addition of 25 μg/ml to the first overlay medium.

*Results.* All viruses except coryzaviruses 11 and 20 and rhinoviruses 41 and 48 showed macroplaques of 2 mm or more in diameter without the incorporation of DEAE dextran. While the addition of DEAE dextran increased the diameter of the above 4 viruses from 1 mm to 2-3 mm, it did not affect plaque size of the other viruses. No increase in number of plaques (titer increase) of any of the 16 viruses tested was observed by addition of DEAE dextran. Table I shows plaque diameter, PFU and $\log_{10}$ $TCD_{50}$ (in WI-38 tubes) of these viruses. Photographs of plaques formed by coryzaviruses types 22 and 25 and rhinoviruses types 42 and 45 originally described by the Merck group are illustrated in Fig. 1.

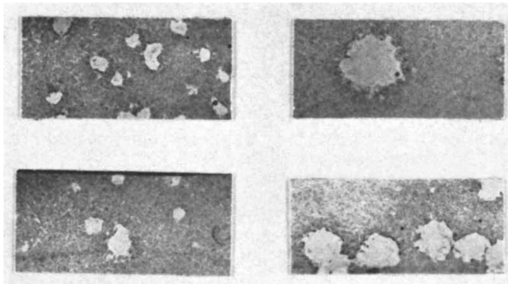

FIG. 1. Plaques of rhinoviruses in WI-38 cells. Magnification: 1×. All plaques were stained by a second overlay containing neutral red (see text). Upper left: Coryzavirus 22; upper right: coryzavirus 25. Lower left: rhinovirus 42; lower right: rhinovirus 45.

*Discussion and conclusions.* The lack of a uniform and reproducible plaque assay for rhinoviruses (now totaling about one hundred types) has handicapped characterization of these important human pathogenic agents. Different procedures involving variations in the optimal cell culture, composition of the overlay medium and incorporation of anti-inhibitor and/or enhancing substances (*e.g.*, DEAE dextran and $Mg^{++}$ ions) have been described by various workers. In an attempt to develop a simple and reproducible procedure for plaquing a substantial number of rhinoviruses, we found the following conditions to be optimal for plaque formation by all 16 rhinoviruses that have been tested: (1) fully grown WI-38 cell monolayers in prescription bottles; (2) an overlay medium consisting of HCAEM containing 10% newborn calf serum and 0.7% Ionagar No. 2; (3) double agar overlay technique with the second overlay (containing neutral red) added 6 days after the first; and (4) incorporation of 25 μg/ml of DEAE dextran to the first overlay medium as an additional means to improve plaque formation.

1. Porterfield, J. S., Nature, 1962, v194, 1044.
2. Behbehani, A. M., Lee, L. H., J. Bact., 1964, v88, 1608.
3. Webb, P. A., Johnson, K. M., Mufson, M. A., Proc. Soc. Exp. Biol. & Med., 1964, v116, 845.
4. Fiala, M., Kenny, G. E., J. Bact., 1966, v92, 1710.
5. Ketler, A., Hamparian, V. V., Hilleman, M. R., Proc. Soc. Exp. Biol. & Med., 1962, v110, 821.
6. ———, ibid., 1964, v116, 976.
7. Wenner, H. A., Classification of Rhinoviruses, First International Conference on Vaccines against Viral and Rickettsial Diseases of Man, Pan American Health Organization, Washington, D. C., May, 1967, 94; see also Nature, 1967, v213, 761.
8. Klinger, E. R., Chapin, M., Dubes, G. R., Proc. Soc. Exp. Biol. & Med., 1959, v101, 829.

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## An Androgenic Basis for the Sexual Difference in L-Ascorbic Acid Biosynthesis in Rats.* (32478)

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A sexual difference in hepatic enzymic activities involved in biosynthesis of ascorbic acid, and in concentrations of total ascorbate in a number of tissues from male and female

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rats has been previously reported(3). It was concluded that the higher enzymic activities in male liver, at the subcellular level, might be the molecular basis for the increased ascorbate concentrations observed at the tissue level. If this conclusion is a valid explanation of the higher ascorbate levels in males, conditions causing diminution of these enzymic activities should result in a similar diminution of the tissue stores of the vitamin. Data obtained from hypophysectomized and castrated animals reported in this paper indicate this to be the case. The results not only give further support to the above conclusion, but also indicate the specific endocrinological basis for the observed sexual differences in ascorbate synthesis and storage.

*Materials and methods.* Mature male and female rats of the Sprague-Dawley strain were used. Operations (hypophysectomy and castration) were performed by the Charles River Breeding Laboratories. All animals were given Purina Laboratory Chow and tap water *ad libitum*. In addition, hypophysectomized animals were supplemented with a 5% glucose solution for at least one week following surgery.

Enzymic assay conditions were essentially as reported previously(2). The two hydrolases, gulonolactone hydrolase (formerly aldonolactonase) and glucuronolactone hydrolase (formerly uronolactonase), were assayed manometrically in Gilmont differential syringe manometers. Evolution of $CO_2$ following hydrolysis of the appropriate lactone to its free acid in the presence of bicarbonate buffer was taken as a measure of the reaction rate. The activity of gulonate NADP oxidoreductase (formerly TPN-L-hexonate dehydrogenase) in reducing glucuronate to gulonate was recorded as the change in optical density (340 mμ) as $NADPH_2$ was oxidized to NADP. The activity of gulonolactone $O_2$ oxidoreductase (formerly gulonolactone oxidase) was determined from oxidation of gulonolactone to ascorbic acid under an oxygen atmosphere at 37°C for 30 minutes. Control vessels containing known amounts of ascorbic acid showed no loss of the vitamin under the conditions of the assay. It is assumed that the analyses represent complete recovery of the ascorbate synthesized enzymatically. Ascorbate produced in this assay and that present in the tissues was analyzed by a modification of the method of Lowry *et al*(1).

*Results and discussion.* The higher enzymic activities in males could be either the result of a stimulatory effect of androgens in the males, or the result of an inhibitory effect of estrogens or progesterone in the females. Changes in enzymic activities following hypophysectomy favor the former of the two possibilities. Fig. 1 illustrates the enzymic activities in intact and hypophysectomized rats of both sexes. Hypophysectomy caused no significant change in the activities of enzymes from female rats with the exception of a considerable decrease in gulonolactone hydrolase, an enzyme known to be dependent upon somatotrophic hormone(2). In both sexes the activity of this enzyme was markedly reduced following hypophysectomy. On the

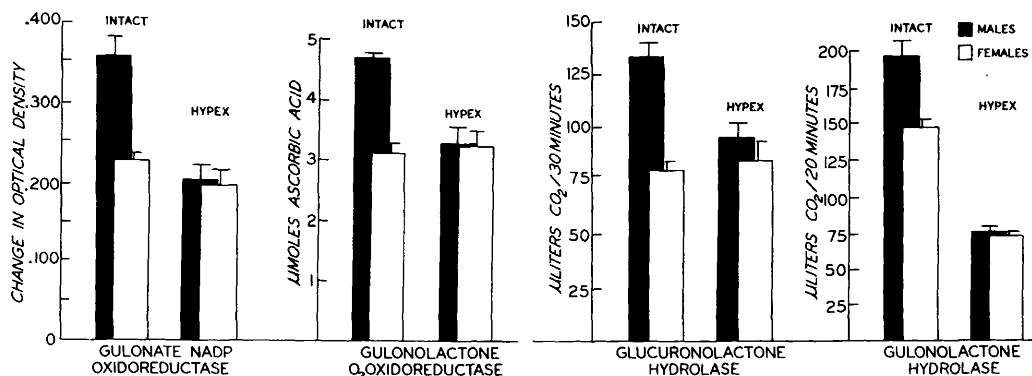


FIG. 1. Comparisons of enzymic activities between intact and hypophysectomized male and female rats. Brackets indicate standard error of the mean.

other hand, the enzymic activities of gulonate NADP oxidoreductase, glucuronolactone hydrolase, and gulonolactone $O_2$ oxidoreductase in hypophysectomized males were significantly diminished to levels characteristic of those found in females ($p < .005$ determined from $t$ test.)

From these data it is concluded that the higher enzymic activities in intact males are androgen dependent, and that the decreased activities following hypophysectomy are the result of decreased secretion of testicular androgens. In order better to demonstrate androgenic requirements, and to exclude the multiple hormonal deficiencies of hypophysectomy, castrated males were studied. Fig. 2

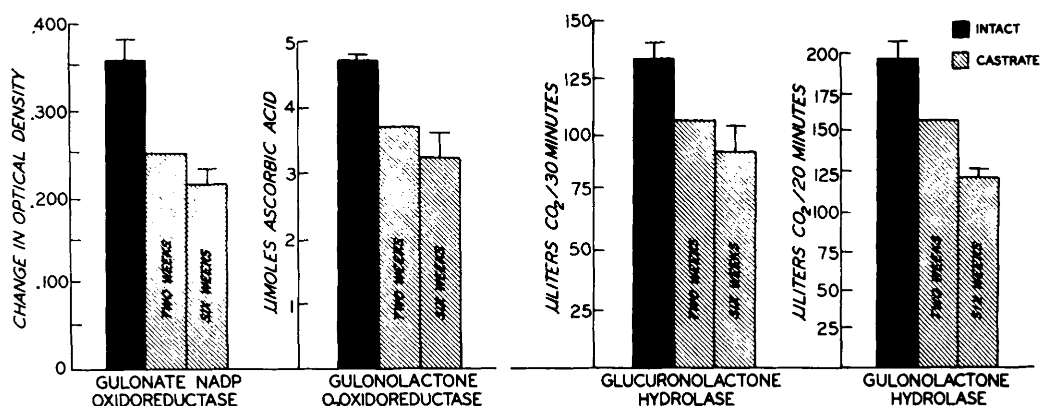


FIG. 2. Comparisons of enzymic activities between intact and castrated male rats. The two groups of castrated animals were operated 2 and 6 weeks before sacrifice. Brackets indicate standard error of the mean.

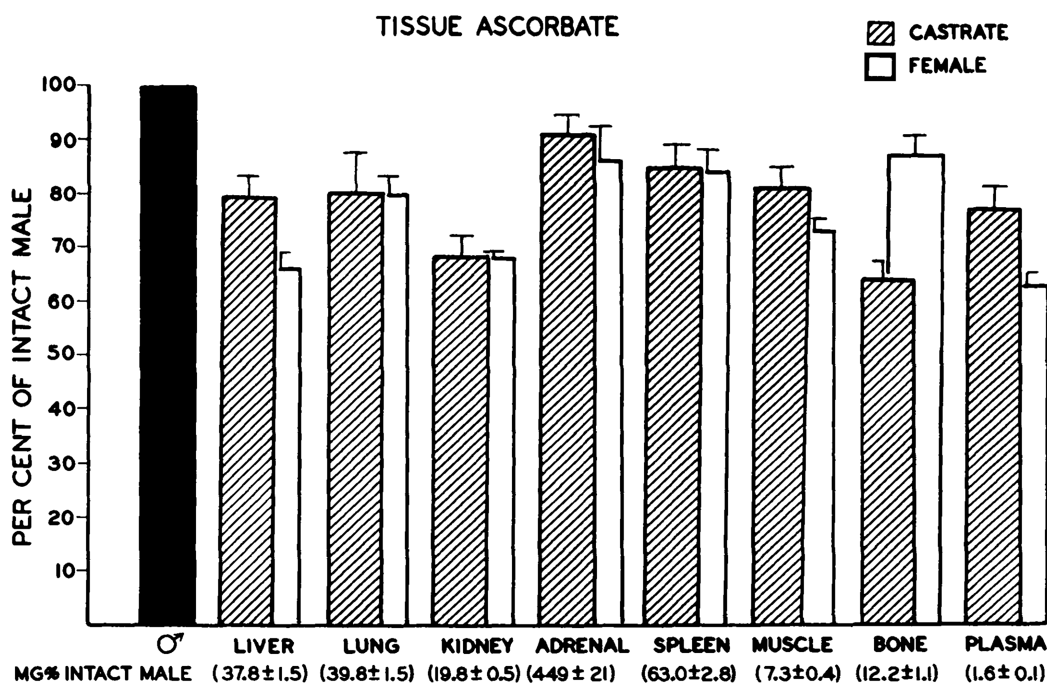


FIG. 3. Tissue ascorbate concentrations of intact females and castrated males relative to intact male levels. Numbers in parentheses are mg of ascorbic acid per 100 g tissue from intact male rats.

illustrates the diminution of enzymic activities following castration for 2 and 6 weeks. The effects of castration are seen to be quite similar to those of hypophysectomy. It is interesting to note that, unlike the marked decrease in activity of gulonolactone hydrolase found in hypophysectomized rats, the decrease in castrated males was of a similar magnitude to that of the other 3 enzymes.

Concomitant with the reduction of male enzymic activities following castration was a decrease in the tissue levels of ascorbic acid. As shown in Fig. 3, male rats castrated for 6 weeks had significantly diminished concentrations of tissue ascorbate which approach, or reach, those typical of females; in one case, *viz.*, bone, the level was even below that in females. Diminished concentrations of ascorbic acid are statistically significant for all tissues with the exception of the adrenals ($p < .05$). These decreased concentrations of tissue ascorbate which were found along with decreased hepatic enzymic activities in castrated males give further support to the hypothesis that tissue levels of ascorbic acid are dependent upon the rate of biosynthesis by the liver. The effects of hypophysectomy and castration clearly indicate a requirement of normal testicular secretion for the maintenance of the typically higher enzymic activities and tissue levels of ascorbic acid in intact males.

*Summary.* Hypophysectomy or castration of male rats results in a diminution of biosynthetic enzymic activities and tissue concentrations of ascorbic acid to levels characteristic of females. The data support the hypothesis that the sexual difference in tissue concentration of ascorbic acid in intact rats is dependent upon a difference in the rate of hepatic biosynthesis. The sexual difference in hepatic enzymic activities is interpreted to be dependent upon testicular secretion of androgens.

1. Lowry, O. H., Bessey, O. A., Burch, H. B., Proc. Soc. Exp. Biol. & Med., 1952, v80, 361.

2. Salomon, L. L., Stubbs, D. W., Biochem. Biophys. Res. Commun., 1961, v5, 349.

3. Stubbs, D. W., McKernan, J. B., Proc. Soc. Exp. Biol. & Med., 1967, v125, 1326.

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## Enteric Bacteria as a Possible Cause of Hemolytic Antibody-Forming Cells in Normal Mouse Spleens.* (32479)

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By the hemolytic plaque assay of Jerne and Nordin(1), 19 S hemolysin producing cells can be individually detected and enumerated (2). With the addition of anti-mouse gamma globulin before addition of complement, the 7 S antibody producing cells can also be detected(3,4). This method can be adapted to study bacterial somatic polysaccharide(5) and other antigens that adhere to sheep red blood cells (SRBC). One perplexing and bothersome aspect of this technique is the variable small number of hemolysin producing "background" plaque-forming cells (B-PFC) against SRBC in the spleens of mice(2), rabbits(5), and humans(6) without prior SRBC injection. These 19 S hemolysin producing B-PFC are also present in small numbers in lymph nodes, thymus(7) and bone marrow(8) as well as following transplantation of isogenic lymphoid tissues to lethally irradiated hosts(9). These B-PFC can be considered as belonging to a clone of cells which produce specific antibodies without prior contact with the antigen according to the clonal selection theory(10). Alternatively, the animal may be responding to other antigens which produce cross-reacting antibodies to SRBC. Naturally occurring hemagglutinins in mouse sera for SRBC and chicken RBC have been described(11). These vary with the strain and sex of mice and seasonally(11,12,

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