pears to be the only known property of endotoxin to which tolerance does not develop. All other effects lead to a diminution of response, *i.e.,* tolerance, on repeated administration of endotoxin. It then appears that endotoxin may be a mixture of at least 2 substances, one producing effects to which tolerance can be produced and the other causing an increase of resistance to infection to which tolerance does not develop. An alternative explanation, which would retain the concept of endotoxin as a single entity, would ascribe the tolerance-producing effects to a common biological action (such as release of a neurohormone) and account for the resistance increasing property by a different mechanism.

Considerable work is being carried out with the aim of producing endotoxin that would increase resistance to infections without possessing other less desirable properties, such as pyrogenicity, lethality, and ability to produce the Shwartzman phenomenon(5,6,7, 8). All these studies have attempted to modify endotoxin chemically. The results reported here suggest that endotoxin may not be a single entity and that attempts to isolate from it the resistance-increasing substance and the tolerance-producing substance may hold greater promise of success than chemical modification of the complex macromolecule.

Summary. The ability of endotoxin to increase the resistance of animals to infection is maintained or increased after repeated administration of endotoxin. This finding contrasts with the development of tolerance to the other effects of endotoxin, such as the diminution or disappearance of the lethal and granulopenic effect of endotoxin on repeated administration. These results suggest that endotoxin may be a mixture of at least two substances, one responsible for the effects to which tolerance is produced and another responsible for the increase in resistance to infections. It is also possible that these divergent effects may be mediated through two different mechanisms. The consequences following from these conclusions are briefly discussed.

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Age Changes in the Plasma Glycoproteins of the Mongolian Gerbil.* (28797)

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Certain unique characteristics of the Mongolian gerbil, *Meriones unguiculatus,* have prompted a number of investigations on the blood of this animal. For example, serum cholesterol levels in the gerbil are easily increased by dietary factors without, however, resulting in any significant atherosclerotic plaque formation(1,2,3). Normal plasma protein values covering the post-natal lifespan of the gerbil (through 28 months of age) have been reported by Ringle and Dellenback(4) using a bromophenol blue staining technique. In view of the importance of glycoprotein values in estimating physiologi-

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cal alterations and disease states(5,6,7,8), age-related changes in plasma glycoproteins were therefore investigated in the Mongolian gerbil. Since plasma glycoprotein values correlated with animal age have not previously been reported for the gerbil (in fact, detailed studies of this type are lacking for almost all species), the values presented here provide a set of normal glycoprotein values for future comparisons.

Methods. Animals were obtained from Tumblebrook Farms or were bred from this original stock. Housing, diet, bleeding and electrophoretic techniques were as previously described(4). Plasma samples (0.02 ml) were applied to paper strips (S & S 2043 A mgl) and run for 18-19 hours at 3.0 ma using a model R Beckman/Spinco paper electrophoresis system (veronal buffer pH 8.6, $\mu = 0.075$). After drying the strips for 30 minutes at 120°C, they were wrapped in aluminum foil and stored in a desiccator until the entire series of gerbils had been bled (storage period extending up to 10 months). The strips were then stained following the periodic acid-Schiff method outlined in Beckman/Spinco Technical Bulletin No. TB6046B. Following fuschin staining the strips were scanned with a Photovolt Densicord scanner equipped with an electronic integrator (kindly provided by Dr. Magnus I. Gregersen).

Results. The plasma glycoprotein values found for the different gerbil age groups are summarized in Table I. Values for the various plasma fractions are expressed as percentages of the total plasma glycoproteins. Albumin, alpha$_1$-, alpha$_2$-, beta-, and gamma-globulins (gamma-globulin values include contributions from fibrinogen) for the male gerbil groups were statistically analyzed (t-test) by comparing these fractions in the 3-month-old group with both the 6-week and 12-month groups, and the 28-month group with the 12-month group.

Albumin. Except for an initially low albumin glycoprotein value at birth, a tendency for decreasing percent concentration in this fraction with increasing age is evident within the male groups. An especially significant decrease ($P<0.05$) in the albumin glycopro-

TABLE I. Plasma Glycoprotein Electrophoretic Fractions of Mongolian Gerbils of Known Ages.

Age and sex	No. of animals	Wt (g) mean ± S.D.	Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin + fibrinogen	A/G*	α_1/β
			(%) mean ± S.D.						
1-3 days (undetermined sex)	1		8.1	20.7	37.4	12.9	21.0	.09	1.6
6 wk (♂)	10	27.4 ± 7.4	18.0 ± 4.7	17.0 ± 2.8	32.5 ± 3.3	19.5 ± 5.3	13.0 ± 4.2	.22	.9
3 mo (♂)	7	52.6 ± 5.1	17.7 ± 3.5	16.9 ± 2.7	30.0 ± 3.0	23.2 ± 5.1	12.2 ± 2.6	.21	.7
3 mo (♀)	5	47.5 ± 7.8	19.3 ± 3.0	15.3 ± 2.2	29.5 ± 2.9	23.4 ± 2.7	12.4 ± 2.4	.24	.6
6 mo (♂)	8	60.9 ± 5.6	17.2 ± 2.3	14.2 ± 2.7	30.8 ± 3.0	20.6 ± 3.0	17.2 ± 2.5	.21	.7
6 mo (♀)	5	49.8 ± 4.9	22.5 ± 2.6	12.9 ± 2.4	29.5 ± 2.5	19.2 ± 2.4	15.9 ± 2.6	.29	.7
12 mo (♂)	10	80.0 ± 9.3	16.9 ± 1.8	12.1 ± 2.3†	30.5 ± 3.4	23.5 ± 3.9	17.1 ± 3.8†	.20	.5
28 mo (♂)	10	89.2 ± 7.3	13.8 ± 4.0‡	10.0 ± 3.7	31.5 ± 3.6	25.0 ± 4.4	19.7 ± 4.2	.16	.4

* A/G ratio includes fibrinogen values as part of the gamma globulin.
† $P < .01$ (12 mo groups compared with 3 mo groups).
‡ $P < .05$ (28 mo groups compared with 12 mo groups).

teins occurred between 12 and 28 months (a drop from 16.9 to 13.8%). Comparison of the male and female groups at 3 and 6 months shows the females to have higher albumin glycoprotein values in both age groups.

Alpha$_1$-globulin. This fraction showed a substantial and progressive drop in percent concentration from birth (20.7%) to 28 months (10.0%). In contrast to findings for the albumin fraction, alpha$_1$-globulin glycoprotein values were lower for females than for males at both 3 and 6 months of age.

Alpha$_2$-globulin. Except for initially high values at birth, aging from 6 weeks to 28 months showed relatively little effect on the percent concentration of plasma glycoproteins in this fraction.

Beta-globulin. Although lower values were found in the younger age groups (1-3 days, 6-week) and higher values in the older age groups (12 and 28 months), values for this fraction showed no marked or consistent pattern.

Gamma-globulin + fibrinogen. Following an initial drop in percent composition after birth, glycoprotein values for this fraction increased from 6 weeks of age (13.0%) to 28 months (19.7%). A comparison of the 3-month value (12.2%) with the 12-month value (17.1%) showed a significant increase ($P<0.01$).

Changes in the glycoprotein fractions with age are perhaps more clearly shown by a consideration of alterations in albumin/globulin ratios. Maximum male albumin/globulin ratios were observed at 6 weeks (0.22), whereas the highest female ratios were found at 6 months (0.29). A comparison of alpha$_1$-globulin/beta-globulin ratios with age changes reveals a distinct pattern in glycoprotein shifts from birth to 28 months of age, a progressive decrease from 1.6 to 0.4.

Discussion. Plasma glycoprotein values reported for adult male gerbils (Table I) are not unlike those reported for sera of normal human subjects (in percentages; albumin, 14.3; alpha$_1$-globulin, 18.4; alpha$_2$-globulin, 29.8; beta-globulin, 20.5; and gamma-globulin, 16.7)(9). Somewhat different percent values for adult human serum glycoprotein fractions have been reported by Wakamatsu (7): albumin, 15.1; alpha$_1$-globulin, 11.5; alpha$_2$-globulin, 27.0; beta-globulin, 24.4; and gamma-globulin, 22.1. In agreement with gerbil glycoprotein values reported here, Wakamatsu(7) noted a percentage decrease in albumin glycoproteins and stable alpha$_2$-glycoprotein values with increasing age in humans. In contradistinction to gerbil plasma glycoproteins, however, an increase in alpha$_1$-globulin and stable gamma-globulin glycoprotein values with increasing age was noted. These differences may be species-related.

Comparison of plasma glycoproteins (Table I) with bromophenol blue-stained plasma proteins(4) in the gerbil shows some similarities as well as some obvious differences in percent changes during aging. In general, the results for glycoprotein values were more variable than results for bromophenol blue-stained proteins (note standard deviations). Thus the relatively modest age-related changes in alpha$_2$- and beta-globulins showed a more definite pattern in bromophenol blue-stained proteins than in the glycoproteins. Alpha$_1$-globulin values which were practically constant for bromophenol blue-stained plasma proteins showed a very marked change in glycoprotein content with age: a steady decrease from birth (20.7%) to 28 months of age (10.0%). Gamma-globulin and albumin fraction changes were generally similar for both staining methods. In the case of female gerbils, however, albumin glycoproteins were generally higher than for male gerbils of the same age group, particularly at 6 months. This sex difference was not seen in bromophenol blue-stained plasma proteins(4). Since the age of 3-6 months coincides with that at which gerbils can begin to breed, it is possible that this apparent rise in albumin glycoproteins in the female may be somehow related to their capacity to bear offspring.

Surprisingly, results reported by Peterson *et al*(8) for pregnant and non-pregnant female hamsters failed to show any glycoproteins in the albumin fraction. Other investigators, however, have reported the occurrence of substantial quantities of glycoproteins in the albumin fractions of various or-

ganisms(6,7,9,10). Further studies should be conducted on the gerbil as well as on other animals to establish age-related modifications in glycoprotein values both in pregnant and non-pregnant females, as well as in males. Since plasma proteins high in carbohydrate content have been found in the embryos of various animals(11), it may be that such proteins are produced by the female for subsequent transfer to developing embryos.

Summary. Age-related changes in the plasma glycoproteins of the Mongolian gerbil *(Meriones unguiculatus)* were studied in animals ranging in age from 1-3 days to 28 months. Analyses were performed using paper electrophoresis and periodic acid-Schiff staining, and glycoprotein percent composition values were calculated for albumin, $alpha_1$-, $alpha_2$-, beta- and gamma-globulin fractions. $Alpha_1$-/beta-globulin glycoprotein ratios decreased with increasing age. Least affected by animal aging were the $alpha_2$- and beta-globulin fractions, whereas the greatest percent changes were shown by $alpha_1$-globulin, which steadily decreased with increasing age. All five plasma fractions contained significant quantities of glycoprotein material.

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Esterification of Free Cholesterol and Hydrolysis of Cholesterol Oleate by Acetone Powder Extracts of Hog Adrenals.* (28798)

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Dailey *et al*(1) failed to observe either esterification of free cholesterol-4-C^{14} or hydrolysis of cholesterol-4-C^{14} esters when they added either one to a hog adrenal gland homogenate preparation in which they were able to show conversion of cholesterol-4-C^{14} to steroids. In this system the endogenous esterified cholesterol remained constant during the steroid synthesis. One explanation offered for these findings was that hog adrenals lacked a cholesterol esterase system. In subsequent studies(2,3) these workers found that homogenates of dog adrenals converted free cholesterol-C^{14} to cholesterol esters and labeled cholesterol esters to free cholesterol under conditions in which these substrates were incorporated into steroids. Dailey *et al* (2,3) stated that the hog adrenal has a much lower ratio of esterified to free cholesterol than does the dog adrenal, and suggested that a relationship might exist between that ratio and the capacity of the adrenal gland of a particular species to interconvert free and esterified cholesterol.

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