increased yields were obtained.

Conclusions. Although at first glance the data may appear rather inconsistent, closer inspection reveals that the results are consistent when consideration is given to the experimental error involved in some of the methods. The variation in the nitrogen determinations of the crude virus suspensions were thought to be due in turn to expected variation in the amount of protein removed in the freezing and thawing steps. Estimations of the degree of purity have been presented in terms of the amount of nitrogen eliminated from the crude sample. Since purified normal brain gave nitrogen values which bore a close similarity to those obtained with purified infected tissue, it can be seen that the so-called purified fractions still contain a large amount of normal brain protein. There is no doubt that the non-viral protein greatly overbalances the viral proteins in the purified fraction. However, a relatively large amount of protein is removed during the procedure, as compared with the amount removed in the ultracentrifugation methods of purification cited by Beard.[1] The activities of crude and purified virus fractions, with a few exceptions, remain fairly constant, indicating that most of the active virus is recovered. Although the titrations may not be accurate enough to determine the exact yield, they indicate that in many of the determinations 100% of the virus appeared to be recovered. The number of infectious units of virus per milligram of nitrogen depends, in part, on the original titer of the virus, and therefore is a relative value. When the methanol precipitate was held at −4°C for 4 hours the yield of virus was greater. Apparently no significant difference resulted from the use of 25% or 30% methanol. Also, the use of buffers at pH 5.1 or pH 5.6 to wash the precipitates did not seem to appreciably alter the results. One step that did result in the reduction of nitrogen was the immediate freezing of the final precipitate at pH 7 before it went into solution. Upon thawing, insoluble protein was removed by centrifugation.

16502 P

Secretion of Fructose and Citric Acid in Transplants of Rat Seminal Vesicle and Coagulating Gland.*

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Earlier investigations[1,2] established fructose and citric acid as secretory products of certain accessory glands of reproduction in several mammalian species; in rats the first is found in the coagulating gland and dorsal prostate, and the latter in seminal vesicle proper and ventral prostate. Further it was demonstrated that the secretion of fructose and citric acid depends upon and is regulated by, the male sex hormone; following castration there was gradual disappearance of both substances though at varying rates, and after treatment with testosterone their level was restored.[3] In the present study more evidence has been accumulated which emphasizes the effect of testosterone upon the accessory reproductive organs as regards their ability to produce fructose and citric acid. Using subcutaneous

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1 Mann, T., *Biochem. J.*, 1946, **40**, 481.

2 Humphrey, G. F., and Mann, T., *Nature*, London, 1948, **161**, 352.

3 Mann, T., and Parsons, U., *Nature*, London, 1947, **160**, 294.

TABLE I.
Fructose and Citric Acid in Transplants of Combined Tissues of Seminal Vesicle and Coagulating Gland.

Male rats, I and II, implanted at the age of 50 days with 15-day-old donor tissue, autopsied 96 days later.

Rat	Wt of tissue (seminal vesicle plus coagulating gland) mg		Fructose, mg%		Citric acid, mg%	
	I	II	I	II	I	II
Organs of host	1210	1329	68	49	58	36
Transplants	1450	1400	43	24	17	60

TABLE II.
Fructose in Transplants of Coagulating Gland.

Donor, days of age	Male host, days of age at		Fructose	
	implantation	autopsy	Coagulating gland of host, mg%	Transplant of coag. gland, mg%
40	40	67	140	147
50	70	107	138	168
47	150	177	200	44

transplants of seminal vesicle and coagulating gland tissue in rats, it was shown that such transplants are capable of secreting fructose and citric acid in response to testosterone propionate, in complete anatomical separation from the rest of the reproductive tract.

The following groups of experimental animals were employed: A. 20 normal control male animals in which the content of fructose and citric acid in the seminal vesicle and coagulating gland was determined in relation to age; B. 30 male and female rats were used for transplantation experiments as hosts—normal, gonadectomized, or gonadectomized and treated with testosterone propionate (200 μg daily). The subcutaneous transplantation technic employed was that previously developed for the cytological study of rat accessory organs.[4,5] Portions of coagulating gland alone or together with seminal vesicle tissue (1-3 mg) from donors aged 15 to 50 days, were inserted into subcutaneous sites on the abdominal wall of the hosts aged 30 to 115 days. They were allowed to grow in the hosts for 16 to 96 days; then they were dissected, weighed and analyzed; for comparison the reproductive organs of the host were also assayed for fructose and citric acid.

Results. A. The accessory organs of reproduction in very young rats such as routinely used as donors, contained low quantities of fructose and citric acid; from about 40 days onward, *i.e.*, shortly before maturation of sperm, the content increased and reached a high level at 3 months of age when spermatozoa were fully active. For example, coagulating gland at 40 days had 40 mg % or 5 μg/organ, at 85 days 166 mg % or 250 μg/organ, of fructose; seminal vesicles at 40 days contained 10 mg % or 10 μg/organ, at 85 days 44 mg % or 300 μg/organ, of citric acid. These data are based in each case upon an analysis of a sample representing an aggregate of gland tissue. Glands from 8 rats were used at 40 days; from 5 rats at 85 days.

B. In transplants of combined coagulating gland plus seminal vesicle tissue, both fructose and citric acid were present (Table I), in those from coagulating gland alone only fructose was found but citric acid was absent (Table II). Coagulating gland transplants in castrated males treated with testosterone propionate for several weeks showed 144 mg %, the host coagulating glands 244 mg %, fructose; however, cessation of hormone treatment for 3 weeks caused a decline in grafts to 12 mg %, in host tissue to 5 mg %. In

[4] Price, D., *Physiol. Zool.*, 1941, **14**, 145.
[5] Price, D., *Anat. Rec.*, 1942, **82**, 93.

spayed or non-spayed females injected with testosterone propionate, coagulating gland transplants contained 102 to 191 mg % fructose; here too, the level in the transplants fell to 3 to 12 mg % fructose when testosterone was withheld for 3 weeks.

Summary. Subcutaneous transplants of coagulating gland and seminal vesicle in rats, were capable of producing fructose and citric acid when grown a) in normal males, b) castrated testosterone-treated males, and c) testosterone-treated non-spayed or spayed females.

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Therapeutic Activity of Bacitracin in Rabbit Syphilis, and Its Synergistic Action with Penicillin.*

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Bacitracin is an antibiotic derived from culture filtrates of certain strains of *Bacillus subtilis*, and first described by Johnson, Anker, and Meleney.[1] As shown in a previous concentration from this laboratory,[2] it has a low renal clearance which approximates the glomerular filtration rate, in contrast to penicillin which has a renal clearance approximating the total renal plasma flow.[3] In consequence, bacitracin is excreted more slowly than penicillin, the rate of excretion remaining at a reasonably constant level for a period of several hours; and the blood level also falls more slowly than does that of penicillin G injected in similar gravimetric dosage.

In vitro, bacitracin has been shown to be actively treponemicidal against the cultured Reiter strain of *Treponema pallidum*.[4] Concentrations of 0.004 units per cc inhibited the growth of the organisms, and 0.1 unit per cc killed 99.9% in 48 hours. Against the pathogenic *T. pallidum in vivo*, doses of 72 units/kg given intramuscularly caused the temporary disappearance of the organisms from rabbit testicular chancres.[4] It may be noted that in both rabbits and man, this dosage provides plasma concentrations in excess of 0.1 unit per cc for approximately 2 to 3 hours, and in excess of 0.01 unit per cc for approximately 6 to 8 hours.[2] This period of exposure was apparently enough to render the chancre temporarily dark-field negative, but was not enough to kill all the organisms. The organisms regularly reappeared in the lesions days, weeks or even months after the injection; and it required single doses of 5,000 units/kg and more to effect the permanent healing of the primary lesion.

The present paper is a report on the therapeutic activity of bacitracin in rabbit syphilis when aqueous solutions were injected intramuscularly once daily for 4 days. Further, data are given which suggest a remarkable synergistic action between penicillin and

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1 Johnson, B., Anker, H., and Meleney, F., *Science*, 1945, **102**, 376.

2 Eagle, H., Newman, E. V., Greif, R., Burkholder, L. M., and Goodman, S. C., *J. Clin. Invest.*, 1947, **26**, 919.

3 Eagle, H., and Newman, E., *J. Clin. Invest.*, 1947, **26**, 903.

4 Eagle, H., Musselman, A. D., and Fleischman, R., *J. Bact.*, 1948, **55**, 347.