different shapes of the curves imply that the effects of various reactions which compete for sulfur shift with changing temperature and Na_2S concentration.

R. H. ARNTSON
Department of Geology,
University of California, Los Angeles
F. W. DICKSON
Division of Physical Sciences,
University of California, Riverside,
and *Institute of Geophysics,*
University of California, Los Angeles
G. TUNELL
Department of Geology,
University of California, Los Angeles

References and Notes

1. This report is paper number 104, Institute of Geophysics, University of California, Los Angeles. The work was supported by grants from the National Science Foundation.
2. F. W. Kuster and E. Heberlein, *Z. anorg. Chem.* **43**, 54 (1905).
3. F. W. Dickson and G. Tunell, *Science* 119, 467 (1954).
4. The value of n in moles of S at 25°C in the analyzed solutions ranged from 3.74 to 3.80, and averaged 3.77; at 50°C, n ranged from 3.60 to 4.48 and averaged 4.03.

28 April 1958

Occurrence of Serotonin in a Hallucinogenic Mushroom

Although some doubt has been cast recently upon the identification of *teonanacatl,* the sacred fungus of the Aztecs, as a species of *Panaeolus* (*1*), members of this genus are well known for their hallucinogenic properties and remain as the classical examples of mushrooms producing mycetismus cerebralis (*2*).

A preliminary chromatographic survey of a number of toxic mushrooms collected in western Washington (*3*) revealed the presence of several compounds in *Panaeolus campanulatus* (Fr.) Quélet (*4*) which gave, with Ehrlich's reagent, color reactions characteristic of indole derivatives. Subsequent investigation revealed that the most abundant of these compounds exhibited properties identical with those of serotonin (5-hydroxytryptamine). Although this compound has previously been detected in animals (*5*) and higher plants (*6*), this is the first report of its occurrence in a fungus. Its dimethyl derivative, bufotenin, has been reported to exist in certain species of *Amanita* (*7*).

It should not be assumed that serotonin, per se, is the hallucinogenic principle in *Panaeolus campanulatus* since Waalkes *et al.* (*6*) have established that large (20 mg) oral doses of the compound do not produce physiologic effects in human beings. The presence of serotonin may be indicative of the presence of related indole compounds, possibly of the type recently isolated from *Psilocybe mexicana* Heim (*8*). This compound, which has been named psilocybin, causes psychotropic effects in human beings following oral administration.

One gram of the dried mushroom was extracted with 70 percent ethanol, the extract was concentrated in a vacuum at 45°C, and the residue was purified by partition between n-butanol and water essentially as described by Udenfriend *et al.* (*5*). The purified extract was concentrated, and the entire quantity deposited as a line on a sheet of Whatman No. 3 filter paper which was subjected to ascending formation with a wash liquid composed of n-propanol and 1N ammonia (5:1). The section of the sheet corresponding to serotonin was eluted with water, concentrated, and again purified by partition between n-butanol and water.

The residue thus obtained was identical chromatographically with serotonin (*9*) in four solvent systems: the n-propanol–ammonia system described above, n-butanol–acetic acid–water (4:1:5), n-butanol saturated with 1N hydrochloric acid and methyl ethyl ketone–acetone–water (20:2:5). It gave reactions identical in all respects with serotonin with Ehrlich's reagent, Pauley's reagent, cinnamic aldehyde followed by hydrochloric acid, and with Jepson and Stevens' reagent, the latter being highly specific for certain tryptamines (*10*).

The ultraviolet absorption spectrum of an aqueous solution of the compound at pH 5.4 had a minimum at 250 mμ, a maximum at 275 mμ, and a shoulder with a point of inflection at 300 mμ. This is in good agreement with the absorption characteristics previously reported for serotonin (*11*). From these data it was concluded that the compound obtained from *Panaeolus campanulatus* was serotonin.

VARRO E. TYLER, JR.
Drug Plant Laboratory,
College of Pharmacy,
University of Washington, Seattle

References and Notes

1. V. P. and R. G. Wasson, *Garden J.* **8**, 1 (1958).
2. W. Ford, *J. Pharmacol. Exptl. Therap.* **29**, 305 (1926).
3. I gratefully acknowledge the assistance of Miss M. McKenny, L. Brady, and J. Wier in collecting the plant material used in this study.
4. The identification of the mushroom was verified by Dr. D. E. Stuntz, professor of botany, University of Washington.
5. S. Udenfriend, C. T. Clark, E. Titus, *Experientia* **8**, 379 (1952).
6. T. P. Waalkes, A. Sjoerdsma, C. R. Creveling, H. Weissbach, S. Udenfriend, *Science* **127**, 648 (1958).
7. T. Wieland and W. Motzel, *Ann. Chem. Liebigs* **581**, 10 (1953).
8. A. Hofmann, R. Heim, A. Brack, H. Kobel, *Experientia* **14**, 107 (1958).
9. Authentic serotonin creatinine sulfate was supplied through the courtesy of Vismara Therapeutici.
10. J. B. Jepson and B. J. Stevens, *Nature* **172**, 772 (1953).
11. K. E. Hamlin and F. E. Fischer, *J. Am. Chem. Soc.* **73**, 5007 (1951).

5 May 1958

Passage of Bacteriophage Particles through Intact Skin of Mice

It has long been known that two highly infectious species of bacteria, *Pasteurella tularensis* (*1*) and *Brucella melitensis* (*2*), can set up systemic infections in experimental animals when suspensions of such organisms are placed in contact with the apparently normal skin of animals. Rickettsia producing Rocky Mountain spotted fever can infect guinea pigs through the unabraded skin (*3*). Since bacteriophage particles are within the size range of most animal viruses but do not undergo either specific adsorption or multiplication in sensitive host tissues and have the added advantage of being assayed by relatively easy and accurate techniques, they lend themselves admirably to studies on the physical interactions of viruses in animals. Recent results from this laboratory (*4*) present evidence that bacteriophage particles can pass rapidly through the gastrointestinal barrier and into the blood circulation of mice. In view of the afore-mentioned results, experiments were initiated to ascertain whether particles of the size of bacteriophage could pass through the intact skin of mice.

The phage strain utilized in this study was derived from *Bacillus megatherium* 899a (lysogenic) and is the clear plaque mutant, strain C, as described by Gratia (*5*). The sensitive indicator strain *Bacillus megatherium* KM growing on a medium containing 2 percent Bacto Peptone was used for the production of phage stock suspensions, and assays were made by the pour plate method. Two different anatomical sites of adult white Swiss mice, strain C.F.W., Carworth Farms, were chosen as experimental areas—namely, the tail and the abdomen. These areas were not manipulated by any means such as depilation, shaving, or clipping of hair. In fact, great care was taken to choose only mice which by gross observation manifested normal and continuous dermis in these areas. In order to minimize the obvious effect that microscopic abrasions would have on these experiments, mice utilized in the tail experiments were isolated for 2 days prior to the experiments.

The mice were anesthetized for the duration of each experiment by the intraperitoneal administration of Pentathal sodium. In the first set of experiments the tail of each mouse was exposed to the virus solution (approximately 1×10^{10} phage/ml) by immersing it in a test tube to a level approximately 1 inch from the body proper. The tail was allowed to stay in contact with the virus solution for 15 minutes. In the second set of experiments, which were concerned with passage of bacteriophage through

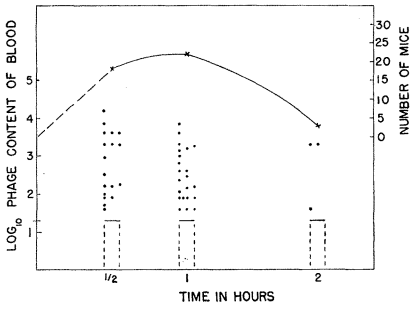

Fig. 1. Passage of *B. megatherium* bacteriophage through the skin of white mice. Numbers of active phage particles recovered from the total blood circulation of 96 mice. Thirty-two mice were tested at each time interval.

the skin of the abdomen, the same techniques were followed with the exception that the Pentathal sodium was administered by the subcutaneous route into the interscapular area of the mice instead of intraperitoneally. Two-tenths of a milliliter of the high-titer phage suspension was applied to the fur of the abdomen and gently spread with the tip of the pipette to effect penetration through the fur and onto the skin. The phage-treated area was equal in size to that of a 2.0 cm circle. At indicated time intervals after the administration of the bacteriophage suspension, the chest area was thoroughly sterilized with iodine and alcohol, and 0.1-ml samples of blood were taken by cardiac puncture with a heparinized 1-ml syringe. Following this procedure, the mice were sacrificed. Full-strength and serial two-fold dilutions of such blood samples were then assayed for the presence of virus particles. Control experiments on the accidental contamination of blood samples with phage were performed by placing 0.2 ml of the high-titer phage suspension on the chest area. After the routine sterilization procedure, blood samples were taken by cardiac puncture. From these as well as from other normal control animals no phage particles were recovered.

The recovery of active virus particles from the circulation of mice thus treated was quite irregular in both the number of positive recoveries and in the amount recoverable from the circulation of each mouse. There was essentially no difference in the rate and quantities of recovery by the two experimental methods. Figure 1 cites the cumulative results of one series of such combined experiments. The quantity of phage particles per 0.1 ml of sample has been converted by a factor of 20 and is cited for an average total blood volume of such mice of 2 ml. Thirty-two mice were sacrificed at each time interval. As can be seen, the number of mice yielding recoverable virus particles decreases with time, for only three mice yielded particles at 2 hours. These results suggest that this effect is possibly due to the immune mechanisms of the experimental animals. In this respect it is worth while to note that Van Vunakis *et al.* (*6*) have reported in vitro inactivation of *Escherichia coli* phage by normal mouse serum. The factor responsible was shown to be the properdin system previously described by Pillemer (*7*). Sulkin and his associates (*8*) reported evidence for the in vivo inactivation of *Staphylococcus* phage by the properdin system in rabbits.

In this study great variations in the recovery of active phage particles from the blood circulation were noted after intraperitoneal inoculations into different strains of normal mice. It may be possible that these variances were due to differences in titer of natural antibodies. Since current work is showing that the rate of arterial disappearance of this bacterial virus from the circulation of dogs is very rapid (*9*), the actual number of particles which were able to pass the skin barrier is possibly of a larger magnitude.

The size of the bacteriophage particles used in this study has been determined (*10*) to be 49 mμ for the width of the head and 330 by 15 mμ for the length and width of the tail. On the basis of the size of the head only, this particle then would be in the size range of the small animal viruses.

It is hoped that this report will stimulate reinvestigations of the possibility that virus infections may occur by penetrations through the intact skin and of the effects that this rather exotic mode of transmission may have in the epidemiology and pathogenicity of virus infections of man (*11*).

ROBERT KELLER*
Department of Microbiology,
School of Medicine,
University of Missouri, Columbia

References and Notes

1. G. C. Lake and E. Francis, *Public Health Repts.* (*U.S.*) 37, 392 (1922).
2. A. V. Hardy, M. G. Hudson, C. F. Jordan, *Infectious Diseases* 45, 271 (1929).
3. R. R. Spencer and R. R. Parker, *Public Health Repts.* (*U.S.*) 154, 60 (1930).
4. R. Keller and F. B. Engley, Jr., *Proc. Soc. Exptl. Biol. Med.* 98, 577 (1958).
5. A. Gratia, *Compt. rend. soc. biol.* 123, 1018 (1936).
6. H. Van Vunakis, J. L. Barlow, L. Levine, *Proc. Natl. Acad. Sci.* (*U.S.*) 42, 391 (1956).
7. L. Pillemer *et al.*, *Science* 120, 279 (1954).
8. S. E. Sulkin, R. A. Finkelstein, E. D. Rosenblum, *Science* 125, 742 (1957).
9. R. Keller and M. L. Zatzman, in preparation.
10. J. S. Murphy, *J. Exptl. Med.* 98, 657 (1953).
11. This study was supported in part by research grant E-752C from the U.S. Public Health Service. Grateful acknowledgement is made for technical assistance to Mary Jo Olds and Paul W. Schaper, the latter serving under a U.S. Public Health Service summer medical student fellowship.

* Present address: Department of Microbiology, Michael Reese Hospital, Chicago, Ill.

19 May 1958

Hemagglutinins in Uterine Secretions

Two kinds of findings have led to the suggestion that the ABO blood group phenotypes may be subject to the action of natural selection. One line of investigation has purported to show that there is a relative deficiency of living children of blood type A among the offspring derived from the "incompatible" mating, mother type O, father A (see, for example, *1, 2*). For a number of reasons considerable uncertainty exists concerning the existence and/or magnitude of this deficiency (*3*). The other line of investigation has suggested that in blood-group incompatible matings, the frequency of abortions is higher and the mean number of living children is lower than in compatible matings (*4*). (An incompatible mating is defined as one in which the male possesses an antigen which is lacking in the female; in the ABO system, the male possesses an antigen for which his spouse has the corresponding antibody.)

There are two possible mechanisms whereby selection due to the ABO blood groups may alter the number of children born with a given blood type. Many have assumed that immune antibodies produced by the mother could damage the fetus and result in its loss (for example, *1*). Another possibility, which formed the starting point for this investigation (*5*), is that selection may be exercised during the preconception period on the spermatozoa themselves. These two mechanisms are, of course, not mutually exclusive.

The postulate of spermatozoal selection involves one or more basic assumptions. One is that human sperm possess the specific blood group antigens of the donor. Previous findings on this point (for example, *6*) need to be reevaluated in light of the possible contamination of blood group substances in the seminal fluid. (Sperm from a nonsecreter donor would obviate such a possibility.) A second is that two antigenically different kinds of sperm are produced by a heterozygous AO male—that is, sperm bearing and sperm lacking A antigen. This antigenic dimorphism in the sperm population of heterozygous males has not, to our knowledge, been demonstrated. A third assumption, aside from the cellular antigens of the sperm, is that the presence of soluble blood group substances in the seminal fluid may itself be the (or one of the) determining agent(s) in spermatozoal selection.

If any of these assumptions is correct, then it becomes conceivable that there may be specific selection of sperm in the female reproductive tract, either by selective impedance of motility, complete inactivation, neutralization of a point of