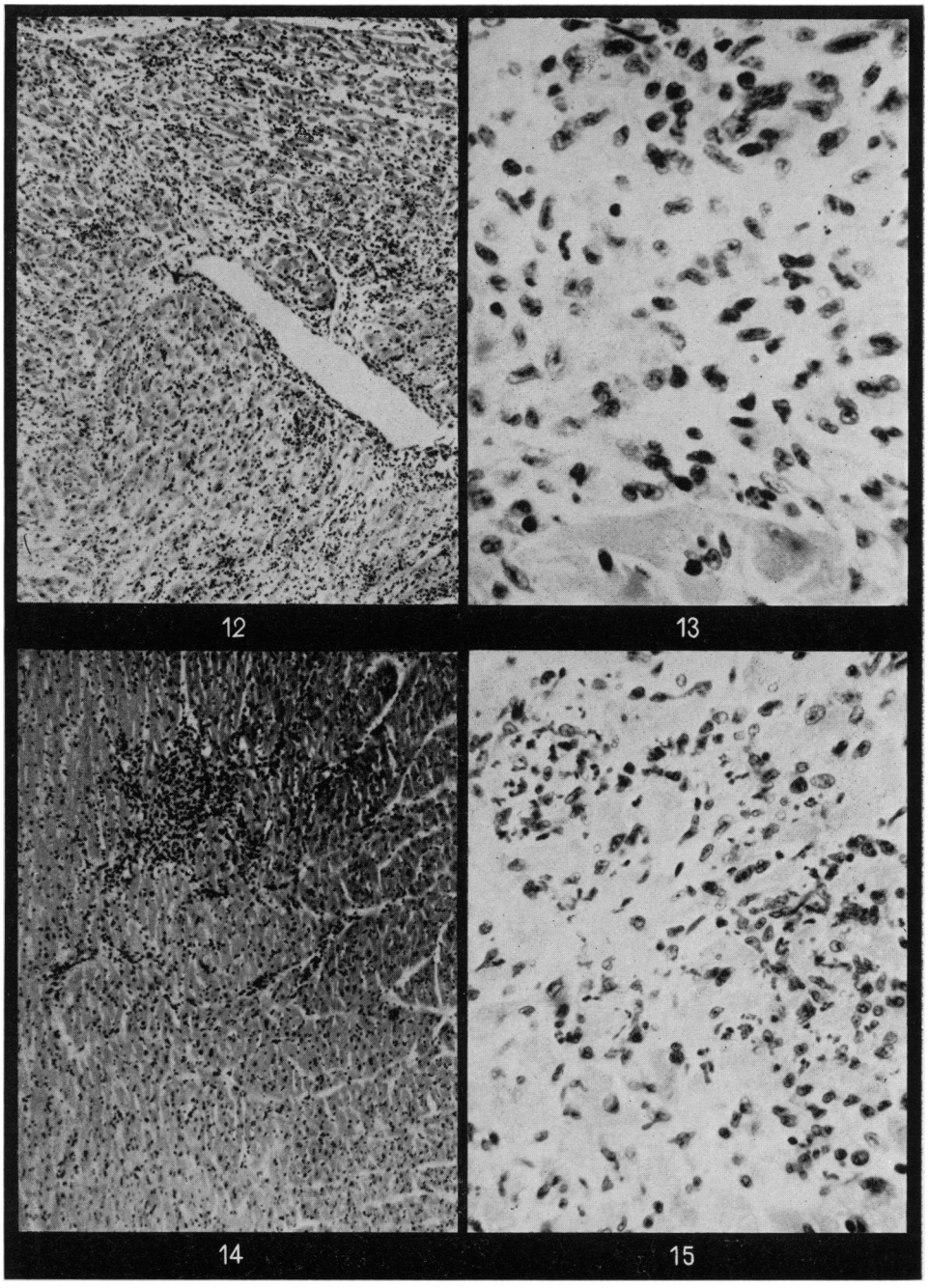

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cells (macrophages), neutrophils, and a few lymphocytes. Large mononuclear cells predominated in focal lesions in control and vitamin B_{12}-deficient animals while there were about as many neutrophils as mononuclear cells in copper- and protein-deficient rats. Mitosis of Kupffer cells was seen frequently in livers of control and vitamin B_{12}-deficient animals but only occasionally in livers of copper- and protein-depleted rats.

Based on the extent of histopathological alterations, blockade of the RES decreased involvement of the liver in all groups with the decrease in severity of lesions most marked in control and vitamin B_{12}-deficient rats. Furthermore, in these 2 groups there was less tendency toward focal accumulation of cells following RES blockade. Although RE cells proliferated after blockade they tended to remain diffusely distributed along sinusoids instead of aggregating into focal areas. RES blockade had little detectable effect on infected copper-deficient rats and only slightly more effect on protein-deficient ones; the response in both groups was about the same with and without blockade.

Although some congestion was present in all spleens, enlargement in response to infection was chiefly a result of RE cell proliferation. The control and vitamin B_{12}-deficient rats exhibited the greatest increase in spleen weight; this increase approximated that observed morphologically in RE cell proliferation. Fig. 5 shows the marked proliferation of RE cells in the control animals; the spleens of vitamin B_{12}-deficient rats were similarly involved. Fig. 6 shows the lesser involvement of protein-deficient rats while Fig. 7 illustrates the rather feeble attempt of the RES of copper-deficient rats to respond to infection.

The lungs were involved to some degree in all groups. Figs. 8 and 9 illustrate the focal and interstitial response to infection of control and protein-deficient rats, respectively. Lung lesions in vitamin B_{12}-deficient rats were comparable to those seen in control animals while copper-deficient rats exhibited an interstitial pneumonitis with little tendency toward focal aggregation. RES blockade had no appreciable effect beyond that observed with infection alone on lesions in the lungs of infected rats in any group.

The kidneys were affected to some degree in most rats in all groups with or without RES blockade. In some animals the organism was not isolated even though the kidneys exhibited histologic lesions. Copper- and protein-deficient rats had more severe involvement of the kidneys than did either control or vitamin B_{12}-deficient animals, a reversal of the pattern observed in the spleen and liver. The characteristic histological change was interstitial nephritis (Fig. 10). The papillary zone of many of the copper- and protein-deficient animals had severe haemorrhagic pyelonephritis (Fig. 11).

The heart of most infected animals exhibited some degree of involvement. In control and vitamin B_{12}-deficient animals there was focal and diffuse myocarditis (Figs. 12 and 13). Copper- and protein-deficient rats had less extensive involvement; however, neutrophils were more abundant than mononuclear forms (Figs. 14 and 15). RES blockade had very little observable effect on either incidence or severity of cardiac lesions.

DISCUSSION

The results of the 30-day study (Table I) indicate that copper deficiency and, to some extent, protein deficiency decrease the resistance of rats to infection with *Salm. typhimurium*. RES blockade had no further effect in copper- or

protein-deficient animals but did further decrease the resistance of control and vitamin B_{12}-deficient rats. Mean survival time of rats fed the control and vitamin B_{12}-deficient diets was reduced by RES blockade to about half that of the control and vitamin B_{12}-deficient infected groups without RES blockade. Equally impressive was the sharp decrease in the number of infected animals with RES blockade that survived the infection. The control group was more severely affected by the RES blockade than was the vitamin B_{12}-deficient group. The decrease in survival time and increase in mortality in these 2 groups were not solely the result of failure of the RES to proliferate since both liver and spleen were enlarged following infection and blockade (Table II).

Either copper or protein deficiency resulted in marked decreases in the number of survivors and the average time of survival. Failure of rats in these 2 groups to resist the infection indicated that these deficiencies altered the RE defensive mechanisms. This effect was probably mediated through the RES since the responses to infection by copper-deficient and, to a lesser extent, protein-deficient rats were not appreciably altered by RES blockade. In addition, the spleens of rats in the copper-deficient groups, with or without RES blockade, failed to enlarge in response to infection and blockade had no further effect on this parameter in the protein-deficient group. It appears, then, that deficiency of copper or of protein has a direct effect on the RES. Blocking an already impotent RES would not be expected to have further appreciable influence on the ability of an animal to respond to infection; this appears to be the case in copper-deficient and, to a lesser extent, in protein-deficient rats.

A number of investigators (Robertson and Doyle, 1936; Watson, 1937; Guggenheim and Buechler, 1949) have shown that protein-deficient animals are more susceptible to *Salm. typhimurium* infection than animals fed normal amounts of protein. The results of this study agree with previous reports and indicate that copper deficiency has an even more profound effect on the ability of the RES to respond to infection. In earlier studies (McGuire *et al.*, 1968) we have found that nitrogen losses are higher in Salmonella-infected rats fed low-protein diets than in those fed higher levels of protein; furthermore, muscle polysome profiles are altered in low-protein infected rats (Young *et al.*, 1968).

The increase in relative weight of both liver and spleen in infected rats results in part from congestion but, more importantly, from an increase in cell population largely derived from the RES. In our study animals deficient in copper and, to some extent, those deficient in protein failed to respond in a positive manner to provide increased numbers of cells. This was particularly striking in copper-deficient rats with or without RES blockade. Thus, copper must be related in some obscure way to RE cell proliferation. The same relationship does not exist, under the conditions of this investigation, for proliferation of myeloid elements; copper- and protein-deficient rats elaborated quantities of polymorphonuclear cells which were observed in large numbers in affected organs and tissues. Copper-deficient rats surviving for longer periods of time in the 30-day experiment developed some enlargement of the spleen. Thus, the difference in response to infection between copper-deficient rats and those in the other groups may be a result of differences in the rate at which they can respond.

Total serum protein levels were lower in both copper- and protein-deficient rats than in control or vitamin B_{12}-deficient animals (Table III). Infection appeared to have little effect on total serum protein in any of the groups but it

had a profound influence on the albumin fraction, decreasing its value sharply in all groups. This change was probably related to the involvement of the liver with infection and necrosis. With large numbers of parenchymal cells destroyed, their function as sites for the synthesis of albumin was lost and the serum levels of this protein fraction decreased. The lowered levels of serum albumin, because of its important contribution to osmotic pressure, were reflected in some infected animals by varying degrees of ascites. Infection in copper- and protein-deficient animals failed to elicit an increase in the globulin content of the serum, thus further reinforcing our hypothesis that both deficiency states (but particularly copper deficiency) resulted in a decreased functional capacity of the RES.

Although it is difficult to draw conclusions from the data on isolation studies (Table IV) we find it interesting that viable organisms were circulating in most animals sampled at days 2 and 5 postinfection. In general, RES blockade and a deficiency of copper or of vitamin B_{12} seemed to prevent the localizing of sufficient organisms to result in positive cultures. Perhaps it is necessary to have an active RES functioning at optimum capacity for bacteria to be rapidly sequestered in the tissues or organs in sufficient numbers to permit isolation by usual culture techniques.

When the function of the RES is suppressed through blockade with colloidal agents such as Thorotrast, the capacity of the animal to withstand the effects of endotoxins (Good and Thomas, 1952; Smith, Thomas and Good, 1953) or of traumatic shock (McKenna and Zweifach, 1956) is diminished. There is also evidence that acquired resistance against the lethal effects of these stresses can be overcome by blocking the RES (Beeson, 1947; McKenna and Zweifach, 1956; Zweifach and Thomas, 1957; Zweifach, Benacerraf and Thomas, 1957). Alterations in macrophage elements of spleen, liver, and lymphoid tissue have been observed during shock and some investigators (Windle, Chambers, Ricker, Ginger and Koenig, 1950; Fine, Frank, Ranin, Rutenberg and Schweinburg, 1959) suggest that changes in bacterial defence mechanisms are important in overcoming the effects of severe injury and shock. More recently, Rapoport and co-workers (Rapoport, Hodoval and Beisel, 1967) have shown that RES blockade in monkeys decreased the rate at which radioiodinated aggregated albumin was cleared from plasma but had no effect on plasma clearance of staphylococcal enterotoxin B. These investigators (Rapoport, Hodoval, Grogan, McGann and Beisel, 1966) had previously shown that staphylococcal enterotoxin B had a rapid rate of clearance, similar to that of endotoxin, from the plasma of experimental animals but that, in contrast to endotoxin, the immune state was associated with a profound delay in removal of enterotoxin B. These observations imply that the role of the RES in an animal's defence against enterotoxin B may be different from that postulated for bacterial endotoxin. A situation analogous to that for endotoxin may exist for infection with Salmonella in that neither the bacteria nor, perhaps, the endotoxins produced by them are cleared efficiently from the plasma of copper- and protein-deficient rats.

Agglutination titres of serum from rats in our experiments indicated little more than the fact that adequately fed animals developed a higher titre after 5 days than any of the other groups. Recently Katz and Plotkin (1967) observed a reduced tolerance in mice fed a protein-free diet and exposed to the virus of herpes simplex. This decreased tolerance was not associated with a difference

in production of antibodies or of interferon between protein-deficient and control groups. An analogous observation was made by Johnson (1964), who found that mice developed with age a " barrier " against the spread of herpes simplex virus to the brain after extraneural inoculation and that the barrier depended on macrophage action. Perhaps in our experiments copper deficiency and protein deficiency resulted in less macrophage activity and therefore less resistance to bacterial infection.

Additional evidence for a dietary effect on response to infection has been recently reported by Siegel, Squibb, Solotorovsky and Ott (1968). These investigators observed that chicks infected with tuberculosis and fed a low-protein diet had a decreased amount of tuberculous tissue and a higher mortality rate than birds fed a normal level of protein. Furthermore, infected chicks fed a high-protein diet had an increase in the amount of tuberculous tissue. The amount of tuberculous tissue was equated with susceptibility; thus, the greater the amount of tuberculous tissue the more susceptible the birds were thought to be to the infection. Although the findings of Siegel *et al.* (1968) parallel our findings in many ways, our interpretations are different. Based on mortality, results of our studies indicate that the greater degree of involvement of the liver and spleen can be equated with a more profound response, a more efficient defence system, and, therefore, a decreased susceptibility to the infection. It must be remembered, however, that we were working with an acute infection in a mammalian species while Siegel *et al.* (1968) used a subacute or chronic infection in an avian species.

We would like to know something about the mechanisms of action of copper deficiency and protein deficiency at the molecular level. Such investigations might reveal an additional metabolic role for copper in the animals' defence system.

SUMMARY

Male rats were fed an adequate diet or a diet deficient in either vitamin B_{12}, copper, or protein and then infected with *Salm. typhimurium*. At the time of infection and again 48 hr. later Thorotrast (thorium dioxide) was injected to block the reticuloendothelial system (RES) of some rats in each infected group. During the 30-day period postinfection mortality was higher and survival time shorter in the copper- and protein-deficient groups. In these 2 groups mortality and survival time were not enhanced by blocking the RES while they were enhanced in the adequately fed and vitamin B_{12}-deficient groups. In another series of rats killed during the first 5 days postinfection there was a significant increase in spleen weight in the control and vitamin B_{12}-deficient groups; no increase was observed in the copper-deficient group while a moderate increase was noted in the protein-deficient group. Spleen weights following infection were not further altered by RES blockade. Infection caused a decrease in serum albumin in all groups and an increase in γ-globulin in the control and vitamin B_{12}-deficient groups. These findings suggest that the RES of adequately fed rats is more capable of responding to infection than is that of either copper- or protein-deficient rats.

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