showed that this treatment did not change the amount of gas when hydrogen, oxygen, or dry air alone was present.

Irradiation with sunlight of the solution in the evacuated reaction vessel was accomplished by disengaging the ground-glass joint just above stopcock 1 after the latter had been closed and by placing the reaction vessel and auxiliary equipment for stirring in a clean place in direct sunlight. The gas produced by sunlight was determined after reassembling the apparatus at the ground-glass joint and evacuating and degassing the apparatus before opening stopcock 1.

The results of these experiments are given in Table 1.

TABLE 1*

Expt	$HClO_4$	$Ce(ClO_4)_3$	$Ce(ClO_4)_4$	A	10^3 B	C	10^3 D
1	2.6	0.14	0.002	0.03	0.8	28	0.8
2	2.6	0.14	0.002	0.02	2.0	34	2.0
3	2.5	0.08	< 0.0001	65†	0.64	28	0.59‡
4	2.5	0.08	< 0.0001	120†	1.0	35	0.91

* Evidence for the photochemical production of a mixture of hydrogen and oxygen gases in Expts 1 and 2 by light of 2536 A and in Expts 3 and 4 by sunlight absorbed by a mixture of cerous and ceric ions in aqueous perchloric acid. The concentrations are formal values in moles/liter of solution. The volume of the solution irradiated was 30 ml in every case. A represents, in units of 6×10^{23}, the quanta of light of 2536 A absorbed, except in Expts 3 and 4; B, moles of noncondensable gas collected in the ignition chamber; C, the pressure in mm Hg at which the noncondensable gas was ignited; D, the decrease produced in the moles of noncondensable gas by the ignition. The experiments were carried out at 25° C.

† Hours in sunlight.

‡ The noncondensable gaseous residue was found to be hydrogen.

The gaseous product of the photolysis is believed to be a mixture of hydrogen and oxygen, because the amount of noncondensable gas decreased significantly when the mixture was ignited by a spark, whereas similar treatment of hydrogen or oxygen alone produced no significant change in the amount of gas; these gases are produced by the photochemical oxidation and reduction of cerous and ceric perchlorates, respectively, when photolyzed separately in dilute aqueous perchloric acid (*1, 2*).

Subsequent photolysis of the solutions employed in Expts 1 and 2 produced a solute that browned the solutions and a gas that was mainly oxygen. The photochemical production of hydrogen may be restored in the following way. The ceric ions can be decomposed thermally at a measurable rate above 30° into cerous ions, with the simultaneous production of oxygen (*3*). This leaves to the light that can be absorbed by the cerous ions the principal task of converting the cerous ions into ceric ions, with the simultaneous production of hydrogen. This method would increase the photochemical efficiency of the net reaction and use the longer wavelengths of sunlight to supply the heat for the thermal reaction and to establish a temperature gradient by means of which the liquid can be circulated. It also makes possible the production of the hydrogen and oxygen in separate parts of the system, where they can be collected largely free from one another.

References

1. Heidt, L. J. *Proc. Am. Acad. Arts Sci.*, **79**, 228 (1951).
2. Heidt, L. J., and Smith, M. E. *J. Am. Chem. Soc.*, **70**, 2476 (1948).
3. Klop, D., and Thomas, H. C. *Ibid.*, **71**, 3047 (1949).
4. Heidt, L. J., and Boyles, H. B. *Ibid.*, **73**, 5728 (1951).

Manuscript received July 11, 1952.

Separation of Synnematin into Components A and B by Paper Chromatography

B. H. Olson, J. C. Jennings, and A. J. Junek

Michigan Department of Health, Lansing

Synnematin, an antibiotic produced by *Cephalosporium salmosynnematum* (*1*), was first described by Gottshall, Roberts, and Portwood in 1949 (*2*). The *in vitro* spectrum of synnematin and the results of some *in vivo* tests were presented by Gottshall *et al.* in 1951 (*3*).

It is the purpose of this report to present evidence that crude culture filtrates of *C. salmosynnematum* contain at least two active components, which will be designated as synnematin A and B, and to present preliminary data about the chemical composition of synnematin B. Brief descriptions are included for the method of production and separation of one of these active components (synnematin B). The detailed description of suitable fermentation media and methods of production and purification of synnematin will be presented in a later report.

Antibiotic activity as expressed below was measured by the *S. typhimurium* serial dilution assay described by Gottshall *et al.* in 1951 (*3*).

The following fermentation medium was used in this study: cornmeal 4%; soya bean meal 4%; ammonium sulfate 0.1%; calcium carbonate 1%; with a pH of 7.4 before autoclaving. The use of this medium has resulted in antibiotic activities of 400 *S. typhimurium* u/ml in shake flasks and 300 u/ml in 30-liter stirred jars.

Several recovery procedures have been used:

1) Aluminum oxide adsorption from culture filtrate containing an equal volume of acetone, followed by elution with water, phosphate buffer, or akaline methanol.

2) Countercurrent extraction from culture filtrate with butanol, plus 15% methanol, and subsequent removal of the active material in a water layer formed by the addition of xylene or benzene.

3) Solvent precipitation of inactive material, followed by removal of the antibiotic in a small volume of water thrown down by the addition of another solvent to the filtrate.

The procedure which has given the most satisfactory results is precipitation of the culture filtrate with 3

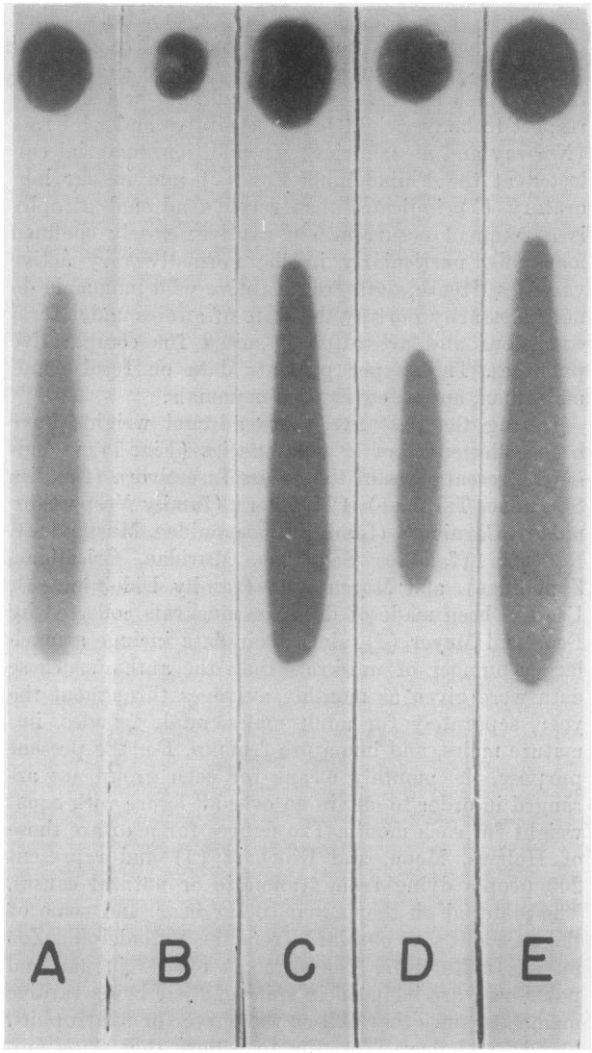

FIG. 1. Paper chromatograms of synnematin culture filtrate (20 units of activity of synnematin A and B) developed for 24 hr with methanol buffered at pH 5.5 with 0.013 *M* citrate. Strips were placed on agar seeded with the following organisms: A, *S. typhimurium*; B, *Staph. aureus*; C, *Proteus vulgaris*; D, *B. subtilis*; E. *Sarcina lutea.*

volumes of isopropanol, removal of the inactive precipitate, and addition of sufficient amyl acetate (usually between 2.5 and 3 volumes) to the filtrate, to give a water fraction of 1/10 to 1/20 the volume of the original culture filtrate. A second water layer is thrown out of the solvent by the addition of either more amyl acetate or water. The volume of the combined concentrates (aqueous layer) should be no more than 1/10 the original volume of culture filtrate. Recoveries of antibiotic activity have varied between 65% and 80%. The concentrate, which is then dried from the frozen state, yields a product with 10–15 u/mg. Other solvents may be used in place of the isopropanol and amyl acetate. Ethanol and acetone have been used in place of isopropanol for precipitation of the inactive matter, and benzene, xylene, and chloroform have been used in place of amyl acetate for precipitation of the active fraction.

For further purification the crude dry product is extracted twice with dry methanol (10 ml methanol/10,000 units crude synnematin/extraction). An inactive precipitate is removed from the methanol extract by the addition of ¼ volume ether. The addition

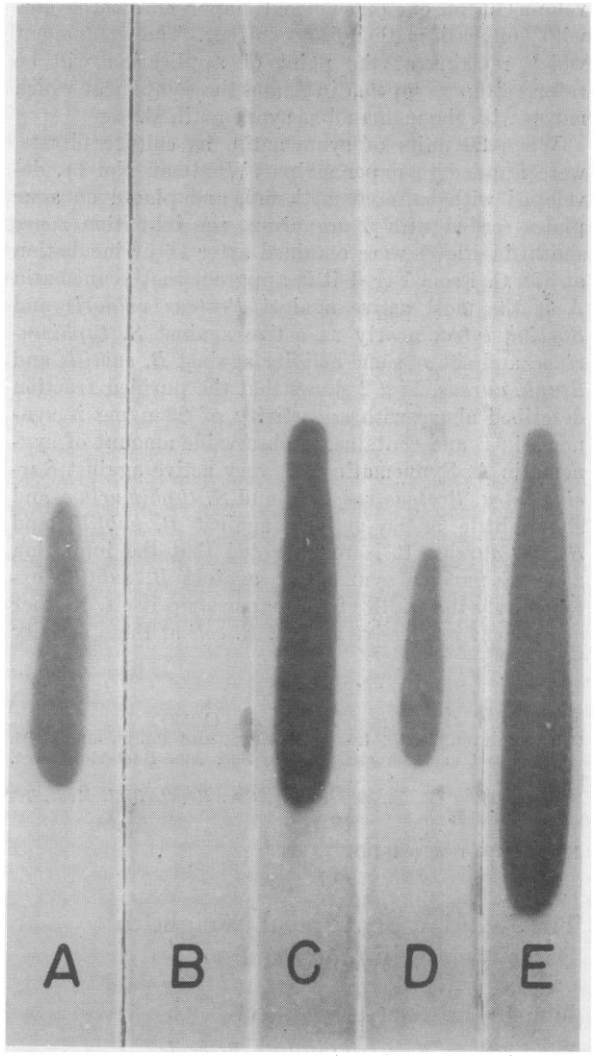

FIG. 2. Paper chromatograms of the purified fraction described herein (80 u/mg). Ten units of activity were used per strip. Results indicate that this fraction contains only one of the active components (synnematin B). The test organisms were: A, *S. typhimurium*; B, *Staph. aureus*; C, *Proteus vulgaris*; D, *B. subtilis*; E, *Sarcina lutea.*

of more ether to give 2 parts ether and 1 part methanol extract precipitates the active antibiotic. The precipitate is washed once with ether and twice with small volumes of absolute ethanol. The residual solvents are removed under vacuum. The white powdery product has an average potency of 70–80 u/mg.

Chemical determinations performed on material of 80 u/mg prepared by solvent precipitation and concentration, and also on material of 70 u/mg prepared

by aluminum oxide adsorption and elution, gave the following positive reactions: polypeptide (biuret and ninhydrin), nitrogen, and sulfur. Negative results were obtained in tests for tryptophan (*p*-dimethylaminobenzaldehyde) and carbohydrate (Molisch).

The antibiotic activity present in the culture filtrate can be separated into at least two components by development of one-dimensional paper chromatograms with buffered methanol as solvent. The component which remains at the point of application will be referred to as synnematin A and the component which moves with the methanol as synnematin B.

When 20 units of synnematin, as culture filtrate, were applied to paper strips (Whatman No. 1), developed with buffered methanol, and placed on agar plates seeded with 5 organisms, the inhibition zones shown in Fig. 1 were obtained after 16 hr incubation at 37° C. From Fig. 1 it is apparent that synnematin A is the most active against *Proteus vulgaris* and *Sarcina lutea,* nearly as active against *S. typhimurium,* and shows some activity against *B. subtilis* and *Staph. aureus.* Fig. 2 shows that the purified fraction described above with an activity of 80 u/mg is synnematin B and contains no observable amount of synnematin A. Synnematin B is very active against *Sarcina lutea, Proteus vulgaris,* and *S. typhimurium* and shows little or no activity against *B. subtilis* and *Staph. aureus.* It is to be noted that the inhibition zone of purified synnematin against *B. subtilis* extends only the width of the paper strip itself. Neither component is effective against *E. coli* at these activity levels.

References

1. ROBERTS, J. M. *Mycologia,* **44,** 292 (1952).
2. GOTTSHALL, R. Y., ROBERTS, J. M., and PORTWOOD, L. M. Presented at Michigan Branch, Soc. Am. Bacteriol. (Oct. 6, 1949).
3. GOTTSHALL, R. Y., *et al. Proc. Soc. Exptl. Biol. Med.,* **76,** 307 (1951).

Manuscript received July 2, 1952.

The Relation of Adrenal Weight to Body Weight in Mammals[1]

John J. Christian

Naval Medical Research Institute, Bethesda,
and *Division of Vertebrate Ecology,*
Johns Hopkins School of Hygiene and Public Health,
Baltimore, Maryland

Most reports concerned with adrenal gland weights have dealt, as a rule, with a limited weight range, with a single species, or with a few closely related species. The adrenal weight is usually expressed as mg/100 g body weight. We are unaware of any reports giving the absolute adrenal and body weights for a large group of species. This is particularly pertinent to data collected from wild mammals. In addition, there usually has been a failure to state whether the data have been collected from captive or feral specimens. Rogers and Richter (*1*) have shown that there is a wide divergence in adrenal weight with respect to body weight between captive and wild rats (Norway and alexandrine). In addition, material collected at the Philadelphia Zoo (*2*) and in our laboratory (*3*) indicates that relative adrenal atrophy is a frequent occurrence in captive, closely confined mammals, particularly in the normally very active varieties. Hence, data from captive wild mammals do not necessarily portray the state of affairs under feral conditions and are often ill-suited for comparative purposes. This paper presents data on feral mammals, man, and a few captive mammals.

During the past five years adrenal weights have been collected from 19 wild species (Fig. 1), including representatives of the orders Insectivora (families Soricidae, Talpidae), Chiroptera (family Vespertilionidae), Carnivora (families Procyonidae, Mustelidae), Rodentia (families Sciuridae, Muridae, Cricetidae, Zapodidae), and Marsupialia (family Didelphiidae). Use has been made of data for muskrats collected by Beer and Meyer (*4*), since their data include a much larger number of muskrats than the author's. These data were given as monthly averages throughout the year, separately for adult males, adult females, immature males, and immature females. For the present purpose, the monthly means for each group are arranged in order to obtain an over-all figure with equal weight for each month. The figures for man are those of Holmes, Moon, and Rinehart (*5*) and represent 200 people dying from traumatic or natural causes. The material on the jaguar, polar bear, and some of the raccoons was obtained from the Philadelphia Zoo by H. L. Ratcliffe. The adrenals of the jaguar and polar bear are included in spite of their being captive mammals, since they fall on the curve for unatrophied glands, and there is reason to believe that these animals were about as active in captivity as their feral counterparts. The published weights for 100 male guinea pigs (*6*) are included, as well as our own, for a smaller series of active animals of both sexes, since they too failed to show evidence of atrophy of the adrenals for similar reasons, although closer confinement is known to produce this effect (*3*).

The wild rats represent captures from the city of Baltimore, Md., and from a rather isolated colony on a farm north of Baltimore. All rats have been lumped together in weight groups for the purposes of this paper. The average combined adrenal weight for the rats in each weight class has been plotted. A similar procedure has been followed for the big brown bats (*Eptesicus*) and the guinea pigs from our laboratory.

With the exception of human, muskrat, guinea pig, jaguar, polar bear, and raccoon data, all weights are after fixation in neutral formalin. The author has found that the weights of adrenals fixed *in situ* and subsequently dissected free are as dependable as fresh weights, and in the case of the small mammals prob-

[1] The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.