however, that the eosinophiles were increased in the 2 patients with elevated blood histamine (31 and 32), although the analysis of the remainder of the data shows little, if any, correlation between blood histamine and the number of eosinophiles. The data also show no constant relationship between the number of neutrophiles and blood histamine. As further evidence may be cited a patient whose blood histamine was 8.8 γ with a white blood cell count of 5,480; after an injection of typhoid vaccine, resulting in an increase of the white cells to 12,650, the blood histamine dropped to 3.7 γ.

Summary. Blood histamine is within normal limits in patients with gastric carcinoma and with peptic ulcer. There is no relationship between blood histamine and the presence or absence of free hydrochloric acid in the stomach.

Blood histamine in man is carried chiefly by granulocytes, but is not directly related to the number of granulocytes in the blood.

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Bacterial Synthesis of *p*-Aminobenzoic Acid.

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Fildes[1] and Woods[2] have postulated that the sulfonamide drugs prevent bacterial growth by interfering with the utilization of an "essential metabolite,"* *p*-aminobenzoic acid (PAB), by virtue of their similarity in structure to this compound. At the time this theory was proposed it was assumed that PAB was an essential metabolite for bacteria, although for technical reasons, it had not yet been isolated from them, nor had it been proved to be a growth factor in the absence of a bacterium which could not synthesize it. Recent investigations have done much to substantiate Fildes' conception of the role of PAB in sulfonamide bacteriostasis.[3,4] PAB has been found to be a growth essential for *Clostridium acetobutylicum*,[5] *Acetobacter suboxydans*[6] and evidence now indicates that it may be a growth factor for certain Lactobacilli[7,8] and Corynebacteria[9] as well.

If PAB is truly an essential metabolite, the fact that it is a growth factor for a few organisms should be supplemented by evidence that it is important in the nutrition of bacteria generally, namely proof of its synthesis by organisms able to grow in PAB-free culture media. Analogy to other growth factors indicates that this is a situation which might well exist. It has been repeatedly observed that while a few organisms may require the presence of growth factors, others (the majority) usually synthesize these vitamins in apprecia-

1 Fildes, P., *Lancet*, 1940, **1**, 955.

2 Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

* According to Fildes[1] an essential metabolite is a substance which takes an essential part in a chain of syntheses necessary for bacterial growth. A "growth factor" which must be supplied in the nutrients is an essential metabolite which the cell cannot synthesize, *e.g.*, nicotinic acid is an essential metabolite for all bacteria but a growth factor for only a few.

3 Green, H. N., and Bielschowsky, F., *Brit. J. Exp. Path.*, 1942, **23**, 1.

4 McIlwain, H., *Brit. J. Exp. Path.*, 1942, **23**, 265.

5 Rubbo, S. D., Maxwell, M., Fairbridge, R. A., and Gillespie, J. M., *Austral. J. Exp. Biol. and Med.*, 1941, **19**, 185.

6 Lampen, J. O., Underkofler, L. A., and Peterson, W. H., *J. Biol. Chem.*, 1942, **146**, 277.

7 Isbell, H., *J. Biol. Chem.*, 1942, **144**, 567.

8 Lewis, J. C., *J. Biol. Chem.*, 1942, **146**, 441.

9 Chattaway, F. W., Happold, F. C., Lythgoe, B., Sandford, M., and Todd, A. R., *Biochem. J.*, 1942, **36**, vi.

ble amounts.[10-13]

The importance of PAB in determining the susceptibility or resistance of bacteria to sulfonamide bacteriostasis, together with the recent availability of an adequate microbiological method for PAB assay[14] led us to investigate its production by bacteria. This report is a summary of data indicating that PAB is, in fact, synthesized by bacteria of many diverse species.

Experimental. The culture medium employed for growth of most of the organisms investigated was synthetic in composition and free of PAB (by test with *A. suboxydans*). The composition of this medium is given in Table I.

TABLE I.
p-Aminobenzoic Acid-Free Culture Medium.

Glucose	1.0	g
Casein hydrolysate, purified	0.5	"
Tryptophane	10	mg
Cystine	10	"
K_2HPO_4	50	"
KH_2PO_4	50	"
$MgSO_4 \cdot 7H_2O$	20	"
NaCl	1	"
$FeSO_4 \cdot 7H_2O$	1	"
$MnSO_4 \cdot 2H_2O$	1	"
Adenine sulfate	500	μg
Thiamine	1	"
Folic acid	0.5	"
Biotin	0.5	"
Riboflavin	20	"
Calcium pantothenate	20	"
Nicotinic acid	20	"
Pyridoxine	40	"
Distilled water to pH adjusted to 7.5	100	cc

As will be indicated below, other culture media were required in special instances; however, most of the tests were made with this medium.

Since the streptococci and pneumococci grew poorly, or failed to grow, in the above medium (Table I), it was necessary to use a special procedure for their cultivation. A medium which was satisfactory in supporting their growth and at the same time free of PAB was prepared by treating a 2% solution of Bacto Tryptose with Darco G-60 charcoal (2% by weight of Tryptose solution) at pH 5.0, bringing to a boil and filtering while hot.[15] The charcoal treated tryptose solution was supplemented as follows:

TTV Medium (Treated Tryptose and Vitamins).

2% Tryptose solution, charcoal treated	100	cc
Glucose	.1	g
Sodium thioglycollate	.01	"
Tryptophane	.005	"
Thiamine	10	μg
Riboflavin	25	"
Calcium pantothenate	25	"
Nicotinic acid	25	"
Pyridoxine	40	"
Biotin	.1	"
Inorganic salts (same as in Table I)		
pH 7.6		

This medium supported growth of streptococci and pneumococci equivalent to that obtainable with meat infusion broth. The anaerobes were cultured in a simplified medium devised by Dr. Sarah E. Stewart of the National Institute of Health. The medium contained purified casein hydrolysate, crystalline vitamins, tryptophane, cystine and pyruvic acid. The pH was adjusted to 7.6.

The organisms listed in Table II were grown in the various culture media indicated and transferred a number of times to minimize the possibility of carrying over any PAB from the original media. The inoculum size was restricted to a minimum. Incubation was at 37°C for 24 hours, except where otherwise indicated. Although it was not possible to obtain optimum growth of all the organisms tested, growth was generally good.

In preparation for PAB assay, half of each culture was filtered through a Seitz filter while the other half was adjusted to pH 5.0 and hydrolyzed by autoclaving at 15 lb pressure for one-half hour. Both filtrate and hydrolysate were adjusted to pH 6.0 and their PAB content determined by microbiological assay. The PAB values for cells given in Table II represents the difference obtained by subtracting the PAB content of the medium from

[10] Snell, E. E., and Strong, F. M., *Enzymologia*, 1939, **6**, 186.

[11] Landy, M., and Dicken, D. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 499.

[12] Burkholder, P. R., and McVeigh, I., *Proc. Nat. Acad. Sci.*, 1942, **28**, 285.

[13] Thompson, R. C., *Univ. Texas Publ.*, 1942, **4237**, 87.

[14] Landy, M., and Dicken, D. M., *J. Biol. Chem.*, 1942, **146**, 109.

[15] MacLeod, C. M., and Mirick, G. S., *J. Bact.*, 1942, **44**, 277.

TABLE II.
Bacterial Synthesis of *p*-Aminobenzoic Acid.

Organism	Medium	Incubation °C	Incubation Hr	*p*-Aminobenzoic acid found (γ per cc culture) In cells	In medium	Total
Salmonella paratyphi	Synthetic (Table I)	37°	24	.001	.019	.020
Salmonella schottmuelleri	"	"	"	.021	.029	.050
Shigella dysenteriæ	"	"	"	0	.018	.018
Eberthella typhosa	"	"	"	0	.018	.018
Escherichia coli	"	"	"	.038	.007	.045
Brucella abortus	"	"	72	0	.013	.013
Proteus vulgaris	"	"	24	.001	.019	.020
Pseudomonas aeruginosa	"	"	"	.010	.050	.060
Aerobacter aerogenes	"	"	"	.017	.028	.045
Bacillus subtilis	"	"	"	0	.019	.019
Bacillus megatherium	"	30°	24	0	.007	.007
Serratia marcescens	"	"	"	.007	.007	.014
Bacillus vulgatus	"	"	"	.041	.030	.071
Klebsiella pneumoniæ	"	37°	"	.010	.032	.042
Alkaligenes fecalis	"	"	48	.001	.005	.006
Corynebacterium diphtheriæ	"	"	24	.070	.171	.241
Staphylococcus albus	"	"	"	.014	.047	.061
Staphylococcus aureus	"	"	"	.004	.063	.067
Vibrio choleræ	"	"	"	—	.022	—
Shigella paradysenteriæ	"	"	"	.006	.011	.017
Mycobacterium stercoris	Long and Seibert	"	96	.003	.019	.022
Mycobacterium smegma	"	"	"	.007	.052	.059
Mycobacterium tuberculosis	"	"	720	—	.200	—
*Saccharomyces cerevisiæ**	Snell, Eakin and Williams	"	16	—	—	.180
Streptococcus hemolyticus	TTV	"	24	.008	.004	.012
Streptococcus salivarius	"	"	"	.005	.005	.010
Streptococcus scarlatinæ	"	"	"	.020	.030	.050
Diplococcus pneumoniæ I	"	"	"	.001	.004	.005
Diplococcus pneumoniæ II	"	"	"	.001	.003	.004
Diplococcus pneumoniæ III	"	"	"	.002	.003	.005
Lactobacillus casei	Landy and Dicken	"	72	—	.016	.016
Lactobacillus arabinosus†	Snell and Wright	30°	"	0	0	0
Clostridium botulinum	Stewart	37°	144	—	—	.004
Clostridium sporogenes	"	"	48	—	—	.003
Clostridium tetani	"	"	"	—	—	.008

* As determined by reversal of sulfonamide inhibition of yeast growth, Landy and Dicken[16] estimated that *Saccharomyces cerevisiæ* No. 139 cultured in the medium of Snell, Eakin and Williams produced "a neutralizing substance equivalent to approximately 1.5 μg of PAB (10 cc of culture)." This is in good agreement with the direct PAB assay which found 0.18 μg PAB per cc of culture.

† Approximately one-half maximum growth (as measured by acid production) was obtained in the Snell and Wright medium prepared with highly purified casein hydrolysate. By microbiological assay this medium contained less than .0001 μg of PAB per cc. Since *L. arabinosus* has been shown to require PAB for growth, sufficient PAB must still have been present in the medium to support the growth obtained. It is significant, however, that the organism did not synthesize any detectable PAB.

that of the hydrolyzed whole culture.

Discussion. The organisms studied included the greatest variety of bacterial types which could be adequately cultured in PAB-free media. It is important to emphasize that the values for PAB synthesis thus obtained apply only to our test conditions. Since it is known that the culture media were not equally satisfactory for the growth of the various organisms studied, it is not justifiable to compare PAB synthesis by different bacterial species. The effect of various nutrients on production of PAB by bacteria is quite unknown and it is possible that under other cultural conditions different assay values might be obtained.

Examination of Table II reveals that most bacteria synthesize PAB in readily measurable

[16] Landy, M., and Dicken, D. M., *Nature*, 1942, **149**, 244.

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