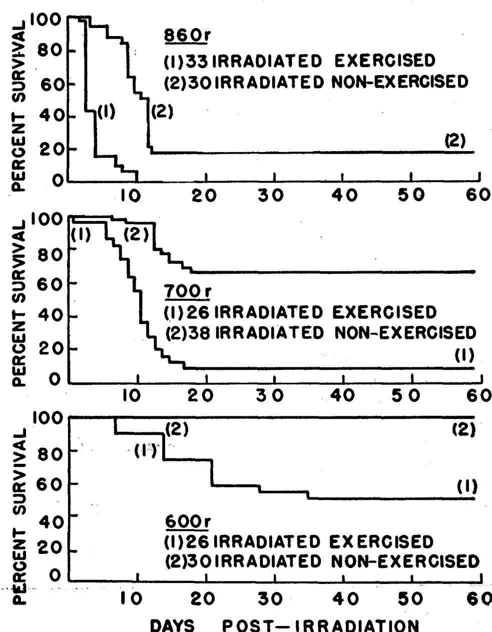

FIG. 1. Effect of exhaustive exercise upon postirradiation survival.

doses (700 r–860 r) that are, to a degree, lethal for nonexercised animals, the exercised animals had a much shorter postirradiation survival time, as well as a higher mortality rate.

As the animals were immersed in water during the period of exercise, it became necessary to know whether exposure to water without exercise would alter the mortality of irradiated rats. After a dose of 860 r, 30 irradiated rats were immersed to the neck in the swimming tanks 20 min daily for 3 weeks in wire containers that did not permit the animals to exercise, without altering the mortality rate from that of nonexposed irradiated rats. To determine whether exercise prior to irradiation would alter the mortality rate, a group of 20 rats, irradiated with 860 r, was exercised exhaustively for ten trials before irradiation and given no further treatment. The mortality rate was similar to that of nonexercised irradiated animals; hence it appears that the mortality rate is altered only if the animals are exercised after irradiation.

The increase in mortality with exercise following irradiation could not be correlated with alterations in total body weight. The time at which minimum body weight had been reached following radiation was similar for exercised and nonexercised animals. The weight loss was not sufficiently greater in exercised animals to provide evidence for a correlation between weight loss and increased mortality.

It is apparent that the lethality of roentgen rays at the doses studied (600 r, 700 r, and 860 r) is increased by the repeated performance of vigorous exercise following irradiation. It is suggested that the increased mortality observed with animals exercised after irradiation provides additional evidence of the relation between radiosensitivity and metabolic level.

References

1. ANDERSON, R. S., and TURKOWITZ, H. *Amer. J. Roentg. rad. Therap.*, 1941, **46**, 537.
2. BLOUNT, H. C., and SMITH, W. W. *Science*, 1949, **109**, 83.
3. COOK, E. V. *Radiology*, 1949, **32**, 289.
4. EVANS, T. C., GOODRICH, J. P., and SLAUGHTER, J. C. *Proc. Soc. Exp. Biol. Med.*, 1941, **47**, 434.
5. ———. *Radiology*, 1942, **38**, 201.
6. HEMPELMANN, L., TRUJILLO, T., and KNOWLTON, N. *Nuclear Sci. Abstr.*, 1949, **3**, 46.
7. KIRSCHNER, L. B., PROSSER, C. L., and QUASTLER, H. *Proc. Soc. Exp. Biol. Med.*, 1949, **71**, 463.
8. MOTTRAM, J. C. *Brit. J. Radiol.*, 1938, **8**, 32.
9. PATT, H. M., and SWIFT, M. N. *Amer. J. Physiol.*, 1948, **155**, 388.
10. SMITH, W. W., *et al.* *Proc. Soc. Exp. Biol. Med.*, 1949, **71**, 498.
11. STRANGEWAYS, T. S. P., and FELL, H. B. *Proc. Roy. Soc. Med.*, 1927, **B 102**, 9.
12. To be published.

Adaptive Production of Amylase and Lipase by Three Species of Fungi[1]

J. J. Goodman

Department of Plant Pathology, University of Minnesota, St. Paul

Recently, it was found that several bacteria and yeasts form certain enzymes adaptively (*4, 6, 9*). The present paper describes what appears to be adaptive production of amylase and lipase by three fungi.

Aspergillus flavus Link, *A. terreus* Thom, and a species of *Penicillium* tentatively identified as *P. notatum* Westling were grown at room temperature in 250-ml Erlenmeyer flasks containing 50 ml of medium. The basal medium for all the experiments was that of Czapek-Dox (*8*). Sucrose and soluble starch were compared as carbon sources of amylase production, and sucrose was compared with Mazola corn oil as a carbon source for lipase production.

Amylase. Twenty milliliters of a 1% agar solution containing 0.1% soluble starch and, as a preservative, 1 part formaldehyde in 1,500 parts of medium, was dispensed into each of several Petri dishes and allowed to harden. Filter paper disks 15 mm in diam were dipped into a solution or suspension of the material to be tested for amylase activity, the excess was drained off, and the disks were placed on the agar. Four disks were applied to each plate, three disks containing active material and one containing material heated in boiling water to serve as a control. The dishes were incubated at 30° C for 24 hr, and then were flooded with iodine-potassium iodide. The diameter of the clear zone around each disk was used as a measure of the amount of amylase present (*5*).

The amylase activities of the mycelium, and of the culture liquid in which the mycelium was growing, were tested. The mycelium was ground in several changes of

[1] Paper No. 2542, Scientific Series, Minnesota Agricultural Experiment Station.

acetone, and given a final wash in ether, as described by Tate (7), and the filter paper disks were dipped in a 1% suspension of this powder in distilled water. The culture liquid was tested without dilution.

Results of such tests with *A. flavus* and *A. terreus*, growing on 3% sucrose and on 2% starch, are given in Table 1.

TABLE 1

AMYLASE ACTIVITY* OF THE MYCELIUM AND CULTURE MEDIUM OF TWO FUNGI GROWN ON SUCROSE OR STARCH AS A CARBON SOURCE

Days after inoculation	*A. flavus*				*A. terreus*			
	mycelium		medium		mycelium		medium	
	sucrose	starch	sucrose	starch	sucrose	starch	sucrose	starch
5	0	+++	–†	–	0	++	–	–
8	0	++	+	+++	0	+	0	++
11	trace	+	–	–	trace	trace	–	–

* Rated 0 to +++ on the basis of size of zone as judged by eye but not measured.
† Not tested.

A commercial sample of Taka-diastase was diluted with distilled water to give solutions of 10%, 1%, 0.1%, 0.01%, and 0.001%, and these were similarly tested. The results are shown in Fig. 1, each point representing an average of 9 disks. The relationship between diameter of clear zone and enzyme concentration appears to be logarithmic.

Basal media containing 3% sucrose and 1% starch were mixed to give the following combinations:

Medium No.	Parts of: 3% Sucrose medium	Parts of: 1% Starch medium
1	100	0
2	80	20
3	60	40
4	40	60
5	20	80
6	0	100

These media were inoculated with *A. flavus*, and the medium and mycelium were periodically tested for amylase. The results for four of the media are presented in Fig. 2. Media 3 and 4 were essentially similar to 5. Amylase activity increased in both the medium and the mycelium as the amount of starch in the medium was increased. In cultures 34 days old, considerable amylase was still present in the medium, but only a trace was detected in the mycelium.

In another experiment, *A. flavus* was grown for 9 days on media containing different proportions of sucrose and starch. The culture liquid was then poured off, the mat was washed several times with sterile distilled water, and a 0.1% solution of starch, in distilled water containing formaldehyde in the proportion of 1: 1,500, was introduced under the mats in the flasks (2). Aliquots of this solution were tested periodically with iodine-potassium iodide. The results, given in Table 2, confirm those obtained when paper disks were used.

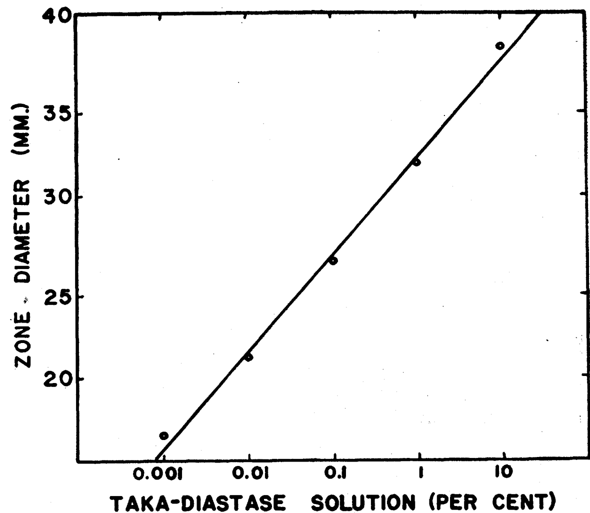

FIG. 1. Relationship between diameter of clear zone and concentration of Taka-diastase using paper-disk assay method.

Lipase. *A. flavus* and *Penicillium* sp. were inoculated into replicate 250-ml flasks containing 50 ml of the following media: (1) Three percent sucrose; (2) 1.5% Mazola corn oil; 0.1% sucrose. The 0.1% sucrose solution was added to the second medium to permit the mycelium

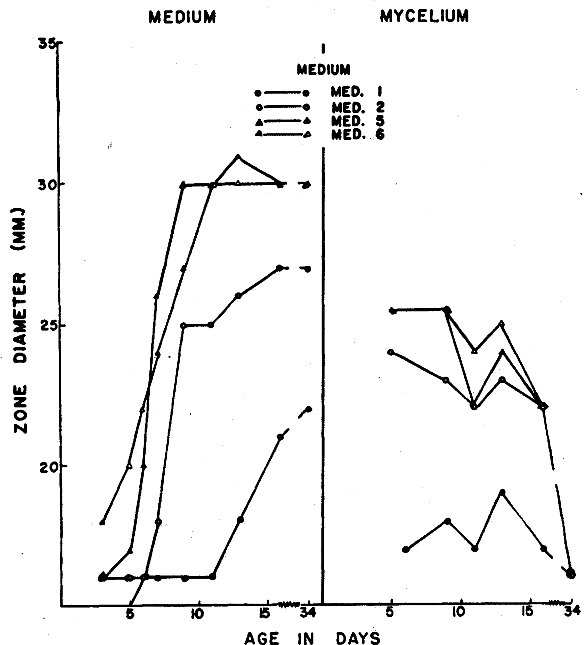

FIG. 2. Amylase activity of the culture medium and mycelium of *A. flavus* grown on media containing different proportions of sucrose and starch. See text for composition of media.

TABLE 2

HOURS FOR STARCH TO SHOW CHANGE FROM BLUE TO VIOLET WITH I-KI

Medium used for growth of fungus				
Percent sucrose	3	2	1	0
Percent starch	0	1	2	3
Hours for replaced solution to show color change	52	52	42	18

to become well established. Agar, 0.25%, was added to both media. The corn oil was emulsified by passing the medium several times through a hand-operated homogenizer, and the agar served to stabilize this emulsion. When a heavy, well-sporulating mat had formed on the surface of the liquid, the mycelium in replicate flasks was harvested and treated as follows:

1. The mycelial mats were ground with ten times their wet weight of 90% glycerol, as described by Fodor (*1*). The ground material was kept at room temperature for 24 hr and then centrifuged to remove most of the cell debris. The centrifugate was stored at 2° C until tested.

2. Acetone powders were prepared as described in the tests for amylase.

3. The mats were air-dried on blotting paper for 24 hr, and then were ground to a fine powder in a mortar. The powder, so prepared, and that prepared with acetone, were kept in a desiccator over $CaCl_2$.

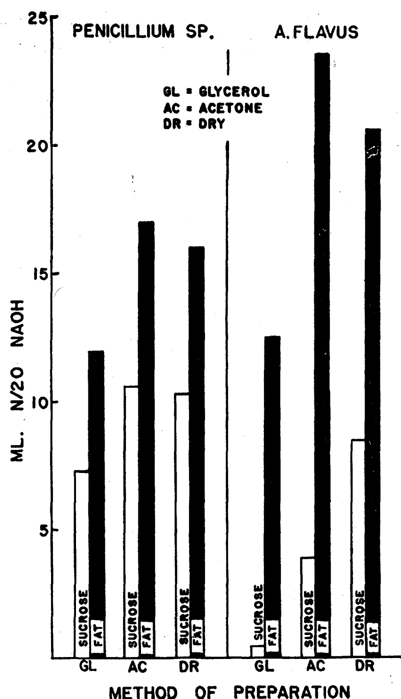

FIG. 3. Lipase activity of the mycelium of two fungi grown on sucrose or corn oil using three methods of preparing the mycelium for testing.

The substrate for testing lipase activity was similar to that described by Peters and Nelson (*3*), consisting of corn oil and 0.25% agar in a citrate-phosphate buffer at pH 6.5. This was dispensed in 10-ml amounts into 125-ml flasks. Three parts of the glycerol extracts were diluted with two parts of buffer before use, and 3 ml of this was added to each 10 ml of substrate. The dry powders were added at the rate of 0.05 g to 10 ml of substrate. Heated controls were included.

The substrate and enzymes were incubated for 48 hr at room temperature (about 22° C). At the end of this period, 50 ml of 1: 1 ether-alcohol was added to each flask, and the fatty acids were titrated with *N*/20 alcoholic NaOH.

The results, representing the average difference between the heated control and the active preparation for three flasks of each treatment, are presented in Fig. 3.

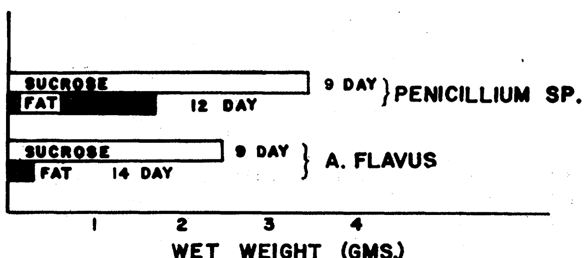

FIG. 4. Weight of mycelium of two species of fungi grown on sucrose or corn oil.

The powders are directly comparable with one another in amounts of fungus material added. It is estimated that the glycerol extracts represent only about ¼–⅓ the amount of fungus material present in the powders. The mycelium of both fungi, in all three methods of preparation, had a much higher lipase activity when grown on the medium containing corn oil than when grown on that not containing corn oil, although an appreciably greater weight of mycelium was produced on the medium not containing corn oil, as shown in Fig. 4.

It has been shown that amylase and lipase are produced adaptively by the fungi used in these tests, considerably more enzyme being produced when the organisms were grown in the presence of the specific enzyme substrated. This is in agreement with the work on adaptive hydrolytic enzymes in bacteria and yeasts. In the present studies enzyme activity was measured in the absence of growth, formaldehyde being used as an antiseptic. It is not known how long the specific activity would be retained had the substrate been changed and growth permitted to continue. The possibility of selection from a heterogeneous population of strains better adapted to a specific medium was not ruled out, and this possibility must be investigated.

The fungi used in these studies are commonly associated with the deterioration of stored grain, and are among several of the organisms being investigated in connection with such spoilage. The results suggest that enzymatic activities of such fungi may be influenced by the substrate on which the fungi are growing, and this may be of some significance in the field of grain deterioration, as well as in the field of commercial production of enzymes.

References

1. FODOR, P. I. *Enzymologia*, 1948, **12**, 343.
2. FOSTER, J. W. *Chemical activities of fungi*. New York: Academic Press, 1949. P. 46.
3. PETERS, I. I., and NELSON, E. E. *J. Bact.*, 1948, **55**, 581.
4. RAHN, O., and LEET, M. *J. Bact.*, 1949, **58**, 714.
5. SHERWOOD, M. B. *J. Bact.*, 1947, **53**, 505.
6. SPIEGELMAN, S. *Cold Spr. Harb. Symp. quant. Biol.*, 1947, **XI**, 256.
7. TATE, P. *Parasitology*, 1929, **21**, 31.
8. THOM, C., and RAPER, K. B. *Manual of the aspergilli*. Baltimore: Williams and Wilkins, 1945.
9. VIRTANEN, A. I. *Svensk Kemisk Tid.*, 1948, **60**, 23.

Determination of Circulating Red Cell Volume by Radioactive Chromium[1]

Seymour J. Gray and Kenneth Sterling[2]

Biophysical Laboratory and Department of Medicine, Harvard Medical School, and Medical Clinic, Peter Bent Brigham Hospital, Boston

Previous investigators (*3–9*) have reported accurate methods of measuring the circulating red blood cell volume by the injection of red cells labeled with radioisotopes. Radioactive iron and radioactive phosphorus were available for these studies. The preparation of red cells tagged with radioactive iron necessitates the administration of radioiron for several weeks to volunteer donors who incorporate it into the hemoglobin of their red cells, which must then be transfused into the experimental subject (*3–5*). Radioactive phosphorus (*6–9*) may be used to label a small volume of the experimental subject's own red cells *in vitro*. However, the phosphate exchanges rapidly between the red cells and plasma, causing the radioactivity of the tagged cells to fall significantly after 1–3 hr (*6–9*).

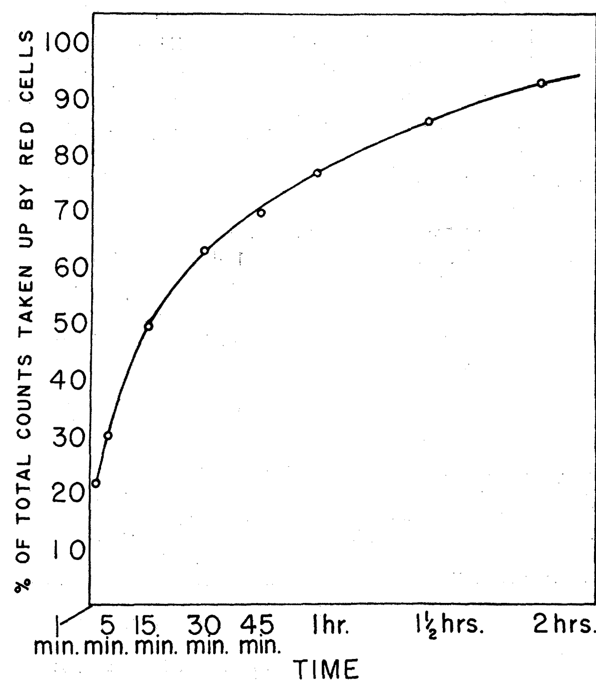

FIG. 1. Uptake of Cr^{51} as $Na_2Cr^{51}O_4$ by human red blood cells; 86 μg chromium added to 2 ml packed red cells suspended in saline at 24° C.

The present study concerns a new biological tracer, radioactive chromium (Cr^{51}), with a half-life of 26.5 days. When this isotope is added to blood *in vitro* as $Na_2Cr^{51}O_4$, it is taken up avidly by the red cells, which retain their radioactivity without significant loss for periods of 1 day or more after injection into experimental animals. The uptake of Cr^{51} by human red cells is illustrated in Fig. 1, demonstrating the marked affinity of red cells for $Na_2Cr^{51}O_4$. Since the exchange of Cr^{51} between red cells and plasma is negligible for 24 hr, this isotope appears ideal for the tagging of red blood cells and the measurement of the circulating red cell volume.

Approximately 50 ml of whole blood was withdrawn from a normal mongrel dog and heparinized. Cr^{51} (7–26 μc),[3] as $Na_2Cr^{51}O_4$, was added to whole blood or a red cell suspension in saline. After an hour, the red cells were washed with either physiological saline or plasma and then resuspended in plasma from the same dog. The plasma radioactivity was less than 1% of the radioactivity of the red cells.

A 4-ml aliquot was removed for counting, and the remaining measured volume of blood containing tagged red cells was injected intravenously into the dog. After allowing time for mixing in the circulation, 4-ml samples were withdrawn for counting at intervals varying from 10 min to 24 hr.

The injected blood and all subsequent blood samples were centrifuged in 4-ml hematocrit tubes, and the plasma was separated. One milliliter of each plasma sample was pipetted into a weighed planchet, dried overnight at 60° C (to constant weight) and counted with a Geiger-Müller counter. The plasma, regardless of the time interval, had no significant counts above background. The packed red cells were dried overnight at 60° C, then ground to a fine powder and counted in weighed planchets, preferably in duplicate. Since Cr^{51} is a soft x-ray emitter, self-absorption corrections were applied to all samples. All counts were corrected for radioactive decay.

[1] This work was supported in part by the U. S. Atomic Energy Commission, the Office of Naval Research, and the National Cancer Institute, U. S. Public Health Service.

[2] The authors wish to thank Arthur K. Solomon and Charles B. Robinson for their assistance in the processing and counting of the radioactive isotopes.

[3] The radioactivity dosage required for this method is well within the tolerance limits for human use. From the disintegration scheme of H. Bradt *et al.* (*1*), the administration of 42 μc of Cr^{51} per kg body wt approximates a total dose of 1 rep (*2*), on the conservative assumptions that there is no excretion and that radiation is localized in the red cells. The amount of chromium injected per kg body wt varied from 1 μg to 20 μg, depending upon the specific activity of the sample. This is well below the toxicity level.

In order to obtain the high specific activity required for this research, samples of chromium, enriched in Cr^{50}, were bombarded in the nuclear reactor. We wish to express our thanks to the Isotopes Division of the Atomic Energy Commission for making this material available.