there is kinetic energy potentially available for mechanically destroying red blood cells. The increased mechanical fragility of red blood cells which have been rendered nearly spherical suggests a teleological reason for the biconcavity of mammalian erythrocytes; namely, the inevitability of rupture, were a nearly spherical cell to be deformed in traversing a narrow capillary. The increase in mechanical fragility with increase in hematocrit may be a factor in limiting the concentration of red blood cells normally in circulation.

The correlation between increased osmotic fragility (spheroidicity) and increased mechanical fragility, already observed under experimental conditions, was found to occur in congenital hemolytic jaundice. In one case in which the osmotic fragility of the erythrocytes was characteristically increased, their mechanical fragility was also augmented, so that with a hematocrit of only 30.2 per cent. the M. F. was 12.8 per cent. At splenectomy, the osmotic fragility of the blood in the spleen was found to exceed that of the peripheral blood. After splenectomy, as the evidence of increased blood destruction diminished, the osmotic and mechanical fragilities of the red blood cells declined progressively, until 39 days after the operation both were approximately normal.

In patients with thermal burns and hemoglobinuria, the erythrocytes have been found to be relatively spheroidal and to be increased in osmotic fragility.[9] In addition, such erythrocytes exhibit increased mechanical fragility, as do those of samples of human blood momentarily heated *in vitro* to from 52° to 58° C. Similarly, the erythrocytes of heated dog's blood are osmotically and mechanically fragile and are rapidly destroyed on re-injection into the animal.[9]

In the absence of increased osmotic fragility and cohesion between erythrocytes, increases in mechanical fragility are presumably on the basis of diminished strength of the cell membrane. This was found to be the situation with respect to patients with pernicious anemia tested prior to treatment with liver extract. Thus, in 3 such cases, osmotic fragility values were normal, but the M. F. values were 3.7, 3.9 and 4.3 per cent. The M.F. of the erythrocytes of a patient in advanced remission induced by liver extract was 2.1 per cent.; that of a normal control was 2.0 per cent. In these experiments the hematocrits were all adjusted to approximately 25 per cent.

In agreement with others,[5,6,7,8] it is suggested that the cohesion of erythrocytes may lead to their prompt mechanical destruction while in motion in the circulation. *In vitro,* increased mechanical destruction was shown to occur in the presence of iso-agglutinins and cold agglutinins, and in experiments with sickled erythrocytes. It has already been suggested[11] that such types of erythrocyte cohesion may cause sequestration of erythrocytes in the spleen and other tissues, with consequent progressive increase in their spheroidicity and osmotic fragility. Incubation of erythrocytes, at least *in vitro,* increases both their osmotic and mechanical fragilities. Consequently, if certain red blood cells temporarily sequestered (incubated) in the spleen, escape before their osmotic destruction occurs, they may still be readily destroyed when re-subjected to the traumatic motion of the circulation, because of their increased mechanical fragility.

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PROGRESSIVE ASCENDING PARALYSIS IN DOGS DUE TO DEFICIENCY OF A VITAMIN B COMPLEX FACTOR FOUND IN YEAST[1]

THE thirty-eight dogs used in this study received a synthetic B complex free diet composed of casein (water and alcohol extracted) 40 per cent., sucrose 36, cotton seed oil 18, cod liver oil 2, mineral salts 4 per cent. This was altered in the case of the positive control animals to contain dried brewers' yeast at a level of 10 per cent. as a source of the B complex. The others had their B complex requirement met by seven or eight of the following synthetic vitamins: (1) thiamine hydrochloride; (2) riboflavin; (3) pyridoxine; (4) nicotinic acid; (5) pantothenic acid; (6) para-aminobenzoic acid; (7) inositol, and (8) choline.[2]

The incidence of paralysis varied considerably on the different deficiencies, but it was greatest in the animals receiving all the synthetic B complex factors listd above where eleven out of twelve animals became paralyzed.

The paralysis comes on gradually, the early signs being a peculiar gait and an arching of the neck; then the hind legs show marked spasticity. There are often several bouts of transient paralysis with spontaneous recovery before the final progressive stage is reached. It is then rapidly progressive and ascending, the hind legs becoming involved first, then the fore legs, then the neck and, finally, the respiratory center. The paralysis is at first spastic and later becomes almost completely flaccid. It is rapidly fatal if untreated.

[11] T. H. Ham and W. B. Castle, *Tr. Asn. Am. Phys.,* 55: 127–132, 1940.

[1] Reported at the meeting of the American Chemical Society, September 12, 1944, New York City.

[2] SCIENCE, 98: 520, 1943. The amounts of inositol and choline were subsequently increased to 300 mg per dog per day.

Paralysis is regularly prevented by brewers' yeast, is cured by a water extract of yeast,[3] and has responded promptly (8–12 hours) to synthetic biotin[4] therapy in seven attacks in four dogs. The biotin was dissolved in physiological saline and administered subcutaneously. The therapeutic dose is approximately 100 gamma per kilo.

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PRELIMINARY NOTE ON THE INACTIVATION OF ANTIBIOTICS

DURING the course of investigations on antibiotic substances of plant origin[1] an antibiotic active against both Gram positive and negative organisms was isolated from *Allium sativum*. During the course of chemical studies of this antibiotic, the reaction with cysteine was investigated. It was found, as is the case with penicillin, that the antibiotic is rapidly inactivated by cysteine.

A number of other antibiotics of thallophyte and spermatophyte origin available in this laboratory were tested in the presence of cysteine. In every case, cysteine gave complete inactivation or marked diminution of antibiotic activity. Gram-positive antibiotc activity is more susceptible to cysteine inactivation than the Gram-negative activity.

The following antibiotics were inactivated: penicillin, citrinin, gliotoxin, clavacin (patulin or claviformin), pyocyanine; the active principles of *Allium sativum, Ranunculus acris* and *R. bulbosus, Erythronium americanum, Asarum reflexum,* Bassica species and *Arctium minus*. The antibiotic principles of *Allium sativum, Erythronium americanum, Asarum reflexum* and *Arctium minus* will be described in greater detail later.

The testing procedure was as follows: Water solutions of each of the antibiotics were divided into two portions. One portion was used as a control and to the other was added solid sodium biocarbonate adequate to maintain a pH of approximately 7 and cysteine hydrochloride. The solutions were allowed to stand for 30 to 60 minutes, then tested for antibiotic activity against *Staphylococcus aureus* and *Bacillus paratyphosus* A by the Oxford cup method.

This antagonistic effect of cysteine was similarly displayed by cysteine esters (methyl and ethyl), but not by S-methyl cysteine, methionine, alanine or serine. Other –SH compounds such as glutathione and thioglycollic acid had either no effect or a much weaker action.

This inactivation is especially unusual in the light of the widely different chemical types of antibiotics involved. The nature of the reaction of cysteine with some of the antibiotics is known; others are being investigated. In the known instances, cysteine reacts irreversibly with the antibiotics. However, this may not be true of all the antibiotics. Quantitative relationships of the antagonistic activity of cysteine and related compounds are being studied and will be reported later. It is suggested that possibly the fundamental mode of action of certain classes of antibiotics involves their ability to interfere with the normal function of sulfhydryl groups in bacterial metabolism. This has already been observed in some specific instances as by Fildes,[2] in his investigation of the mode of action of mercury as an antibacterial agent; by Eagle,[3] who observed that the anti-spirochetal action of arsphenamine could be counteracted by cysteine; and by Atkinson[4] in her work with penicidin.

That the sulfhydryl group is essential to cell proliferation has been demonstrated and discussed by Hammett.[5,6]

This note is published with the desire that other investigators having access to different antibiotics will test such substances for inactivation by cysteine.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ENHANCED PRODUCTION OF PENICILLIN IN FLUID MEDIUM CONTAINING CELLOPHANE[1,2]

THE observation was made that young colonies of *Penicillium notatum* in fluid medium show a tendency to develop nearer the side walls of the vessel than

[3] Kindly supplied by Dr. C. N. Frey, of the Fleischmann Laboratories, Standard Brands, Inc., New York, N. Y.

[4] Kindly supplied by Dr. D. F. Robertson, of the Merck Company, Inc., Rahway, N. J.

[1] This work was begun before the appearance of the article by Osborn, *Brit. Jour. Exper. Path.*, 24: 227, 1943, and as a result, many of the plants tested have been duplicated.

[1] From the Laboratories of Bacteriology, The Mount Sinai Hospital, New York, N. Y.

[2] The author wishes to acknowledge thankfully the accurate and capable assistance of Miss Alice Fisher.

[2] Fildes, *Brit. Jour. Exper. Path.*, 21: 67, 1940.

[3] Eagle, *Jour. Pharmacol.*, 66: 436, 1939.

[4] Atkinson, Stanley, *Australian Jour. Exper. Biol. Med. Sci.*, (4)21: 249, 255, 1943.

[5] Hammett, Hammett, *Protoplasma*, 15: 59, 1932.

[6] Hammett, Chapman, *Growth*, 2: 223, 297, 1938.