TABLE 1. Adrenal ascorbic acid depletions by bacterial extracts.

Organism	Ratio: vol suspending fluid/ vol packed cells	Depletion in mg/100 g adrenal tissue	
		Individual values	Av
*Bacillus licheniformis**	3	159, 162, 85, 112, 129	129
Bacillus subtilis (rough)	4	135, 129, 136, 122, 102	125
Serratia marsecens (pigmented)†	3	116, 71, 67, 110	91
Lactobacillus leichmanii, 7830§	10	131, 77, 60, 100	92
Escherichia coli, 9637	10	63, 55, 106, 82	77

* Bacitracin-producing strain.
† Grown at room temperature.
§ Required 100 mg Tween 80 (Atlas Powder Company) per 100 ml broth for growth.

in diluting samples for the Sayers assay, may result in adrenal ascorbic acid depletions up to 40 mg/100 g. (b) As a result of extensive collection of data by our bioassay group, an average depletion of 60 mg/100 g adrenal tissue may be estimated to represent about 0.1 milliunit (U.S.P. provisional standard) of ACTH activity and represents a minimum value acceptable for quantitative estimation. (c) The depletions reported in Table 1 were measured at only one dosage level, frequently without accompanying standards. For these reasons, we have regarded average depletions of less than 70 mg/100 g of adrenal tissue as of doubtful significance. Thus, *Mycobacterium smegmatis*, in 3 vol of suspending fluid, gave a depletion of 54 mg/100 g tissue. The nonpigmented form of *S. marsecens* was definitely without activity when tested at the same dilution as its pigmented variety. Similarly, *L. casei* was inactive under conditions comparable to those under which *L. leichmanii* demonstrated its activity.

Since some of the bacteria first examined and found to produce the ACTH-like activity were also known producers of vitamin B_{12}, a single experiment was carried out to determine whether other recognized sources of the vitamin might also yield the ACTH response. A 5-g portion of sardine fish meal was autoclaved in 100 ml of 0.1 normal sulfuric acid and the insoluble residue was removed by centrifugation. The supernatant solution was adjusted to *p*H 2.2 with sodium bicarbonate and was administered to three hypophysectomized rats at 0.5 ml/100 g body weight. Adrenal ascorbic acid depletions of 109, 103, and 71 mg/100 g of adrenal tissue (av, 94) resulted. The activity is not due to the vitamin itself, as was found by administering a B_{12} concentrate. Whether there is some relationship between the vitamin and the hormonal activity of these nonmammalian sources is not known at this time.

The small quantities of low-purity material that have been available have not permitted an independent characterization of the ACTH activity to establish whether or not all the physiological properties of the pituitary hormone are reproduced. In a further survey of the specificity of the Sayers procedure, however, it was found that neither *d*-carvone nor *dl*-dihydroxyphenylalanine, compounds having a relationship to ascorbic acid metabolism (*6, 7*), caused any depletion at a dosage of 5 mg/100 g body weight.

On the basis of the data obtained by the Sayers procedure and of the similar chemical behavior of the observed active principle to pituitary ACTH, we suggest that there is present in various nonmammalian organisms, particularly bacteria, a constituent possessing adrenocorticotropic activity.

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Effect of Hypoxia on DNA Synthesis in the Bone Marrow and Spleen of the Rat[1]

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The constancy of the desoxyribonucleic acid (DNA) content of nuclei in cells of a given species (*1*) permits the use of DNA phosphorus (DNAP) as a measure of cellularity of the bone marrow and spleen. The rate of synthesis of DNA which may be estimated from the incorporation of radiophosphorus into the DNAP may be employed as an index of mitosis rate (*2*). In the present investigation, these techniques have been applied in a study of the effects of hypoxia on the cellular activity of the bone marrow and spleen.

Sprague-Dawley strain male rats, 3 to 4 mo old with an average weight of 264 g, were used. The rats were injected intraperitoneally with 2 μc of carrier-free $NaH_2P^{32}O_4$[3] per 100 g of body weight and sacrificed 4 hr later.

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[3] Obtained on allocation from the U.S. Atomic Energy Commission.

TABLE 1. Influence of 30 hr of intermittent hypoxia on the phosphorus fractions of the bone marrow and spleen.

Group	No. of animals	Phosphorus content mg/g tissue		Specific activity*	
		ASP†	DNAP	ASP	DNAP
		Bone marrow			
Control	15	0.966	1.320	0.481	0.199
σ‡		± 0.088	± 0.295	± 0.064	± 0.034
30-hr hypoxia	6	1.059	1.458	0.608	0.383
σ		± 0.070	± 0.116	± 0.078	± 0.086
P§		< 0.98	< 0.80	> 0.99	> 0.99
		Spleen			
Control	15	0.969	1.294	0.468	0.034
σ		± 0.107	± 0.244	± 0.011	± 0.002
30-hr hypoxia	6	0.949	1.454	0.676	0.287
σ		± 0.020	± 0.112	± 0.080	± 0.036
P		< 0.50	< 0.90	> 0.99	> 0.99

* Percentage of dose of P^{32} injected/mg. P in fraction.
† Acid-soluble phosphorus.
‡ Standard deviation of the mean.
§ Probability.

Animals were subjected to hypoxia in a low-pressure chamber at an oxygen concentration of 7.75 percent. The methods of obtaining marrow and spleen samples, the tissue fractionation procedures, and the counting techniques have been described (*3, 4*).

The data on 15 control animals and 6 animals subjected to intermittent hypoxia for 10 hr a day for 3 consecutive days are presented in Table 1. The experimental animals were injected with radiophosphorus 15 hr after return to ground level. There was no weight loss during the experimental period.

Striking changes were found in the specific activity of the DNAP in the bone marrow and spleen. Hypoxia produced a stimulation of mitosis in both organs. The specific activity in the spleen reached 8 times that of the control, while there was a doubling of the control value in the bone marrow.

Although there was not a marked change in the DNAP concentration or cellularity in either organ, the increase in total erythropoietic mass in the spleen was notable. The spleen weights of animals exposed to 30 hr of intermittent hypoxia increased 175 percent. Taken together with the 500 percent increase in the cells of the erythropoietic series observed in the differential counts from the spleen imprints, an almost tenfold increase in the total erythropoietic tissue resulted from the increased mitotic rate following the hypoxic stimulus.

Unlike the spleen, which may increase in size to accommodate a larger number of cells, the bone marrow is restricted to the marrow cavities, which in the rat are almost entirely filled with red marrow. This would be expected to prevent large increases in the total number of cells. The small increase found was due almost entirely to hyperplasia of the red cell series. The importance of the spleen in the erythropoietic response to hypoxia in the rat is clearly shown by these experiments.

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Detection of Microbially Produced Gaseous Hydrocarbons Other than Methane

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Attempts to detect microbially produced gaseous hydrocarbons other than methane have usually failed. Notable exceptions are reports of ethylene being formed by fungi (*1, 2*). Where other gaseous hydrocarbons such as ethane have been reported there has usually been a question of the actual source, due to the use of municipal sewage in experiments, for example.

Buswell (*3*) states that efforts to detect gaseous hydrocarbons other than methane in microbial fermentations in his laboratory have failed. Furthermore, in data furnished to him by A. V. Grosse (*3*) and reported at the 113th Meeting of the American Chemical Society but not included in the abstract (*4*), a mass spectrometer analysis of purified sewage gas indicating 99.2 percent methane failed to reveal ethane or propylene. The limit of sensitivity in the measurement was 20 ppm for those gases.

By increasing sensitivity still further to the order of 0.05 ppm, we have detected numerous gaseous hydrocarbons other than methane produced in microbial fermentations, and also by the fungus, *Penicillium digitatum* A.T.C.C. No. 10030, growing upon potato-glucose agar. Details of the modification of a Westinghouse Type LV mass spectrometer to obtain high sensitivity will be reported elsewhere.

For hydrocarbon measurements in the parts per million range, the C_2 and heavier hydrocarbons were condensed in a trap at liquid nitrogen temperature while the major portions of noncondensable atmospheric gases and methane were pumped off and discarded. Carbon dioxide was removed by KOH absorption. The trapped C_2 and C_3 hydrocarbons were admitted to the mass spectrometer through a trap at $-155°$ C, which retains most of the C_4 and all the heavier hydrocarbons. The use of 500-ml samples of microbially produced gas permitted measurements of individual C_2 and C_3 hydrocarbons to within ± 0.05 ppm of total sample.

Cow dung was selected as an inoculum free from

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