COMMENTS
by Readers

A note in this journal (*Science*, 1946, **104,** 426) cautions against use of sulfuric acid-dichromate mixtures in cleaning glassware for microbiological experiments because of the possible toxic action of the traces of dichromate that often remain on or in the glass, even after repeated rinses. Figures showing retarded growth of various microorganisms and inhibition of enzyme systems in the presence of extremely low concentrations of dichromate are cited. In view of this note and the relatively high degree of toxicity generally attributed to chromium and dichromates, the following observation is of special interest, since it shows that under certain conditions traces of dichromate might also distort experimental results, not by exerting an inhibitory action but by causing stimulation.

During the course of a study of biosynthesis of penicillin by *Penicillium chrysogenum* X-1612 in shake flasks, we attempted to formulate the simplest synthetic medium, composed exclusively of compounds readily available in industrial quantities, that is capable of yielding reasonable concentrations of penicillin. It was found that chromium, furnished as $K_2Cr_2O_7$, was beneficial in this solution (*Science*, 1945, **102,** 482). We were interested to observe the necessity of adding Cr, and probably Al, for biosynthesis of penicillin in the basal synthetic medium where penicillin had to be synthesized from minerals, and from carbon supplied in the form of lactose, starch, glucose, and acetic acid.

Later experiments showed that when the concentration of Cr (supplied as $K_2Cr_2O_7$) was raised from 1 γ/l. to 20 γ/l., the maximum titer of penicillin in the crude liquor was increased from 50 Oxford units/ml. to about 85. Solutions containing 7.5 and 10 γ Cr/l. produced titers of about 70 and 75 units/ml., respectively. Solutions containing 100 γ Cr/l. yielded 85 units/ml., and those with as much as 200 γ Cr/l. permitted good growth of the mold and produced about 55 units of penicillin/ml. In these experiments the concentration of Al (furnished as Al acetate) was 3.6 γ/l. Virtually no penicillin was produced in the basal medium prepared with distilled water when no $K_2Cr_2O_7$ was added. The penicillin potencies were determined by the standard cylinder-plate method using *Staphylococcus aureus* NRRL 313 as the test organism and a standard of calcium penicillin G.

Strictly speaking, Cr and Al should not be designated "essential," since it has not been determined that no other elements can substitute satisfactorily for them. However, in our experiments penicillin could not be detected in the solution in their absence, and no other elements tested (Mo, Ce, Co, Ni) replaced them satisfactorily. This suggests that perhaps Cr and Al may be effective in catalyzing cyclizations and condensations involved in the biosynthesis of penicillin as they are in chemical synthesis (*Ind. eng. Chem.* (Ind. ed.), 1945, **37,** 356, 1038). The biocatalytic activity of Cr may be exerted through a stimulating effect on enzyme systems such as has been reported previously (*J. biol. Chem.*, 1939, **128,** 251). (ROBERTSON PRATT and JEAN DUFRENOY, *University of California College of Pharmacy, San Francisco.*)

Devotion of a rather large amount of space (*Science*, 1946, **104,** 373–374) to the description of what today may be fairly termed a relatively crude device suggests quite strongly that enough of our scientific colleagues bedeviled by the problems of circuit control are not fully oriented with modern resources.

Before the advent of modern relays I made many enforced practical studies on their lack of function, especially troublesome with heater circuits where thermostatic failure adds its contribution. Certain practical points garnered from years of experience may therefore be worthy of record for the benefit of those less interested in such matters. The key to accurate thermostatic control is a sensitive thermostat. This is almost inevitably associated with an inability to handle heavy currents, or sparking occurs at the contacts, leading to eventual failure. The natural corollary is that the associated relay must operate from small currents. In practice this should be less than 10 Ma. As a-c relays are now available, it is generally better to use them, since the sparking at contacts is less than an equivalent d-c load and consequently corrosion and sticking are minimized. Of the many and varied attempts to eliminate sparking at contacts, none has been entirely successful from a practical standpoint, in which expense is one real factor. Unless care is taken to match the circuit load correctly with the appropriate size of condenser, the use of these alone to suppress sparking may be quite unsatisfactory. In actual practice the condenser large enough to suppress nearly all visible sparking is too large, for its own charge, when released by contact closure, may be, and indeed often is, heavy enough to fuse the points and cause troublesome sticking.

For heavy loads such as heater circuits, I have found the use of a simple high-resistance bridge to be as effective and easier than the use of condensers. This is a small lamp that also has signal value. Where condensers are used, it is still advisable to use such a bridge so that they may be slowly discharged. This saves a good deal of contact "make" sticking. For a-c circuits a choke coil could be used. As the modern "wipe" silver contacts work well with heavy loads—5–10 amp. or more—the advantages of the more expensive, clumsy but sealed in, mercury contacts, except in very dusty or humid environments (stoker furnace controls) is now less apparent. Moreover, they will operate only at slow speeds, due to the internal sloshing of the mercury that may eventually set up a continuous arc. Their use inevitably leads to a bulkier apparatus. The mercury contacts are being replaced for many purposes by the remarkable little 4-ounce pressure switch, a highly sensitive switch operating off a slight pressure and short throw. These are admirably incorporated into relay controls and are also sealed in. They may be found operating the cheap but serviceable "brooder" thermostatic controls, as well as the more expensive room thermostats. The standard ones available

cost a few cents and will handle some 5 amp. at 115 volts (a-c).

For all ordinary purposes a visit to the local radio store will uncover a variety of relays (both a-c and d-c types), of quite remarkable sensitivity and endurance, costing in the order of $5.00 or less. A slightly more expensive instrument and one that I have found by experience to be an almost ideal laboratory tool, is the type 29XAX in the collection of fine relays made by Struthers-Dunn, of Philadelphia. This compact device operates on 5 Ma. at 115 a-c and is rated to carry 2 amp. at that same voltage. It actually carries heavier loads quite comfortably, providing circuit interruption is not too frequent. This type of relay has the advantage of working directly off the house current. If for some reason the high voltage is objectionable at the control point, a similar relay, wound for a lower voltage used with a step-down transformer, can be used. Both relays and transformers are now readily available and obviate very largely the use of batteries. (O. S. GIBBS, *1544-46 Netherwood, Memphis, Tennessee.*)

Reflection on the mechanism of action of chemotherapeutic drugs has led to the concept of specific bacterial enzyme inhibition. The exact mechanism of the inhibition is not yet known [see reviews by Henry (*Bact. Rev.*, 1943, **7,** 175), Frieden (*Texas Rep. Biol. Med.*, 1945, **3,** 569), and Mudd (*J. Bact.*, 1945, **49,** 527)].

Obligate intracellular organisms are dependent on some of the enzyme systems of the host cells, and their growth is affected and can be influenced by varying enzyme metabolism of the host cells. It has been shown by Greiff, Pinkerton, and Moragues (*J. exp. Med.*, 1944, **80,** 561) that rickettsial growth is depressed by the host cell enzyme activator, p-aminobenzoic acid (PABA). Presumably, the metabolic stimulation of the host cells by PABA makes it an unfavorable environment for rickettsial proliferation, which proceeds at an accelerated rate under conditions of lowered cellular metabolism as produced by sulfonamides, sodium fluoride, or deficiency of riboflavin.

For the control of rickettsial infections it is desirable to increase cell metabolism, inasmuch as rickettsial growth is increased in slowly metabolizing cells whether produced by vitamin, protein, or oxygen deficiencies or following radiation trauma. PABA has been found effective in endemic and epidemic typhus, Rocky Mountain spotted fever, and scrub typhus [see review by Anigstein and Bader (*Texas Rep. Biol. Med.*, 1946, **4,** 260)].

Sprunt (*J. exp. Med.*, 1942, **75,** 297) confirmed Rivers' clinical impression that vaccinia virus "is less able to multiply in the poorly nourished cells than in the well nourished one."

Foster, Jones, Henle, and Dorfman (*Proc. Soc. exp. Biol. Med.*, 1942, **51,** 215; *Science*, 1943, **97,** 207; *J. exp. Med.*, 1944, **79,** 221; 1944, **80,** 257) demonstrated that deaths from poliomyelitis virus (Lansing strain) and especially paralysis decreased in mice subjected to thiamine deficiency, restricted food intake, or both. Rassmussen, Waisman, Elvehjem, and Clark (*J. inf. Dis.*, 1944, **74,** 41) reported similar findings for the Lansing strain of poliomyelitis virus as well as for Theiler's virus. Presumably, the host cell metabolism (cocarboxylase) is so inhibited as to be insufficient to support poliomyelitis virus growth, although it seems to be sufficient for cell survival in most instances.

It seems to date that the therapeutic implications of these observations have not been sufficiently emphasized and investigated, although Mudd (*J. Bact.*, 1945, **49,** 527, footnote 2) implies the use suggested below. An attempt might be made to produce a vitamin (coenzyme) deficiency in the early stages of the disease which will make host cells an unsuitable environment for further virus proliferation. Possibly this is analogous to the action of sulfonamides in certain infections with the ornithosis and lymphogranuloma group of viruses (although a direct effect on the virus is difficult to exclude, since virus does not multiply demonstrably apart from living cells).

A thiamine deficiency may be produced by feeding such homologues as pyrithiamine, 2-n-butyl thiamine, or o-aminobenzyl-methyl thiazolium chloride. Possibly this deficiency in susceptible cells might be brought about rapidly, severely, and safely enough in the early stages of infection, thereby depressing further multiplication of poliomyelitis and possibly other neurotropic viruses (increasing "natural resistance") until the acquired immunity mechanisms are brought into operation.

Other intracellular infections might respond to vitamin-deficiency-producing drugs. It has been shown by Seeler and Ott (*J. inf. Dis.*, 1944, **75,** 175) that riboflavin deficiency in chickens produces lighter infections with *Plasmodium lophurae* malaria than in normal controls. In this case galactoflavin or isoriboflavin may be efficient in producing such riboflavin (flavoprotein dehydrogenase enzyme) deficiency. Mudd has also pointed to the structural similarity of riboflavin and atabrine, the antimalarial drug.

Some of the other vitamin antagonists (homologues, vitagonists) are pyridine-3-sulfonic acid and β-acetylpyridine for nicotinic acid; 4-desoxypyridoxine for pyridoxine; desthiobiotin, biotin-sulfone, and imidazolidone caproic acid for biotin; phenylpantothenone and pantoyltaurine for pantothenic acid; dicumarol, iodinine, and salicylic acid for vitamin K (see Woolley, *Science*, 1944, **100,** 579; *Adv. Enzymol.*, 1946, **6,** 129; Roblin, *Chem. Rev.*, 1946, **38,** 255).

Species differences with respect to the response to vitamin deficiencies have been observed. Rats could be protected against a hemolytic streptococcus by pantoyltaurine, whereas mice, whose blood pentothenate level is 5–10 times higher, could not be so protected (McIlwain and Hawkins, *Lancet*, 1943, **1,** 449). Thiamine deficiency did not significantly effect poliomyelitis infection (Lansing strain) in cotton rats (Weaver, *Amer. J. Dis. Child.*, 1946, **72,** 6), whereas mice were markedly protected by such deficiency. This is perhaps significant, inasmuch as the Lansing strain from primates must be passaged through cotton rats before it produces infection in mice. Perhaps the enzyme systems in the cotton rat and monkey support poliomyelitis virus proliferation more easily; there is a larger "margin of survival" with correspondingly decreased possibilities to effect a critical degree of inhibition.

The enzyme system of the host cells upon which each particular intracellular organism depends must be identified and inactivated by enzyme inhibitors. Metabolic studies such as those by Kabat and others (*J. exp. Med.*, 1944, **80,** 247; 1942, **76,** 579) may point the way. Host cell enzyme inactivation can be achieved biologically (virus interference) as well as chemically (vitagonists, amino acid homologues). Viral enzyme inactivation can be effected by penicillin and possibly sulfonamides. An approach along these lines, although hypothetical, may be in a promising direction. (J. K. FRENKEL, *University of California Medical School, San Francisco.*)