unchanged, even when the liberator was administered repeatedly. When rats were given Compound 48/80 in increasing dosage during 8 days, they were entirely resistant to edema normally produced by other liberators. These animals, however, excreted as much 5-HIAA after injection of reserpine as did the untreated controls. These observations do not militate in favor of a serotonin mediation in development of anaphylactoid edema in rats.

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Metabolic Effects of *m*-Dinitrobenzene in *Aspergillus niger*.* (26184)

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m-Dinitrobenzene (DNB) is a potent growth inhibitor for molds; concentrations as low as 2.5 x 10^{-5} M produce complete stasis of *Aspergillus niger* growth. Casein hydrolyzates and amino acid mixtures reversed inhibition but it was not possible to establish mechanism of action for these phenomena(1). The present communication reports metabolic observations which are consistent with the proposal that DNB inhibits mold growth by indirectly depressing amino acid synthesis during early growth phase.

Methods and results. Culturing procedures for *A. niger* (Mulder strain)(2) and growth rate methods were as described previously (1). DNB solutions were prepared fresh weekly since activity is gradually lost on standing in stoppered glass containers for longer periods. Although growth inhibitory properties of DNB were overcome by various amino acid mixtures(1), leucine and proline were the only amino acids which, when omitted individually from such mixtures, prevented this reversal. These 2 compounds were, in fact, nearly as effective as the whole mixture of 19 amino acids when substituted on an equal weight basis. Hydroxyproline was without effect, but aspartic and glutamic acids were partially effective substitutes for proline. These results are in accord with known metabolic interconversions of amino acids. It is significant that these 4 effective amino acids constitute 50% of the casein molecule.

In addition to amino acids, certain intermediates of carbohydrate metabolism were also effective in reversing DNB growth inhibition (Table I) and these effects were most pronounced between 5th and 6th day of growth. (Decrease in weight of controls is due to autolytic changes after 5th day). An equivalent amount of a mixture of citric, α-ketoglutaric, succinic and oxalacetic acids

TABLE I. Reversal of DNB Growth Inhibition in *A. niger*.*

Additions†	Incubation time (days) 5	6	7
None	836	751	667
1.0 μmole DNB	17	23	59
Idem + leucine + proline‡	194	342	479
" + citric acid	240	811	837
" + succinic acid	39	457	727

* mg dry wt of mycelium (50 ml medium/flask).
† 50 mg of each acid listed.
‡ Added at expense of isonitrogenous amount of NH_4Cl.

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produced essentially same response as 50 mg of citric acid. Various additions had no effect on pH of medium nor did they increase growth over control when DNB was omitted.

Ketoacid content of mycelia grown under various conditions was determined in tissue rapidly frozen at −70°C. Ketoacids were converted to 2,4-dinitrophenylhydrazones, extracted and separated by means of their acid functions, and then isolated by paper chromatography(3). Identification was made by comparison of mobilities in at least 2 solvent systems in addition to characteristic colors produced by NaOH location reagent. Appreciable quantities of pyruvate and α-ketoglutarate were found to be normal constituents of young (up to 4 day) mycelia, but at 5 days no pyruvate was demonstrable, thus suggesting rapid metabolism of pyruvate by mature mold. However, when DNB (2.5×10^{-5} M) was added during first 48 to 72 hours of growth, resulting 5 day mycelia showed marked accumulation of pyruvate and little or no α-ketoglutarate. Thus it appeared that in the early growth phase pyruvate piled up because the oxidative pathway and cell synthesis were not maximal. Consequently, it seemed likely that the action of DNB was either (a) to prevent further metabolism of pyruvate, or (b) by preventing cellular proliferation, to produce an accumulation of pyruvate similar to that appearing in younger (slower growing) cultures. It is a well known characteristic of fungi to accumulate various carbohydrate breakdown products in presence of a relatively high concentration of substrate at a time when cell synthesis cannot, or is not permitted to, proceed at maximal rate(4).

To evaluate the interrelationship of certain carbohydrate metabolites and DNB, the dominant pathway of glucose utilization in the mold under these conditions was investigated. It was possible to obtain mycelial preparations with very low endogenous respiratory activity by using cultures incubated at 26°C for 53 hours on wrist-action shaking machine. Medium was the same as that already described except the glucose content was reduced to 0.5% and medium was 0.2 M with respect to phosphate buffer, pH 7. Mycelial pellets were harvested with suction, washed with water, pressed between blotting paper, and frozen for several hours at −20°C. (Both frozen mycelia and homogenates prepared therefrom were stored at −20°C for several days without loss of activity). Frozen material was ground with 3 volumes cold 0.1 M phosphate buffer, pH 6.0 in a Duall glass homogenizer and 0.5 ml added to Warburg vessels which contained 20 mg glucose in side arm and 0.2 ml 10% KOH on filter paper in center well. Total volume was made to 3.0 ml with buffer. After 10 min equilibration in atmosphere of air in 37°C bath, manometers were closed and, after a second 10 min period, glucose was tipped in from side arm and readings made at 10 min intervals for 1 hour. Oxygen uptake was linear for more than 1 hour under these conditions and in 60 assays averaged 1.55 μl per mg mycelium per hour. Oxygen uptake by identical preparations without glucose was less than 0.04 μl.

Glucose oxidation was unaffected by 0.01 M iodoacetate, iodoacetamide, fluoride, arsenite, malonate, cyanide or azide. The following substances were not oxidized: pyruvate, acetate, citrate, aconitate, α-ketoglutarate, glutamate, succinate and oxalacetate. Also, citrate and succinate did not alter rate of glucose oxidation. Fructose, ribose, arabinose and xylose were not oxidized. There was essentially no effect on observed oxygen consumption when the KOH trap for CO_2 was omitted from flasks (Table II). Dialysis at 1°C in pH 6 phosphate for as long as 22 hours did not affect activity nor did subsequent additions of DPN, TPN, thiamine pyrophosphate, ATP and cytochrome c. The molar ratio of glucose metabolized(5) to oxygen utilized was consistently close to 2. Lactic acid(6) was not formed. These same observations obtained with growth medium in place of phosphate buffer in Warburg flasks. Gluconic acid was identified as the main metabolite of glucose oxidation by subsequent chromatographic analysis of reaction mixtures (7). The principal acidic component of the mixture migrated with authentic gluconic acid in two different solvent systems and also reduced ammoniacal silver.

It seems clear, therefore, that metabolism of glucose proceeded overwhelmingly by

TABLE II. Glucose Oxidation under Various Conditions by *A. niger* Preparations. (Means ± stand. error.)

System	No. of exp.	Oxygen used (μmoles/hr)	Glucose metabolized (μmoles/hr)	Glucose / Oxygen
Control	16	9.51 ± .35	17.20 ± 1.57	1.84 ± .17
" minus KOH*	8	8.38 ± .53	20.50 ± 1.92	2.46 ± .18
Dialyzed control†	6	9.24 ± .09	23.87 ± 2.71	2.60 ± .31
Idem + cofactors‡	10	9.54 ± .11	23.38 ± 1.87	2.46 ± .21
DNB-treated cultures§	8	8.77 ± .71	18.47 ± 2.92	2.12 ± .22
Idem minus KOH*	8	8.53 ± .68	19.37 ± 2.97	2.31 ± .30

* KOH in center well replaced by H_2O.
† Preparations dialyzed 3 to 22 hr *vs* 0.05 M phosphate, pH 6.0 at 1°C.
‡ DPN, TPN, thiamine pyrophosphate, ATP, cytochrome c: 10 mg each/flask.
§ Cultures brought to 5.0×10^{-5} M DNB conc. 7 hr before harvesting.

either an Entner-Doudoroff pathway(8) or by direct conversion to gluconolactone and gluconate. Cleland and Johnson(9) have shown by use of labeled substrates that another strain of *A. niger* under similar conditions also converted glucose rapidly to gluconate and this was in turn slowly metabolized *via* pentoses to oxalate. Of further interest are data of Behal(10) which support the view that growth of *A. niger* is not dependent on the citric acid cycle and that this cycle constitutes only a minor respiratory mechanism in this mold.

Oxygen utilization and glucose/oxygen ratios of these preparations were not affected by DNB *in vitro* nor by addition of a growth depressing level of DNB (5×10^{-5} M) *in vivo* 7 hours before harvesting mycelial pellets (Table II). Activity of purified glucose oxidase (Sigma) was not influenced by DNB.

Discussion. An enzymic site for locus of DNB action is still obscure. It has been established that compounds such as isophthalate (*meta*), *m*-nitrobenzoate and *m*-halogenobenzoates function as competitive inhibitors of glutamic dehydrogenase due to their possession of required stereochemical and polar properties for inhibitor-enzyme bonding (11). Suggestion of a similar function for DNB would indeed be attractive because of critical importance of this enzyme in linking amino acid and carbohydrate metabolism. However, DNB had no influence on either forward or reverse reactions catalyzed by highly purified beef liver glutamic dehydrogenase.

Although dinitrophenol was about as effective as DNB in inhibiting growth of *A. niger* in the acid culture medium(1), inhibition due to the former compound was not affected by levels of carbohydrate intermediates which reversed DNB inhibition. Neither was dinitrophenol inhibition alleviated by concentrations of casein hydrolyzate or amino acids which completely overcame DNB growth inhibition. Results with media of different hydrogen ion concentration suggested that mycelial wall was impermeable to dissociated form of 2,4-dinitrophenol (pK = 4.0) since the phenol produced no inhibition near neutral pH. DNB was equally effective regardless of pH.

In view of apparently limited activity of citric acid cycle during growth in *A. niger* it is well to remember that inhibitory action of DNB is restricted to molds and its relative potency increases with decreasing age of cultures(1). These fungi assimilate remarkable quantities of glucose during growth and lay it down as structural polysaccharide. Therefore, it is thought that the action of such compounds as citrate, α-ketoglutarate, succinate and oxalacetate might be to supply metabolic intermediates possibly produced at an inadequate rate from glucose in presence of DNB. Furthermore, it seems a reasonable assumption that rate of formation of carbon rests *during growth* is critical even in absence of DNB because of near absence of citric acid cycle activity. Although these points require verification, evidence is taken to indicate that growth inhibition produced by DNB is mediated by way of depression of a normally marginal rate of biosynthetic formation of in-

termediates for amino acid synthesis.

Summary. Data suggest that major pathway of glucose oxidation in growing cultures of *A. niger* was *via* gluconate. The citric acid cycle played a minor role in young mycelium and inhibitory action of DNB appeared to involve further depression of formation of citric acid cycle intermediates with consequent decrease in rate of amino acid biosynthesis.

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Effects of Androsterone on Atherogenesis and Plasma Lipids in Cockerels. (26185)

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Testosterone propionate has been reported to inhibit coronary atherogenesis with simultaneous reductions in plasma cholesterol, phospholipid and cholesterol/phospholipid ratios in cholesterol-fed pullets(1). In the male bird Katz *et al.*(2) observed no effects on serum lipids and atherogenesis with small doses of testosterone. Higher doses significantly inhibited hypercholesterolemia, although aortic and coronary atherogenesis were not influenced(3). The effect on blood lipids, but not on atherogenesis, was in agreement with previous workers(4,5). The latter investigators reported decreased atherogenesis in testosterone-treated cockerels. The recent report by Hellman *et al.*(6) on hypocholesterolemic effects of androsterone in hyper- and normo-cholesterolemic patients stimulated further studies with androgens in experimentally-induced atherosclerosis. The present report compares effects of androsterone and testosterone propionate on atheroma and blood lipid shifts induced by cholesterol feeding in cockerels.

Methods. Three groups of Hy-line cockerels, 6½ weeks of age, were used for the study. Group 1 (control) was fed an atherogenic diet consisting of mash containing 2% cholesterol and 5% cottonseed oil. Daily subcutaneous injections of corn oil were given in volumes equivalent to those given drug treatment. Group 2 was fed similarly and injected daily with 3 mg/kg of androsterone dissolved in corn oil; Group 3 received the diet and 3 mg/kg of testosterone propionate in the oil solvent. Dosage of both compounds was adjusted each week to the changing weight of the birds. Plasma cholesterol and phospholipid concentrations, comb indices and food consumption were determined at 2-week intervals during the experimental regimen. Total cholesterol was determined by the method of Zlatkis *et al.*(7). The lipid phosphorus, determined by Sperry's method (8), was multiplied by the factor 25 to give the amount of total phospholipids. All birds were sacrificed and autopsied at the end of 8 weeks. The aortas were cut open longitudinally, fixed in 10% Formalin and stained with Sudan IV. Degree of aortic atherosclerosis was graded visually into 5 groups(0-4), according to the area of intima involved by le-