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Relationship of Burned Area in Rats to Incidence of the Anti-Globulin Reaction (Coombs' Test).* (29670)

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In recent experiments in our laboratory(1) positive anti-globulin reactions (Coombs' tests) were observed in 51 of 56 rats 4 days after they had received full thickness 37% body surface burns. The positive test was observed to persist in some animals and disappear in others by the end of the 3 week experimental period. No correlation was found between the several parameters studied at regular postburn intervals (Coombs' tests, hematocrit, reticulocyte values, serum protein levels) and death or survival. Thus it seemed desirable to determine whether: (a) the positive Coombs' test is directly related to the percent body surface burned, (b) a decrease in percent of burn area would effect a change in the postburn time at which a positive Coombs' test could be detected, (c) the other parameters studied including survival would be significantly altered if animals were subjected to reduced area burns, and (d) such changes could be correlated with changes in the incidence of the Coombs' test. In this report 3 groups of animals receiving 26%, 20% and 14% burns, respectively, have been studied in relation to these questions.

Materials and methods. Male Sprague-Dawley rats (190-210 g) were subjected to back burns in water at 90°C for 40 seconds under deep ether anesthesia. Each animal except the normal controls received intraperitoneally in 3 injections immediately postburn (p.b.) and at 5 and 10 hours (p.b.) 2% of the body weight of a 1/6 M sodium lactate solution and 7% of the body weight of a 1.4% solution of NaCl. The average percent body surface burns were as follows: group A (18 rats) 25.5%, SD=1.72; group B (13 rats) 20.2%, SD=0.99; and group C (14 rats) 14%, SD=2.5. Six non-burned injected controls and 4 non-burned, non-injected controls were also studied. All blood determinations were performed on lateral tail vein blood. Hematocrits were done by the standard microhematocrit method and reticulocyte values were determined by the method of Brecher(2). Serum protein electrophoretic studies were performed using the Spinco Model R electrophoretic system with Veronal buffer pH 8.6, ionic strength 0.075. Anti-globulin tests were performed on thrice washed erythrocytes of each animal preburn and postburn at 4, 7, 10, 14, 21, 28 and 35 days using rabbit anti-rat gamma globulin. Sixty blood samples from animals in groups A and B and 70 samples from group C animals were streaked on blood agar plates and incubated at 37°C. At autopsy the spleens and thymi were excised and weighed to the nearest milligram.

Results. Survival. Postburn (p.b.) survival numbers were not significantly different

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at 35 days for groups A and B, 46 and 54% respectively. All rats in group C survived.

Coombs' reaction. Fifteen of 18 rats (83%) in group A developed positive tests; in 40% of the 15 animals the positive tests were observed later than 4 days p.b. Seven of the 9 rats (67%) which remained positive survived through 35 days p.b. Three of 5 animals that remained or became negative (60%) survived to the end of the test period. In group B, 9 of 13 animals (69%) developed positive reactions and of the 9, five (56%) developed the positive tests later than 4 days p.b. Four (67%) of the 6 positive animals remained positive through 35 days p.b. Three of those that remained or became negative (75%) survived to the end of the test period. None of the control animals developed a positive Coombs' test.

Hematocrit and reticulocyte percents. At 4 days p.b. survivors which developed positive Coombs' reactions in groups A and B showed similar average hematocrit depressions from 42% to 33% and reticulocyte increases from 6% to 23%. Hematocrit averages and reticulocyte number averages approximated initial values in both groups at 35 days p.b. Average hematocrit depressions at 4 days for the negative survivors were similar in groups A and B (43% to 38%) and were slightly less than those for the positive survivors. Elevation of reticulocyte values was similar to that of the positive survivors, reaching a peak at 4 days p.b. and decreasing to approximately initial values at 35 days p.b. The group C hematocrit values at 4 days p.b. showed only a slight depression; at 35 days p.b. these values were above preburn levels. The reticulocyte increase for group C rats was approximately half of that of the other groups. A return to initial values was observed by the end of the test period. Among the infused non-burned controls the average reticulocyte numbers were approximately half the initial level at 35 days p.b. and hematocrits were slightly higher than initial. (Both changes reflect normal growth trends.)

Serum protein values. In all Coombs' positive and negative p.b. survivors the average serum albumin levels showed marked declines

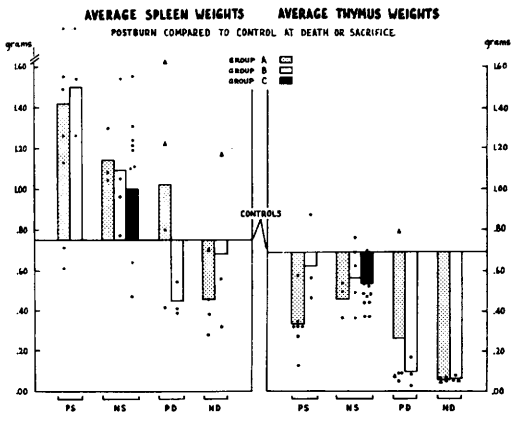

FIG. 1. Horizontal base lines for the group bars represent average control values. Round dots indicate individual organ weights, triangles indicate individual organ weights of animals which died after 14 days postburn. Animals are grouped according to Coombs' reaction and death or survival accordingly: PS (positive survivors), NS (negative survivors), PD (positive deaths) and ND (negative deaths).

from approximately 65% to 35% at 7 days p.b., although the decrease in these values in the C group occurred later than in groups A and B. At 35 days p.b. the serum albumin levels of the Coombs' negative survivors in groups A, B and C were 50%, 60%, and 57% of initial, respectively. The $alpha_1$ serum globulin values for all groups including both positive and negative survivors approximately doubled during the early postburn period, then declined slightly but remained significantly higher than the control values through 35 days p.b. $Alpha_2$ values were similar. The positive and negative beta globulin values for groups A, B and C showed an early increase and achieved levels of 157%, 140% and 170% of initial values at 35 days p.b. The gamma globulin values showed a marked increase in all groups; in the Coombs' negative survivors at 35 days p.b. levels of 160%, 230%, 330% and 590% of initial were observed in the controls and groups C, B and A, respectively.

Spleen and thymus weights. A comparison of the average spleen weights at death or sacrifice (Fig. 1) of positive and negative animals in each of the 3 groups showed that the splenomegaly, observed in all survivors, was pronounced in those that were Coombs' positive. Spleens of rats dying before 14 days

p.b. were generally smaller than those of the controls; spleens from those animals dying late in the test period were generally as large (1.0 g) as those of the survivors. A comparison of thymus weights showed that all thymi from experimental animals were smaller than those of the controls; surviving animals in group A had generally smaller thymi than did those from groups B or C. Atrophy was nearly total (wts. of less than 100 mg) in 14 of 15 animals dying before 35 days p.b.

Blood agar plates. All plates from group C animals were negative. Seven of the samples from groups A and B were positive. An enteric form *E. coli* was identified in 3 cultures and *P. aeruginosa* and *P. mirabilis* were each found in 2 cultures.

Discussion. The data above have shown differences in percent survival and incidence of Coombs' tests related to burn area, whereas response in other parameters tested could not be equated to extent of area burned. The serum protein values in all 3 groups were markedly similar, with the exceptions of higher gamma globulin levels in Coombs' positive survivors in groups A and B. Whether this indicates the existence of a specific immune system in these animals has not yet been determined. Hematocrit and reticulocyte response in the 3 groups and in positive and negative animals within each group were also generally similar with the exception of the reticulocyte response in group C. Involvement of reticulocytes in "false" positive Coombs' tests has been cited(3); it could be suggested therefore that the absence of positive tests in group C was due to the lower reticulocyte response in this group. However, animals in groups A and B with negative tests were seen to experience reticulocytoses as marked as those animals that were positive.

Several investigators have pointed to the significance of bacteremia in postburn sequelae. Millican(4) has demonstrated the significance of infection in the delayed death of mice following extensive burns. Matter *et al*(5) found indications that specific antimicrobial antibodies may be present in the blood of animals recovering from infected burn wounds. Simonart(6) has reported that bacteria can be found in the blister fluid of burned rabbits. It could be suggested that postburn bacteremia may be involved in the development of the positive anti-globulin reaction. Weeden(7) has reported that bacteria (a) may adhere to red cells and be agglutinated by the appropriate antibodies in Coombs' sera, (b) may under certain conditions render red cells pan agglutinable, and (c) may produce hemagglutinins. However, in our studies Coombs' tests were found to be positive in 4 and negative in 3 of the 7 blood samples which contained bacteria; therefore no correlation existed between the positive test and bacteremia.

The quantitative relationship between area burned, bacteremia and the positive Coombs' test as well as the delayed and transient nature of the Coombs' test in some animals leads to several questions with regard to the earlier suggestion(8) that the positive Coombs' test indicates the development of an auto-immune or auto-toxic response to severe thermal trauma. Additional studies using more sensitive techniques both for detection of the presence of anti-bacterial antibodies and for characterization of the red cell coating globulins are needed before the etiology of the postburn positive Coombs' test can be fully defined.

Summary. Male Sprague-Dawley rats were subjected to full thickness burns of 26%, 20% and 14% body surface, respectively. A reduction in burned area was correlated with (a) increased survival, (b) decreased incidence of positive Combs' tests and (c) a lengthening in the postburn time at which the Combs' tests could be detected. Hematocrits, reticulocyte numbers and serum protein values showed only partial correlation with the percent of area burned. Postburn splenomegaly was greater in positive survivors than in those that were negative. Blood samples cultured on blood agar plates showed a low incidence of bacteremia in the A and B groups; all C group animals were negative.

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Methionine Deficiency and Fatty Liver in Male and Female Rats.* (29671)

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Liver fat accumulation has been reported in rats made deficient in tryptophan(1), phenylalanine(2), histidine and threonine(3) and methionine(4).

A recent study from our laboratory(5) demonstrated that isoleucine-deficient rats also developed fatty livers resulting exclusively from an accumulation of triglycerides. Investigations into possible reasons for the excessive triglyceride deposition indicated that an appreciable amount of the fat must have risen from increased hepatic fatty acid synthesis.

Fatty liver in rats made deficient in a single essential amino acid seems, therefore, to be a frequent occurrence. Since the condition may arise by a change in one or more of a number of processes involving synthesis of, mobilization to and from, and utilization of the liver fatty acids, an experiment was conducted to see whether a deficiency of another essential amino acid (methionine) would affect liver lipid metabolism as had isoleucine deficiency.

Materials and methods. Because it had been reported previously(6) that fatty liver developed only in methionine-deficient adult female rats, but not males, both male and female Long-Evans rats were used in the following experiments.

Average weight of the females at the beginning of the experiment was 160 g and that of the males 180 g. A complete diet(5) containing purified amino acids in the proportion and amount necessary to approximate 18% of casein was fed to control groups of both sexes. Methionine was omitted from the amino acid mix in the experimental groups. Both control and deficient groups were force-fed (in 2 separate feedings) about 10 g of diet daily for 10 days. During that time, control rats gained weight, whereas deficient animals lost weight. By the end of the experimental period, most of the deficient animals also exhibited unkempt fur, hyperirritability and porphyrin staining about the whiskers and forepaws. On the eleventh day, rats of each group were fed 5 g of their respective diets. Two hours later they were decapitated, and blood was collected and allowed to clot. Serum was then extracted with alcohol:ether (3:1 v/v)(7), and total cholesterol(8) and phospholipid(9) were determined.

Livers were removed and weighed. About 500 mg of slices, 0.5 mm thick, were incubated with 0.3 μc of sodium acetate-2-C^{14} for 3 hours at 37°C in 4.5 ml of Krebs-Ringer bicarbonate buffer (pH 7.3). Gas phase was 95% O_2 and 5% CO_2. Following incubation, the slices were thoroughly washed, saponified with KOH, acidified and extracted into chloroform. An aliquot of the extract was plated and counted in a gas-flow counter (10). A part of the unincubated liver was

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