

In 1933 Leriche and Fontaine (3) foresaw the value of free muscle grafts for the replacement of myocardial infarcts. However, their work was very limited and was never taken up by others.

Basic studies on the regeneration and reconstitution of free muscle grafts are indicative of the applicability of this method to clinical surgery. The regeneration of small sections of mammalian striped muscle grafted in situ and/or transplanted at right angles to its original orientation was studied by LeGros Clark (2). Reconstitution of muscle was manifested by fine plasmodial outgrowths from the ends of the surrounding muscle and by sarcoplasmic buds from the muscle graft. The initial fibroblastic union between recipient muscle and the donor graft was replaced rapidly by young muscle fibers crossing in parallel formation into the graft. The directional pathway was determined by the structure of the degenerating transplant rather than the course of the original muscle fibers. After 18 days the majority of new muscle fibers had penetrated the graft. Thereafter, maturation was rapid, and the usual skeletal striations were present histologically. These experimental studies have provided evidence in support of the practicability of the transplantation of free muscle grafts.

The present investigation consisted of an attempt to graft free skeletal muscle onto the intact myocardium of dogs. Nine dogs of variable age, and ranging in weight from 10 to 17 kg., were used. The animals were anesthetized by intravenous injections of sodium pentobarbital. Positive pressure in the lungs was maintained when the thoracic cage was opened. The muscle graft, rectangular in shape and averaging 7 × 4 cm., was obtained from the anterior abdominal wall (internal oblique) or from the lower extremity (vastus lateralis). It was placed around the heart and anchored thereto by means of three or four fine cotton sutures. The epicardial surface of the heart was not scarified. The pericardium was sutured over the graft, the latter being usually included in the suture line. The Emerson respirator proved a valuable adjunct in re-establishing voluntary respirations immediately following the operation.

Except in the infected cases, the postoperative course of the dogs was accompanied neither by shock nor by severe disability, and within two or three days they were up and about in their kennels. After recovery, the dogs were exercised daily. The animals showed no signs of cardiac incompetency or depletion of cardiac reserve. After a period of 10 to 15 weeks the animals were anesthetized and the cardiac transplants examined.

In this series of nine dogs, six animals were sacrificed and the other three preserved for special studies. In the former group two completely success-

ful muscle-graft "takes" were found, while in a third, islands of regenerating muscle were present. In the other three dogs there was total absorption of the muscular elements of the graft with replacement by a connective tissue layer not unlike that of fascia. Signs of intrapleural, pulmonary, and pericardial infection were evident in these cases. The three dogs still alive have shown no signs of postoperative infection. One of them was reoperated upon after 15 weeks, and a branch of the left coronary artery was ligated. Upon opening the pericardium the graft was found to have taken completely.

Examination of the dogs with the free muscle-graft "takes" revealed several significant facts. The grafts were well fixed to the myocardium with little or no mobility. The transplant was easily identified over the ventricle and showed practically no shrinkage. However, the skeletal muscle graft had lost its reddish color and, on section, appeared tan-yellow with white trabeculae of connective tissue. Between the graft and myocardium the epicardium was thicker than normal with a rich vascular network. Sections taken through the graft showed a normal muscle histology with no histopathological variations in either the nuclei or the muscular components. The usual cross striations characteristic of skeletal muscle were present. On examination of the heart with the thoracic cage open, no deleterious effect on cardiac function and blood circulation from the standpoint of cardiac dilatation, extrapericardial adhesions, or intrapericardial pressure was observed.

Sufficient evidence has been accumulated from these experiments to state that free muscle transplants may successfully be grafted upon the myocardium of the dog's heart.

References

1. BECK, CLAUDE S. *Ann. Surg.*, 1943, **118**, 788.
2. CLARK, LEGRS W. E. *J. Anat.*, 1946, **80**, 24.
3. LERICHE, R., and FONTAINE, R. *Bull. Mem. Soc. nat. Chir.*, 1933, **59**, 229-232.
4. O'SHAUGHNESSY, L. *Brit. J. Surg.*, 1936, **23**, 665.

Effect of Penicillin on Seed Germination

WALTON J. SMITH¹

Chas. Pfizer & Company, Inc., Brooklyn, New York

Many clinical and biochemical differences have been reported between pure, crystalline penicillins and the crude, yellow grade of therapeutic penicillin used for clinical purposes. Lewis (2) reported a growth inhibition toward cancer tissues of therapeutic penicillin, but purified penicillin showed no such inhibition. It was hoped that seed germination tests could be used to measure this anticarcinogenic activity. Work done

¹The author wishes to express his appreciation to Dr. George S. Avery, Jr., of the Brooklyn Botanic Garden, for his valuable advice in connection with this work.

by the author over a year and a half ago showed that while therapeutic penicillin inhibited seed germination, crystalline sodium penicillin G had little such effect. This antigermination activity was shown to be due to aromatic acids of the indole-3-acetic acid type, which are commonly present in crude penicillin preparations.

The work recently reported by Ribeiro (3), in which penicillin was found to be exceedingly active in inhibiting germination, suggested that impure penicillin was used. The author did not describe the nature of the penicillin used.

In the present study of sodium penicillins G, K, and dihydro F (constituents of commercial penicillin), all were found to be relatively inactive in inhibiting germination of radish seeds. Streptomycin sulfate, whether purified or crude, was also relatively inactive when treated in the same way.

Phenylacetic acid, indoleacetic acid, and furoic acid had been found in all lots of the therapeutic preparation tested and were therefore tested separately for antigermination activity. Each of the three substances was active against germinating seeds; however, most of the inhibiting property of therapeutic penicillin could be accounted for by the indole-3-acetic acid present. At concentrations at which the other substances permitted nearly normal elongation, indole-3-acetic acid permitted only 7 per cent of the normal root elongation in germinating wheat seeds (4). Analysis of the particular lot of therapeutic penicillin used for the germination tests (Pfizer lot 755) by the method of Holt and Callow (1) showed 2.1 per cent indoleacetic acid.

The germination tests were made in 90-mm. Petri dishes, the seeds being placed on filter paper dampened with 5 ml. of solution. They were allowed to germinate for several days in the dark, after which time the percentage of germination was observed. A more quantitative measurement of growth was the length of the shoots and roots of the germinated seeds.

The penicillins used were extensively purified² by means of silica gel chromatographs saturated with phosphate buffers. They were further purified by recrystallization.

Most of the experiments were carried out with radish seeds (var. Sparkler), but similar results were observed with spring wheat when therapeutic penicillin was compared with crystalline sodium penicillin G. Therapeutic penicillin and indole-3-acetic acid have been tested with many different kinds of seeds and found inhibitory to all.

Since therapeutic penicillin was inhibitory to about the same degree reported for "penicillin" by Ribeiro, against lettuce, it may be concluded that Ribeiro actually used therapeutic penicillin for his tests and that his findings were not due simply to the use of a different variety of seed.

The nature of the stunted growth of roots caused by the impurities in therapeutic penicillin and by the aromatic acids was very similar. Instead of normal root elongation, there was a marked enlargement of the hypocotyl. Sunflower seed from which the seed coat was removed was affected similarly; in addition, the cotyledons were curled away from one another. The cucumber behaved similarly. Of the seeds tested, cabbage was the most sensitive to indoleacetic acid.

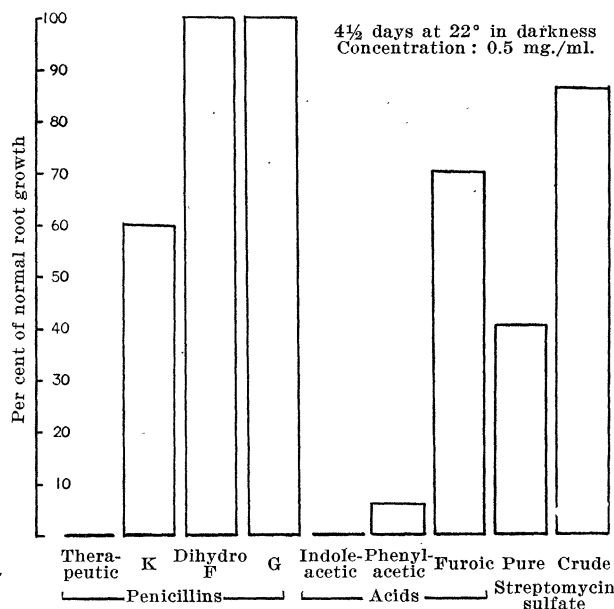


FIG. 1. Germination tests on radish seeds.

The accompanying chart (Fig. 1) illustrates the comparison of the action on seeds at one concentration of each of the substances tested. The data included in the chart are representative of those at other concentrations.

Data on streptomycin in pure and crude form have been included for comparison. The rather low toxicity of streptomycin and penicillins to seed germination and growth suggests their possible use in antiseptics for seed treatment and plant sprays.

Summary. The substances present in therapeutic penicillin which cause inhibition of germination and root growth are represented by the indole-3-acetic acid type of compound. Since this and phenylacetic acid were known to be present in the penicillin tested, it is concluded that they are responsible for the inhibition activity against seeds.

² The purified antibiotics were made available through the courtesy of Dr. R. Pasternack; the penicillins were purified by Dr. V. Bogert, and the streptomycin by Mr. I. A. Solomons, all of Chas. Pfizer & Company.

None of the crystalline penicillins tested appreciably retarded germination.

References

1. HOLT, P. F., and CALLOW, H. J. *Analyst*, 1943, **68**, 351.
2. LEWIS, M. R. *Science*, 1944, **100**, 314.
3. RIBEIRO, D. F. *Science*, 1946, **104**, 18.
4. SCHMID, H. *Helv. Chim. Acta*, 1944, **27**, 1197.

A New *Helminthosporium* Blight of Oats¹

FRANCES MEEHAN

Botany and Plant Pathology Section
Iowa Agricultural Experiment Station

H. C. MURPHY

Bureau of Plant Industry, Soils and Agricultural
Engineering, U. S. Department of Agriculture

A new *Helminthosporium* disease affecting mainly oat varieties and selections possessing the Victoria-type resistance to crown rust has become widespread in most oat-growing regions of the United States (1, 2). Although the first isolation from oats was made in November 1944, the organism was previously isolated from timothy seed. Numerous field isolations were obtained in 1945, and in the 1946 oat season infection was so severe in many areas as to cause serious reduction in yields. The fungus is known to have been present in 19 states in 1946, from Texas to New York and from Florida to Idaho.

Plants infected in the seedling stage were characterized by necrosis of the basal portions, and striping or reddening of the leaves, progressing upward from the lower leaves. The same symptoms were evident on plants in later stages of maturity, but the basal stem and root-rot became the primary factors in identifying the disease, since striping and discoloration of leaves may be due to a number of causes. The leaf striping is believed to be a secondary toxic effect of basal infection. Mature plants in the field were blackened at the nodes with abundant sporulation of the fungus, and the lower internodes showed a characteristic brownish translucence. Culms weakened by severe infection broke over near the ground line and at the lower nodes, and excessive lodging made harvesting of many fields difficult.

The species of *Helminthosporium* responsible for this destructive disease of oats resembles three other members of the genus in several respects: *H. setariae* Sawada, *H. sacchari* Butler, and *H. sativum* Pam. King and Bakke. These similarities will be discussed in detail in a subsequent paper. Since, however, no

description of a species of *Helminthosporium* which corresponds satisfactorily to this species has been found in the literature, it is proposed that it be recognized as a new species under the name *Helminthosporium victoriae*. This specific name is suggested because of the potential importance of this parasite as the cause of a foot-rot and leaf-stripe disease of oat varieties and selections possessing the Victoria resistance to crown rust (*Puccinia coronata avenae* (Corda) Eriks. & E. Henn.).

HELMINTHOSPORIUM VICTORIAE SP. NOV.

Conidiophoris erectis, simplicibus, pallide olivaceis usque brunneis, 60–280 × 5.8–10 μ , 4–10 septatis, apicibus geniculatis 30–80 μ ; conidiis pallide olivaceis subcurvatis, elongato-ellipsoideis parte superiori plerumque angustiori, hilis aliquantulus protrudentibus, 40–130(70) × 11–25(15) μ , 4–11(8) septatis, muris modice tenuibus, tubulo uno e quaque cellula terminali germinantibus.

Hab.—In radicebus et culmis *Avenae sativae* L. (typus) et *A. byzantinae* C. Koch et hybridis inter eas parasiticis; et in plantis variis saprophyticis vel leniter parasiticis.

Conidiophores form velvety growth on lower nodes and sparse fructifications on basal leaf sheaths of mature oat plants. Conidiophores are erect, simple, emerging usually singly or occasionally in clusters of 2 to 5 from stomata or from between epidermal cells of infected culms, and measure 60–280 μ in length × 5.8–10 μ in width with 4–10 septa, mostly 120–160 μ × 6.5–7.8 μ with 6–8 septa; they are light olivaceous to medium brown and have a rather closely geniculated apical spore-producing area, 30–80 μ in length.

Conidia are fuliginous to dark olivaceous but typically light olivaceous, slightly curved, rounded at the base, widest near the center, and tapering to a rounded tip. Normal conidia measure 40–130(70) μ × 11–25(15) μ with 4–11(8) septa, have moderately thin walls, and germinate by one polar germ tube from each terminal cell, the basal germ tube emerging adjacent to the slightly protruding hilum. Conidia produced on water agar approach normality but are somewhat smaller and have fewer septa. Weathered spores at bases of mature plants in the field frequently are atypical, dark brown, irregular in shape, and with thick exospore. Typical cultures form a light- to medium-gray tufted colony on oat agar. One saltant, a profusely sporulating strain, produces a dark greenish-black colony.

The fungus is evident chiefly on the basal portions of *A. sativa* L. and *A. byzantina* C. Koch and hybrids between them, producing necrosis of roots and lower stem parts. On immature plants, it causes reddish-

¹Journal Paper No. J-1396 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 72: cooperative investigation between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture, and the Botany and Plant Pathology Section of the Iowa Agricultural Experiment Station.