very consistent and produced an almost complete reduction of the number of parasites for 20 hours. Penicillin K, on the other hand, was of very low activity even if a four times higher dose was given. The presence of 30 per cent penicillin K in mixtures of G and K was evident not only by the lower initial reduction but especially by the increase of parasites after 20 hours. The ratio t 3 : t 20 was in all instances greater than 1.1. If 50 per cent K were present in the mixtures, a still greater drop in activity was observed. In the untreated controls the number of parasites increased steadily and was generally 50–100 per cent higher after 3 hours and three to five times higher than the initial count after 20 hours. Similar results were obtained with the other Borrelia strain.

TABLE 1

ASSAY OF PENICILLINS G AND K AND MIXTURES OF G AND K IN *B. novyi* INFECTIONS OF MICE
(Initial count/100 fields: 798 ± 160; single subcutaneous treatment)

Penicillin		Dose units/kg.	Reduction from initial parasite count (%)		Ratio t 3 : t 20
% G	% K		t 3*	t 20†	
100	0	25,000	98.7 ± 1.8	92.5 ± 3.6	1.06
70	30	25,000	93.4 ± 5.3	75.6 ± 9.2	1.22
50	50	25,000	84.3 ± 6.9	46.0 ± 2.3	1.83
0	90	100,000	76.0 ± 3.7	53.7 ± 11.8	1.43

* 3-hour interval.
† 20-hour interval.

From these experiments the conclusion was drawn that 25,000 units/kg. of a penicillin containing more than 70 per cent penicillin G would reduce the initial number of *B. novyi* by not less than 95 per cent (usually more) within 3 hours, the reduction lasting 20 hours.

Although there are indications that this *in vivo* assay technic might be developed to a method of higher sensitivity, it seems that approximately 30 per cent K could be determined in a mixture of active penicillins with the present procedure.

Routine assays with penicillin mixtures from production batches demonstrated that the Borrelia test carried out with crystalline G and an artificial mixture of 70 per cent G and 30 per cent K as standards was sufficiently sensitive for practical purposes, e.g. for the study of the influence of precursors.

Whether the *in vivo* assay will be preferable to the differential assay with *Bacillus subtilis* R and *Staphylococcus aureus* (*3*) for the purpose of production control cannot yet be decided. In case of artificial mixtures there was a fairly good agreement of the *in vitro* and *in vivo* determinations.

The question arose whether other *in vivo* assay methods, e.g. with bacterial infections, could be used for the determination of penicillin K. The low activity of penicillin K in pneumococcal and streptococcal infections as demonstrated by Eagle and Musselman (*2*) seemed to suggest such a possibility. It may be seen from Table 2 that the presence of 50 per cent K in an artificial mixture of pure penicillins could easily be detected; the presence of 30 per cent K did, however, not interfere significantly with the antibacterial activity.

TABLE 2

ACTIVITY OF PENICILLINS G AND K AND THEIR MIXTURES IN EXPERIMENTAL INFECTIONS OF MICE WITH 1,000 MLD OF TYPE 1 PNEUMOCOCCI (STRAIN 6301) AND β-HEMOLYTIC STREPTOCOCCI (STRAIN #4)

Penicillin		Total dose units/kg.	Organism	Number of mice survivors		Survivors (%)
% G	% K					
100	0	3,000	type 1 pneumococci	20	14	70
75–70	25–30	3,000		10	9	90
50	50	3,000		10	2	20
25	75	3,000		10	3	30
0	90	6,000		10	1	10
0	90	12,000		10	4	40
..	..	Controls		20	0	0
100	0	1,000	β-hemolytic streptococci	10	9	90
70	30	1,000		10	7	70
50	50	1,000		10	4	40
..	..	Controls		10	0	0

Even if it would be possible to increase the sensitivity of an *in vivo* assay method in bacterial infections sufficiently for the determination of smaller quantities of penicillin K, these methods would always require at least a five-day observation period before a definite result could be obtained.

The advantage of the Borrelia assay technic is that it requires no more time than the *in vitro* methods.

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Apparatus for the Prolonged Sterile Culture *in Vitro* of Whole Plants or Excised Plant Tissues

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An apparatus for prolonged sterile culture *in vitro* of whole plants or excised plant tissues should meet

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the following requirements: (1) The nutrient medium should be easily removable for renewal or analysis without disturbing the cultures; (2) adequate aeration of roots or isolated tissues should be afforded; and (3) the fluid medium should be kept in circulation to ensure an even supply of nutrient. These requisites can be met by the type of culture vessel shown in Fig. 1, used in conjunction with the apparatus shown in Fig. 2.

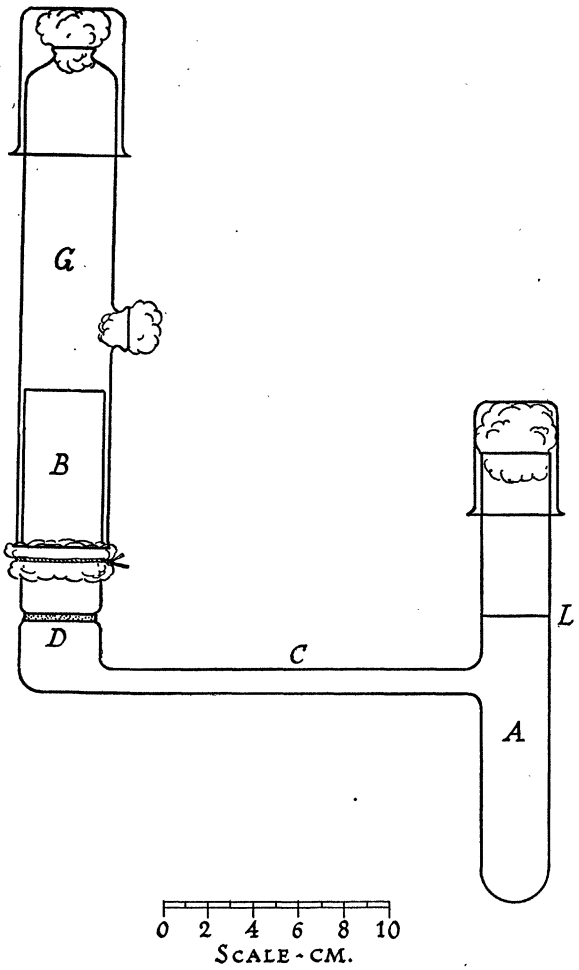

FIG. 1

The culture vessel consists of two main parts: the nutrient medium reservoir, A, and the growing chamber, B. These are connected by a sidearm tube, C. At the base of the growing chamber is a sintered glass disc, D, on top of which can be placed the substrate, a layer either of washed quartz sand or of small glass balls, depending upon the nature of the culture material. On this layer is placed the material to be cultured. The reservoir, A, is filled with sterile nutrient medium to level L, which ensures that, when the vessel is held upright, the medium in B reaches the level of the sintered glass disc. The liquid nutrient medium is brought into contact with culture material by slowly tilting the culture vessel so that the position of B is lowered while that of A is raised. As a result of this change of position, the fluid nutrient medium rises through the sintered glass disc to the desired level in the growing chamber. The rise of the fluid level in B can be adjusted either to immerse the material completely or merely to flood the substrate on which the tissue culture is placed. A detachable tube, G, is fitted over the open top of the growing chamber and rests on a layer of cotton, wrapped and tied securely around the growing chamber. The height of the tube, G, with the cotton wrapping supporting it, can be adjusted to allow increased space for growth increment

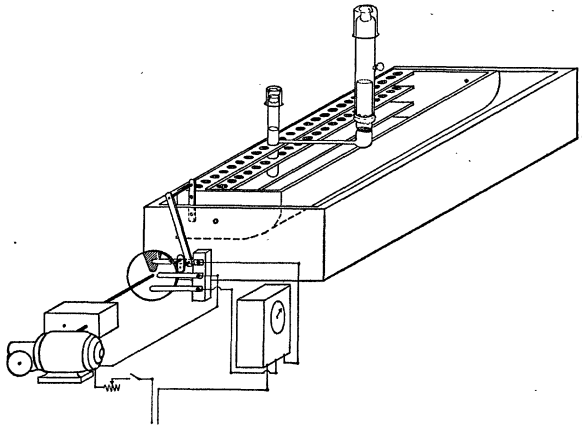

FIG. 2

of the culture. Side and top openings in the detachable tube make possible the manipulation of the culture material without exposing it to the atmosphere.

The apparatus presented in Fig. 2 consists of a rectangular wooden frame within which fits a rack attached to the frame by means of a pivot at each end. The A tube of the culture vessel is fitted into the rack which, when in the horizontal position, holds the culture vessel upright.

At one end of the frame and on a platform attached to it is secured the mechanism which furnishes the motion for tilting the rack. The power is supplied by an electric motor working through a reduction gear and attached, by a shaft, to a cam. A small and a large operating arm, joined by a rod, convert the rotating motion of the cam to a reciprocating motion which tilts the rack. The small operating arm is attached to the camshaft, and the large operating arm to a rod secured to the rack at one pivoted end. A time switch, which activates the electric motor, is also attached to the cam. An insulated strip inserted in the brass cam breaks the circuit to the motor when the rack has been tilted to the necessary angle. It

remains thus tilted until the motor is again activated, the circuit being broken by the insulated strip when the rack has returned to the horizontal position. By this means the culture material can be maintained in contact with the nutrient medium for any given period.

The size and form of the growing chamber, B, can be made to conform to the requirements of the material to be cultured. Entire plants, such as sunflowers, require a vessel 35×200 mm. in diameter, containing quartz sand to a depth of 170 mm. For the culture of small fragments of plant tissue the upper tube, G, can be dispensed with, and the fragments cultured in a tube 25×200 mm. Molds also can be cultured by this means, provided that the fluid medium level is so arranged as to ensure the maintenance of a layer of liquid between the mycelium and the sintered glass disc, which must be of sufficient fineness to prevent spores from being washed back into the nutrient medium reservoir. Optimum frequency of immersion must be determined separately for each type of tissue. In general, plant tissues grow most satisfactorily if their immersion is infrequent. One immersion of 5 minutes duration in a period of 24 hours was sufficient for sunflower stem tissue if supported on quartz sand. The nutrient medium can be changed as often as is desired by emptying vessel A with a sterile pipette and introducing fresh nutrient to level L.

A High-Capacity Sensitive Relay

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A high-capacity relay is often needed for the control of heating elements in constant-temperature cabinets and water baths that will operate on a minimum amount of current. The sensitivity of most thermostats is usually affected by the arcing at the contact points. Such a relay has been developed to reduce this arcing to a minimum.

By using a solenoid instead of an electromagnet it is possible to operate an arm bearing a mercury contact tube with a minimum amount of current. Mercury contact tubes are available with rated capacities of 5–35 amperes.

The assembled relay is diagramed in Fig. 1. The base for the relay was constructed from seasoned oak (K). The mounting for the solenoid was made from a brass plate (A). Four small holes were drilled in the base for roundheaded brass screws. The holes were made slightly larger than the screws in order to permit final adjustments in the position of the solenoid in relation to the arc described by the end of the arm. A hole ¼ inch in diameter, of the same size as the brass casing of the solenoid (B), was drilled in the center of the brass mounting plate, to which the solenoid casing was then soldered. The bearing support (F) for the mercury tube was made by cutting a rectangular brass bar into three pieces, two pieces 2 inches long for uprights and one 3 inches long for the base, and soldering them together to form a U.

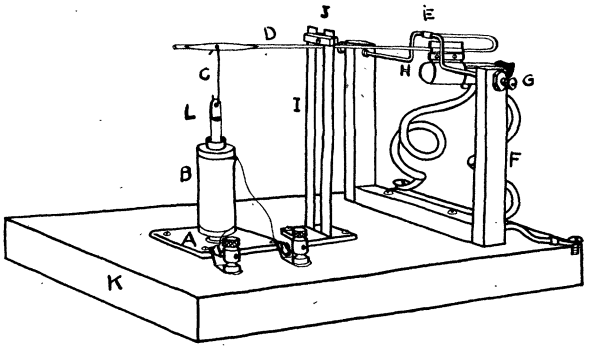

Fig. 1

It was necessary to make a wide support for the mercury tube to allow free movement of the connecting wire. Holes (G) ⅛ inch in diameter were drilled ½ inch from the top of the support and threaded with an 8–32 tap. Through these holes were screwed two ½-inch brass bolts (G), the ends of which were drilled to a depth of 1/16 inch to provide a bearing for the relay arm assembly. Each bearing was held in a fixed position by a hexagonal lock nut.

The arm (D) for the mercury tube was made from 12-gauge, galvanized-iron wire about 9 inches long and bent into the shape shown in the diagram. Both ends were flattened, one for drilling the hole for the link to suspend the soft-iron plunger and the other end to solder to E. A second piece of 12-gauge wire (E) was bent to form a U, 1 inch in width and height, with projections to be used as a shaft. The U wire was then soldered to the arm (D) 4 inches from the hole drilled for the iron plunger (L). The holder for the mercury tube was made from light-gauge tin plate. The mercury tube and holder (H) were then clamped to the arm by small machine bolts. This type of clamp provided a final adjustment of the instrument and made it possible to reverse the action of the tube for special requirements. The movement of the arm was controlled by means of an adjustable stop made of two small pieces of heavy brass (J) fastened with small machine bolts to two strips of brass (I) soldered to a brass base. The connecting link (C) for the soft-iron plunger was made from a piece of 16-gauge steel wire after assembly of the instrument. The soft-iron plunger was made from a piece of 6-gauge iron wire,