drogenase and the eye color of the adult. In vivo, ry^2 is lethal in larvae reared at high temperatures (30°C), but, if reared at lower temperatures, larvae with ry^2 survive. Connected with this is our finding that cells isolated from *ma-l* embryos exhibited the same growth rate and optimum temperature as those from embryos of wild-type *D. melanogaster*, while cells from ry^2 exhibited a lower optimum temperature (25°C) and grew more slowly, with a mean generation time of 42 hours in the log phase.

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15 June 1964

Mycotoxins: Aflatoxin Isolated from Penicillium puberulum

Abstract. Penicillium puberulum *Bainer was found growing on a sample of moldy peanuts. It also grows on shredded wheat, potatoes, and laboratory culture media such as wort, potato dextrose, and Sabouraud agars, and synthesizes aflatoxin on these substrates. Thin-layer chromatograms of the chloroform-soluble toxin produced by the mold when grown on shredded wheat show fluorescent bands with* R_F *values identical with those of the fractions* B_1, B_2, G_1, *and* G_2 *of the toxin produced by* Aspergillus flavus. *This extract produces typical bile duct proliferation type of liver damage in 2- to 3-day-old Peking white ducklings.*

Initially aflatoxin was isolated by extraction from toxic peanut meal contaminated with *Aspergillus flavus* Link ex Fries, as described by Austwick, and identified by the strain number V-3734/10 (*1*), in our nomenclature, M-3. For some time, the toxin was considered to be a unique product of these particular strains of *A. flavus*. Now there is evidence (*2*) that certain other molds produce aflatoxin, such as *A. parasiticus* (Austwick, strain number IMI 15, 957 ii). The *A. parasiticus* is a subculture of the original strain isolated and described by Speare (*2*). We now report that *Penicillium puberulum* Bainer (Hodges, strain number M-56) produces aflatoxins. This strain of *Penicillium* was isolated from a sample of rejected moldy peanuts.

Mold mycelia were found on the inner surface of the cotyledons of many nuts. Samples of the different mycelia were transferred with a sterile needle to wort agar medium in a petri dish, and after 2 to 3 days of incubation at room temperature, mold colonies were transferred to Czapek agar slants. One of the mold cultures was initially recognized as a *Penicillium* species (*3*) and was later identified as *P. puberulum* Bainer (*4*).

This strain of *P. puberulum* has been cultured on sterilized potato plugs, moist shredded wheat, Sabouraud's agar slants, and potato dextrose agar slants. Chloroform extracts of all these cultures exhibited a strong fluorescence when excited by 365 mμ of light. A substrate of shredded wheat was chosen for toxin production after a preliminary evaluation of the relative intensity of fluorescence of the extracts from the several cultures.

The chloroform-soluble extract (M-56) was a light tan amorphous powder, (*5*). Analytical tests were performed on this preparation with ascending thin-layer chromatography on silica gel G-HR (distributed by Brinkmann Instrument Co.). The chromatograms were developed with a mixture of chloroform and methanol (95 : 5) in sealed, lined tanks. The chromatographed M-56 extract exhibited four characteristic blue and green fluorescent spots with R_F's identical to known B_1, B_2, G_1, and G_2 aflatoxins. Thin-layer quantitative analysis of the extract, with pure aflatoxins B_1 and G_1 as standards, indicated that the extract is composed of 15 percent each of aflatoxins B_1 and G_1 and 1 percent each of aflatoxins B_2 and G_2 (by weight).

A propylene glycol solution of the M-56 extract, when tested by our qualitative screening test in 2- to 3-day-old Peking white ducklings, produced the typical bile duct proliferation type of liver damage that is characteristic of the aflatoxin effect. The oral acute toxicity of the extract in similar ducklings was measured, ten birds being used for each dose, and may be expressed in milligrams per kilogram with 95 percent confidence limits (*6*) as follows: The 50-percent lethal dose, $LD_{50} = 2.30$ (2.12 to 2.48); slope = 1.14 (0.05 to 1.39).

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20 July 1964

Reduction of Cardiac Stores of Norepinephrine in Experimental Heart Failure

Abstract. *Constriction of the ascending aorta in the guinea pig resulted in ventricular hypertrophy and congestive heart failure. In animals with heart failure which were killed 5 to 40 days after constriction, the norepinephrine stores in both ventricles were strikingly reduced; the extent of reduction was related to the severity of the constriction.*

Marked reductions of the norepinephrine concentration in the atrial appendage have recently been found in some patients with chronic congestive heart failure (*1*). This finding has led to the suggestion that there may be depletion of the norepinephrine stores during heart failure which could interfere with sympathetic nervous transmission. In view of the important positive inotropic effects on the heart of activity of the sympathetic nervous system (*2*), such interference with adrenergic function could possibly further impair cardiac performance in heart failure. To study this problem in a controlled experimental situation, we have produced heart failure in guinea pigs by graded aortic constriction and have examined its effects on the stores of norepinephrine in each ventricle and in the kidney.

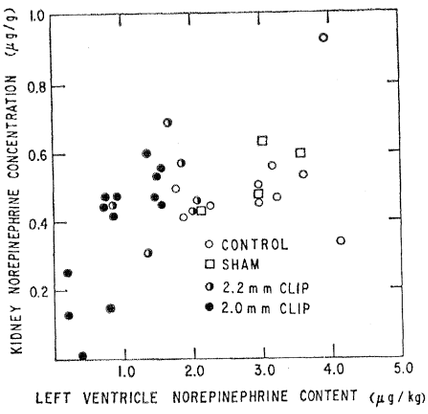

Fig. 1. Relation between norepinephrine concentration in the kidney and the total content in the left ventricle of control guinea pigs, sham-operated guinea pigs, and two groups of animals with aortic constriction.

By means of a technique similar to one previously described (*3*), a constricting ring of polyethylene-covered wire was aseptically placed around the ascending aorta of adult guinea pigs, weighing 700 to 900 g, that had been anesthetized with intraperitoneal sodium methohexital (50 mg/kg); respiration was maintained meanwhile by applying intermittent positive air pressure to the pharynx, after effecting esophageal occlusion by means of an indwelling balloon catheter. The aortic lumen was reduced to an average of 10 percent of its normal size by constriction with a clip having an internal diameter of 2.0 mm; to 25 percent with a 2.2-mm clip. Sham-operated animals also were prepared by the same operative technique, with a nonconstrictive clip placed around the ascending aorta.

Mortality due to the aortic constriction was approximately 50 percent. Five to 40 days after the operation the heart and kidneys were removed under sodium methohexital anesthesia from 13 surviving animals with the 2.0-mm clip, 14 animals with the 2.2-mm clip, and from 5 sham-operated animals; these organs were also removed from 15 control guinea pigs. Organs of the guinea pigs that died spontaneously of heart failure were not analyzed for norepinephrine content because of the variable changes which occur in the norepinephrine concentration after death. The atria were detached from the heart, and the free wall of the right ventricle was removed; right and left ventricles and the kidneys were weighed and immediately frozen with dry ice. For the determination of norepinephrine, each tissue was homogenized with at least 10 times its volume of 5 percent trichloroacetic acid. The catecholamine in this tissue extract was adsorbed on aluminum oxide, eluted with acetic acid, and oxidized to the trihydroxyindole. Norepinephrine was measured spectrofluorometrically, two different wave lengths of activation and fluorescence being used in order to distinguish norepinephrine from epinephrine (*4*). The total norepinephrine present in the right and left ventricles individually was expressed in terms of the preoperative body weight.

The animals with aortic constriction developed persistent heart failure, evidenced by pulmonary congestion with pleural effusion, hepatic enlargement with ascites, and, in the animals more severely ill, cyanosis and peripheral edema. These abnormalities were more prominent in the animals with the 2.0-mm clip. Significant hypertrophy of the left ventricle developed in the 27 animals with aortic constriction; the left ventricular weights averaged 2.40 ± 0.38 (S.D.) g/kg of body weight, while those of controls averaged 1.80 ± 0.15 g/kg ($p < .001$). The right ventricles showed a small increase in weight in the animals with aortic constriction, averaging 0.55 ± .12 g/kg, compared with 0.50 ± .05 g/kg in the controls; this difference was not statistically significant.

Norepinephrine in the ventricles was depressed in the animals with aortic constriction and heart failure, and the magnitude of this change was related to the degree of the constriction (Table 1). Concentrations in the left and right ventricles averaged, respectively, 1.82 and 2.13 μg/g in the controls; 0.35 and 0.84 μg/g, respectively, in animals with a 2.0-mm aortic clip. These concentrations were 0.98 μg/g, and 1.43 μg/g, respectively, in the guinea pigs with a 2.2-mm aortic clip. These reductions in concentration are not due merely to a dispersion of the norepinephrine-containing nerve endings in a hypertrophied muscle mass, but reflect an absolute decrease in the total ventricle content of norepinephrine. Thus norepinephrine content in the left and right ventricles averaged, respectively, 3.01 and 0.93 μg/kg of body weight in the control animals; 0.94 and 0.42 μg/kg, respectively, in animals with a 2.0-mm aortic clip; and 2.14 and 0.70 μg/kg, respectively, in animals with a 2.2-mm aortic clip.

The concentration of norepinephrine was measured in the kidneys removed from 20 of the animals with heart failure and averaged 0.41 ± 0.03 μg/g, a value significantly lower than the control, 0.53 ± 0.09 μg/g ($p < .05$). The depression of renal norepinephrine concentration was directly related to the degree of aortic constriction and to the level of norepinephrine depression in the heart. In all four guinea pigs in which the renal norepinephrine concentration was below the lowest normal value (0.34 μg/g), there was a striking reduction of the left ventricular norepinephrine content to less than 0.90 μg/kg. Norepinephrine stores were normal in the hearts and kidneys of the sham-operated animals (Fig. 1).

These studies show that congestive heart failure produced in the guinea pig under controlled experimental conditions causes a decrease in cardiac norepinephrine stores. Both the concentration and content in both ventricles were reduced, and the extent of these reductions was related to the severity of the aortic constriction. The underlying basis of the biochemical lesions responsible for this depletion remains to be established. It is conceivable that the prolonged overactivity of the sympathetic nervous system, which is

Table 1. Norepinephrine stores in the heart after experimentally induced heart failure.

Condition of animal	No. of animals	Left ventricle		Right ventricle	
		Concentration (μg/g)	Total content* (μg/kg body weight)	Concentration (μg/g)	Total content* (μg/kg body weight)
Control	15	1.82 ± 0.37†	3.01 ± 0.67	2.15 ± 0.46	0.93 ± 0.15
Sham	5	1.55 ± 0.49	2.94 ± 0.49	1.88 ± 0.62	0.89 ± 0.35
2.0-mm aortic clip	13	0.35 ± 0.19‡	0.94 ± 0.50‡	0.84 ± 0.59‡	0.42 ± 0.26‡
2.2-mm aortic clip	14	0.98 ± 0.14‡	2.14 ± 0.70§	1.43 ± 0.30	0.70 ± 0.09

* Total content of norepinephrine expressed on the basis of preoperative body weight. † Mean, ± standard deviation. ‡ Mean significantly lower than control ($p < .001$). § Mean significantly lower than control ($p < .01$).

known to occur in congestive heart failure, may contribute to the reduction (*5*). This view is supported by the finding of a reduction of renal norepinephrine concentration in those animals in which the left ventricular concentration was most strikingly reduced. In addition, it must be considered that interference with the binding or synthesis of norepinephrine, or both, may be involved. It is clear that the operative procedure itself does not mechanically interfere with innervation of the heart; no significant change in the cardiac norepinephrine stores occurred in the sham-operated animals.

In 8 of the 13 animals with congestive heart failure due to a 2-mm aortic constriction, the concentrations of norepinephrine in the left ventricle were reduced to between 5 percent and 19 percent of the normal mean value. These changes are comparable with those achieved with certain pharmacologic agents whose anti-adrenergic action is mediated through their ability to deplete neurotransmitter stores (*6*). Therefore, the possibility must be considered that the profound decrease in cardiac norepinephrine which occurs in congestive heart failure may be associated with an abnormality in adrenergic function. The very important role of the cardiac sympathetic nerves in increasing myocardial function is well documented (*2*). In the heart in which myocardial performance is already impaired, interference with such an important compensatory mechanism could contribute to further deterioration of cardiac function. Thus, it is possible that these studies provide an approach to the understanding of an important aspect of the congestive heart failure state.

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21 July 1964

Blood Groups of Chimpanzees: Demonstrated with Isoimmune Serums

Abstract. *Of five chimpanzees injected with blood of other chimpanzees in Freund's adjuvant, three produced antiserums for simian blood factors designated* $\mathbf{A}^c$, $\mathbf{B}^c$, *and* $\mathbf{C}^c$, *respectively. Factors* $\mathbf{A}^c$ *and* $\mathbf{B}^c$ *determine four blood groups which were distributed among 60 chimpanzees in conformity with the triple-allele hypothesis. Factor* $\mathbf{C}^c$ *appears to belong to an independent blood group system. All three simian blood factors are distributed independently of sex. An antithetical relation exists between the simian blood factor* $\mathbf{A}^c$ *and blood factor* $\mathbf{N}^v$, *the latter being a blood factor shared by all human N cells. Since the simian blood factors* $\mathbf{A}^c$ *and* $\mathbf{B}^c$ *are also related, the three blood factors* $\mathbf{A}^c$, $\mathbf{B}^c$, *and* $\mathbf{N}^v$ *must belong to one and the same blood group system, which is named the V-A-B blood group system of chimpanzees. The V-A-B blood group system of chimpanzees appears to be the counterpart of the M-N-S blood group system of man.*

Studies on blood groups of apes and monkeys have so far been carried out almost exclusively with reagents originally prepared for typing human blood (*1*). With such specific antiserums, we have investigated numerous species of nonhuman primates during the past two years (*2*). Another method of study is by immunizing laboratory animals with simian red cells (*3*), a technique successful for demonstrating blood groups in rhesus monkeys (*4*). It has been postulated (*3*) that by immunization with red cells of closely related species, as well as by isoimmunization, antibodies may be produced which demonstrate individual differences within the species. In fact, individual differences in chimpanzee red cells have been demonstrated with chimpanzee antiserums to human red cells (*5*).

In the present investigation, five chimpanzees were each injected intramuscularly in multiple sites with whole chimpanzee blood mixed with an equal amount of complete Freund's adjuvant (*6*). Each chimpanzee received the blood of a single donor selected at random. The injected animals all developed large abscesses which persisted for 6 months or longer. For convenience the animals were bled after 6 or 7 months, at which time three of them had isoantibodies in their serums. All three antiserums gave different reactions and were therefore arbitrarily designated as anti-$\mathbf{A}^c$, anti-$\mathbf{B}^c$ and anti-$\mathbf{C}^c$, respectively (*7*). The superscript "c" for chimpanzee has been added to the symbols, in order to distinguish these antiserums from blood-grouping reagents currently being used for other species. The three antiserums detect three corresponding blood factors, $\mathbf{A}^c$, $\mathbf{B}^c$ and $\mathbf{C}^c$, which we designate as "simian" blood factors, because in contrast to the human-like blood factors described in our previous papers, these blood factors were initially detected in apes with isoimmune serums.

The antiserums to factors $\mathbf{A}^c$ and $\mathbf{B}^c$ reacted clearly only by the antiglobulin technique at 37°C. For these tests, rabbit antiserum to human blood serum, prepared for human blood-grouping tests, was used, after further absorption with chimpanzee red cells. The chimpanzee antiserums to $\mathbf{A}^c$ and $\mathbf{B}^c$ factors also reacted by the saline agglutination method, but those reactions were much weaker and were poorly reproducible. The anti-$\mathbf{C}^c$ reagent reacted by the antiglobulin technique, but the titer was higher by the ficinated cell technique, which proved not to be suitable for the anti-$\mathbf{A}^c$ and anti-$\mathbf{B}^c$ reagents. None of the serums gave clear reactions by the acacia or albumin techniques or in tests at refrigerator temperature, nor were they isohemolytic.

A series of 60 chimpanzees from the large primate colony maintained at the Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia, has been included in the present investigation. All have been tested not only for the simian blood factors $\mathbf{A}^c$, $\mathbf{B}^c$, and $\mathbf{C}^c$, but also with anti-$\mathbf{N}^v$ lectin (*Vicia graminea*), as well as with the usual A-B-O, M-N, and Rh-Hr human typing reagents. The pertinent findings are summarized in Table 1.

Theoretically, the four factors, $\mathbf{N}^v$,