amounts. It will be observed that in most instances the bulk of the PAB produced is found in the culture medium. Few of the species retain any great amount of the PAB in their cells. The quantity of PAB made by bacteria appears to be variable since it was observed that strains of a given species cultured under apparently identical conditions did not necessarily yield the same amount of PAB. These results are, therefore, only indicative of the ability of bacteria to elaborate PAB and are not intended to convey any quantitative comparison of various organisms.

The present significance of PAB synthesis by microörganisms is twofold. (1) It is additional evidence for a general biological phenomenon observed in the case of other growth factors, namely, synthesis by bacteria of vitamins when grown in a medium free of such compounds. (2) It has been suggested that the "sensitivity" of a bacterium to sulfonamides would depend, at least in part, upon whether it could synthesize PAB readily or not.† An organism with little synthetic ability would be more sensitive to sulfonamides than one with greater synthetic powers.[2,3] The finding that bacteria synthesize PAB in variable degree appears to make this suggestion a definite possibility. In any case it is likely that the PAB synthesis by a given organism would play some part in the degree of its resistance to sulfonamide action.

That PAB is generally important in bacterial metabolism is indicated by the fact that the organisms tested, of many diverse species, all‡ synthesized PAB. It is not likely that so many unrelated types of organisms would all make this compound unless it is an "essential metabolite" as defined by Fildes. It is likely that PAB is synthesized by many other organisms as well, organisms for which we have been unable to devise suitable PAB-free culture media for test.

Summary. Representative cultures of many bacterial genera were grown in culture media of known composition free of *p*-aminobenzoic acid. Culture filtrates and hydrolized whole cultures were assayed for *p*-aminobenzoic acid content employing the *Acetobacter suboxydans* microbiological method. All organisms successfully cultured in the *p*-aminobenzoic acid free media elaborated *p*-aminobenzoic acid in readily measurable amounts, indicating that *p*-aminobenzoic acid is truly an "essential metabolite" synthesized by many bacteria.

† This association would appear to have been established in at least one instance. We have observed[17] that sulfonamide resistant *Staphylococcus aureus* cultures synthesize far more (approximately 70 times as much) PAB than their parent (susceptible) strains. On the other hand, we could not detect any significant difference in PAB production by sulfonamide susceptible and resistant strains of *Escherichia coli, Shigella dysenteriæ, Vibrio choleræ* and *Diplococcus pneumoniæ,* Types I, II and III.[18]

[17] Landy, M., Larkum, N. W., Oswald, E. J., and Streightoff, F., *Science,* 1943, **97**, 265.

[18] Landy, M., Larkum, N. W., Oswald, E. J., and Streightoff, F., unpublished data.

‡ See footnote † to Table II.

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Influence of Pregneninolone and Pregnenolone on Spermatogenesis in Hypophysectomized Adult Rats.*

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An androgenic action is ascribed to the steroid pregneninolone on the basis of its ability to increase the size of the capon's comb,[1] to stimulate the preputial glands and clitoris of the female rat,[2] to increase the weight of the seminal vesicles, prostates and coagulating glands of both the prepubertal and adult castrate rat[3] and to cause precocious

assumption of male characters in Lebistes.[4] The maintenance of spermatogenesis with androgens following hypophysectomy is well established[5-7] and it was deemed of interest to test pregneninolone in hypophysectomized male rats to ascertain the action on the reproductive system.

Another steroid, pregnenolone, though shown by Selye and Albert[8] to be practically inactive as an androgen, in both the immature and adult castrate rat, was included in this investigation because it was felt that this substance might have a gonadotropic effect. Previous investigations have demonstrated that pregnenolone will inhibit testicular atrophy induced by both testosterone and estradiol[9,10] and recently Selye[11] indicated but presented no data that this substance prevented testicular atrophy following hypophysectomy.

Adult male rats ranging from 120 to 200 days in age were hypophysectomized and treated for a 20-day period starting the day of operation. All rats were autopsied on the 21st day, and organ weights of fresh tissues were taken. Seminal vesicle weight included the coagulating gland and any contained fluid. All pituitary capsules were serially sectioned, stained with Mallory's connective tissue stain and examined microscopically. Data from only completely hypophysectomized rats are presented.

Pregneninolone (Δ^4-Pregnene-in-20-ol-17-one-3) was initially tested by implanting subcutaneously, on the day of operation, 2 pellets of crystalline hormone weighing 20 mg each. After 20 days the recovered pellets weighed 18.0 to 18.5 mg indicating only slight absorption. The testes from the 3 rats were markedly atrophic and spermatozoa were absent.

In view of the small amount of hormone absorbed from the pellets, the steroid was then tested by the daily subcutaneous injection of 10 mg suspended in 0.2 cc of peanut oil. This was accomplished with some difficulty and usually involved the addition of another 0.2 cc of oil in an attempt to obtain all of the hormone from the ampul. After a 20-day injection period the testes had regressed virtually to control weight and the seminiferous tubules were devoid of spermatozoa. It should be noted that seminal vesicle weights are greater than those of the operated control rats, but this can only be suggestive of stimulation because the number of rats tested was small (Table I).

Pregnenolone (Δ^5-Pregnene-ol-3-one-20) was also administered to hypophysectomized rats as a daily subcutaneous injection of 10 mg suspended in 0.2 cc of peanut oil. Testis weight was well maintained during the 20-day test period, being more nearly comparable to that of the normal rat than of the hypophysectomized control rat (Table I). Seminiferous tubules were large and spermatozoa were numerous. However, desquamated cells were observed in the lumina of numerous tubules suggesting that maintenance of spermatogenesis was not completely normal. Interstitial tissue was atrophic.

Pregnenolone was also tested by injecting 10 mg subcutaneously every other day. The administration of this 100 mg total dosage did not maintain testis weight quite as well as the 200 mg dosage but spermatogenesis was maintained, spermatozoa being numerous.

In view of the marked effect of pregnenolone on the testis it was interesting to note that seminal vesicle weight was not influenced by this steroid (Table I). Furthermore, the typical loss in adrenal and thyroid weight resulting after hypophysectomy was not influenced by the injection of either steroid.

The disappearance of the adrenal X-zone

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10 Albert, S., and Selye, H., *J. Pharm. Exp. Therap.*, 1942, **75**, 308.

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TABLE I.
Influence of Steroids on Hypophysectomized Male Rats.

Treatment total Dosage, mg	No. of rats	Body wt., g st.,	Weight end, g	Avg organ wt (mg) Testis	Sem. ves.	Thyroid	Adr.
			Pregneninolone.				
40	3	305	232	887	203	14.8	9.6
200	3	276	207	901	249	13.0	5.2
			Pregnenolone.				
100	3	274	222	1645	145	14.3	6.8
200	6	303	242	1944	151	16.0	6.8
Operated controls	6	281	200	708	133	13.7	7.2
Normal controls	6	240	277	2723	813	25.9	28.2

of the mouse can readily be produced with testosterone propionate. Indeed, Starkey and Schmidt[12] have shown that a single injection of 0.4 mg of this hormone will cause the disappearance of the X-zone at the 6th day. To further test pregnenolone for androgenic activity, eleven 25-day-old female mice were spayed and tested 7 days later. Three mice received a single subcutaneous injection of 10 mg of pregnenolone, 4 mice received 5 mg and 4 mice served as controls (caged separately). All mice were autopsied 10 days later and the weights of the uteri, adrenals and kidneys were nearly identical with those of the control mice. Furthermore, the adrenal X-zone was present and large in all mice indicating the absence of androgenic activity under the experimental conditions cited.

Summary. Pregnenolone is capable of maintaining spermatogenesis for at least 20 days following hypophysectomy. However, this steroid does not influence the seminal vesicles, since the loss in seminal vesicle weight simulates that observed in the hypophysectomized control. Pregnenolone therefore appears to be gametogenic in action.

A single injection (10 mg) of pregnenolone did not influence uterine weight or the adrenal X-zone of the spayed mouse.

Pregneninolone, which has been shown to exhibit androgenic activity, failed to maintain spermatogenesis in the hypophysectomized rat.

[12] Starkey, W. F., and Schmidt, E. C. H., *Endocrinology*, 1938, **23**, 339.

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Vitamin A Absorption in Infantile Eczema.

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It is commonly observed that while most infants with eczema are overnourished, a few intractable cases appear to be underdeveloped and underweight. In most instances, this malnutrition is probably due to prolonged secondary infections, but occasionally this lack of growth and development seems to be primary and perhaps associated, according to some authors, with a disturbance in the physiology of gastro-intestinal tract.

During a period of several months, 4 intractable cases were seen in this hospital. The course of each was characterized by (a) retarded development, (b) malnutrition, (c) severe and more or less generalized eczema (d) pronounced lymphadenopathy (e) marked eosinophilia (between 25% and 35%), (f) protracted respiratory infections with frequent episodes of broncho-pneumonia (low grade and present for several months in 2 cases), and finally (g) lack of response to topical and dietetic treatment.