of inorganic salts. In the tests utilizing inorganic salts, which were added singly to the phage-water suspensions in various concentrations, it has been possible to obscure the oxygen effect or to bring it out sharply, as indicated in the few examples given in Table 1. A discussion of the implications involved with the salts will be presented in detail later.

The results of these experiments raise questions of fundamental importance in radiation biology: Is this effect specific for bacteriophage T1, or is the basic phenomenon reported here obscured in organisms of more complex organization? Does the presence or absence of oxygen alter the phage particles in some way, or is the effect to be explained in terms of suppression or enhancement of certain products of irradiated water? These and related problems are under consideration.

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Adrenocorticotropic Activity of Nonmammalian Origin

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In the course of establishing the specificity of their now standard bioassay for the adrenocorticotropic hormone (ACTH), Sayers, Sayers, and Woodbury (*1*) administered extracts of liver, brain, and spleen of hypophysectomized rats without affecting the adrenal ascorbic acid concentration of the assay animals. Subsequently, Richards and Sayers (*2*) found that the kidney of intact rats contains a substance which depletes the adrenal ascorbic acid content of hypophysectomized rats. They also found that the kidney accumulates hormonal activity following intravenous administration of rat ACTH. Jailer and Knowlton (*3*) and Opsahl and Long (*4*) have presented evidence, including measurements of adrenal ascorbic acid depletions, for the presence of a type of ACTH activity in human placenta. The presence of adrenal ascorbic acid depleting activity in human placenta was confirmed by one of us (JWN). Recently, it has been observed in this laboratory that ACTH-like activity is present in extrapituitary, nonmammalian sites. About 140 source materials, mostly of microbial origin, have been examined,[1] and we wish to report one of the most interesting developments at this time.

This study was initiated as a result of the finding that the administration to hypophysectomized rats of a 1 mg/100 g body weight dose of an antibiotic feed supplement derived from bacitracin fermentation residues[2] caused a significant depletion of adrenal ascorbic acid concentration. On successive days, average depletions of 84 and 80 mg of ascorbic acid/100 g adrenal tissue were obtained; five rats were employed in each assay.[3]

That there was a chemical resemblance between the ACTH-like activity found in the feed supplement and the pituitary hormone was indicated by the fact that the activity could be extracted and concentrated by the acid-acetone method of Lyons (*5*). This is probably the most commonly employed method for extraction and initial purification of pituitary ACTH. The acid-acetone powder so obtained could then be fractionated with ammonium sulfate to achieve a tenfold concentration of activity.

The ACTH-like activity of the feed supplement was not due to bacitracin polypeptides, judging by the fact that fractions representing stages of increasing bacitracin purity were less potent in adrenal ascorbic acid depletion. When it was noted that an approximately quantitative extraction of the activity from the source material could only be achieved when the solids were first autoclaved with dilute sulfuric acid (a procedure that does not inactivate mammalian ACTH) prior to the acetone treatment, it seemed probable that the ACTH-like activity might be associated with the bacterial organism of the fermentation. Consequently, it was decided to examine some isolated bacterial cells for ACTH-like activity.

Procedure. The organism to be studied was transferred from a stock culture to 100 ml of beef-extract-peptone broth and placed in an incubator at 37.5° C for 1 day. An aliquot (1 or 2 ml) of suspended growth was transferred to 200 ml of fresh broth, and the incubation was continued 4 to 5 days. The cellular material was then collected by centrifugation and suspended in dilute sulfuric acid (0.01 or 0.1 normal), employing 3 to 10 vol of acid per volume of packed cells. The resulting suspension was autoclaved at 15 lb pressure for 20 min. After cooling, the suspension was centrifuged, and the supernatant solution was administered in the Sayers bioassay at 0.5 ml/100 g body weight.

In this bioassay, one adrenal is first removed from the test animal (a hypophysectomized rat) prior to the administration of the sample to be tested. The test dose is then administered and, after a suitable time interval, the paired adrenal is removed. Ascorbic acid determinations are then performed on each adrenal, and the depletion resulting from the substance administered is the difference in concentration of ascorbic acid between the animal's own adrenals.

Results. The data obtained from the bioassays are presented in Table 1.

In interpreting these data, the following considerations should be kept in mind: (a) In our experience, administration of acidified saline solution, as employed

[1] Dr. G. M. Savage of our Antibiotics Research Department supplied many of the antibiotic beers which were studied.

[2] We are indebted to Dr. W. G. Bywater, of the S. B. Penick Co., who provided the spray-dried residues obtained from filtration of the fermentation broth.

[3] We wish to thank S. C. Lyster and his group for the bioassays. John Karnemaat assisted in the bacteriological work.

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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[2] Markle Scholar in Medical Science.

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