blood stream after lethal doses of this toxin (3), the selective stabilization of hepatic lysosomal particles in endotoxin-tolerant animals would seem to represent an important component of tolerance to this agent. Such an interpretation is also favored by the observation that Thorotrast increased the fragility of liver lysosomes but did not similarly affect mitochondria. Thus, an agent which overcomes tolerant states exhibited a selective labilizing action upon lysosomal membranes in our test system.

*Summary.* Carbon tetrachloride-induced leakage of 2 acid-hydrolases from mouse liver lysosomes, *in vitro,* was inhibited by chronic pretreatment of the mice with endotoxin. Cortisone-pretreatment inhibited the release of one of the enzymes. Pretreatment of the mice with Thorotrast, a reticulo-endothelial-blocking agent which neutralizes tolerant states, had the opposite effect and caused accelerated release of acid-hydrolases from liver lysosomes of tolerant as well as normal animals. None of the 3 agents studied produced comparable changes in the rate of leakage of soluble dehydrogenases from mouse liver mitochondria. It is suggested that stabilization of hepatic lysosomal membranes in endotoxin-tolerant animals represents a selective response of these particles to repeated small doses of the toxin and plays an important role in the development of tolerance to this agent.

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## Serum Haptoglobin Types in Rheumatoid Spondylitis.* (28774)

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In the few years since Smithies separated by starch gel electrophoresis a number of hemoglobin binding proteins (haptoglobins), a large body of information has accumulated regarding the genetic control of these substances(1-4). Although the early studies indicated that 3 haptoglobin phenotypes (1-1, 2-1, and 2-2) were representative of 3 genotypes produced by a single pair of allelomorphic genes ($Hp^1$ and $Hp^2$) more recent studies have described other rarer phenotypes and techniques of subtyping have been introduced(5).

Since haptoglobins and other serum protein factors are inherited and since other blood substances (especially blood groups) have been associated with specific disease states, an analysis of the relation that might exist between haptoglobin types and rheumatoid spondylitis was carried out. The latter disease was chosen because there is good evidence that genetic mechanisms play a role in its genesis(6).

*Methods and materials.* Forty-five adult

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TABLE I

| Population | No. tested | | Phenotypes 1-1 | 2-1 | 2-2 | Gene frequency p(1-1) | q(2-2) |
|---|---|---|---|---|---|---|---|
| Caucasian males with rheumatoid spondylitis | 45 | Observed | 10 | 21 | 14 | .456 | .544 |
| | | Expected | 9.45 | 22.95 | 13.05 | | |

TABLE II

| | | Haptoglobin types 1-1 | 2-1 | 2-2 | Total |
|---|---|---|---|---|---|
| 1. Rheumatoid spondylitis (present study) | Observed | 10 | 21 | 14 | 45 |
| | % | 22 | 47 | 31 | 100 |
| 2. Seattle control (8) | Observed | 24 | 98 | 78 | 200 |
| | % | 12 | 49 | 39 | 100 |
| 3. Rheumatoid arthritis (9) | Observed* | 3.5 | 23.5 | 8 | 35 |
| | % | 10 | 67 | 22 | 100 |

* Calculated from original data which were reported in percent.

male patients with "pure" rheumatoid or ankylosing spondylitis were studied. These patients, as defined for this study, had pain and stiffness in the back, limited back motion and radiologic evidence of bilateral sacroiliac disease. None of the patients had symptoms or objective findings in the peripheral joints and no rheumatoid nodules were present. The latex fixation test for rheumatoid factor was negative in all 45 individuals.

Starch gel electrophoresis was performed on a freshly obtained serum from each individual according to the technique of Smithies (2). Hemoglobin was added to the serum to facilitate the identification of the haptoglobins. Gels were made in 0.025 M boric acid-0.01 M sodium hydroxide buffer at pH 8.5. The bridge solution consisted of 0.3 M boric acid-0.06 M sodium hydroxide pH 8.3. A voltage gradient of 5 volts per centimeter was applied for 17 hours. After electrophoresis, matching sections of the gel were stained with amido black and with benzidine, then photographed.

*Results.* Of the 45 patients, 10 (22%) had a 1-1 phenotype, 21 (47%) 2-1 and 14 (31%) demonstrated the 2-2 phenotype. The gene frequency was determined using the Hardy-Weinberg equation ($p^2 + 2pq + q^2$) (7) and was found to be 0.456 for p(1-1) and 0.544 for q(2-2). The calculated expected phenotypic frequencies (Table I) showed a good correlation with the observed frequencies.

The haptoglobin frequencies were then compared by chi square analysis with those found in 200 Caucasian individuals in Seattle (8) and with the one reported series of 35 patients with peripheral rheumatoid arthritis (9) (Table II). The chi square value comparing the phenotypes of the rheumatoid spondylitics with the controls was 3.516 while that obtained when the phenotypes of the spondylitics were compared with those of the patients with peripheral rheumatoid arthritis was 4.972. With 2 degrees of freedom, $p = 0.135\text{-}0.223$ for the former and 0.082-0.135 for the latter.

*Discussion.* Increasing numbers of papers are appearing on the chemistry, genetics and geographic distribution of serum haptoglobins. Although it is clear that the exact mechanisms of haptoglobin inheritance have not been determined (*i.e.,* does a third allele exist(5) or is there a control gene that activates or represses structural gene activity (10)), it would appear that for population studies a search for 1-1, 2-1, 2-2 phenotypes as well as ahaptoglobinemic individuals would be reasonable.

In the studies carried out to date, variations in haptoglobin phenotype have been reported in a number of population groups (8), although there has been consistency in

haptoglobin frequencies among Caucasian populations in various parts of the world. In general, the 1-1 type frequency has been in the 10-18% range. In the present study the control group 1-1 frequency was 12% while that of the patients with rheumatoid spondylitis was increased to 22%. There is roughly a 10-20% probability that this is a chance correlation and therefore this difference cannot be called significant. Almost the same figures pertain to the comparison of the patients with rheumatoid arthritis and rheumatoid spondylitis; that is, the latter have an increased frequency of 1-1 phenotype, decreased 2-1 and increased 2-2 phenotype. The p value here is roughly at the 10% level.

Studies of haptoglobins in disease have generally been concerned with states of red blood cell destruction during which the haptoglobin level is known to be diminished(8) or studies of chronic inflammatory disorders in which increases in amount of circulating haptoglobin have been documented(9,11). There are few investigations other than that on rheumatoid arthritis which have attempted to relate haptoglobin phenotypes to specific diseases. Studies of diabetic patients in Canada(12) and of hemophiliac patients in Scotland(13) have not demonstrated correlation with haptoglobin phenotypes. In the present study, the borderline significance found suggests that further evaluation of large populations could ultimately demonstrate significant correlations. It is also likely that haptoglobin subtyping utilizing the method of cleaving the polypeptide chain of the native haptoglobin would be of interest (5).

*Summary.* It was found that in 45 patients with rheumatoid spondylitis, 22% had a 1-1 haptoglobin type, 47% a 2-1 and 31% a 2-2 phenotype. These results were compared with published phenotypes of a normal population and a population with peripheral rheumatoid arthritis. Differences of borderline significance were observed.

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## Differences in Antibody Response of Rabbit to Enterobacterial Antigen After Intravenous and Subcutaneous Injection with Adjuvant.* (28775)

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Recently, Kunin *et al*(1) described an antigen common to many species of enteric bacteria. It was shown that antisera against *E. coli* 014 and a few other serogroups cause agglutination of red blood cells modified with this common antigen (CA) from *Escherichia, Aerobacter (Klebsiella), Salmonella, Shigella,* and *Proteus.* CA has several unusual fea-

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