period of compound punishment: the tactual stimulus continued, a 200-watt light came on, and then the animal was drawn up in a pipette and returned to the starting point of the maze. The maze was composed of eight individual Y's assembled in such a way that they formed an octagon. Thus, when an animal made a correct choice it was not necessary to remove it from the maze in order to start the next trial. Two continuous mazes were employed, one orientated to the left to be used where a left turn was positive, and one orientated to the right where a right turn was positive. The water in the mazes was always at room temperature.

The animals used in this study were four diploid and four triploid full-grown *Triturus* larvae between 100 and 104 days old. The animals were coded in such a manner that the experimenters had no knowledge of the type of larva being trained until the completion of all tests. The criterion for learning was 10 consecutive errorless trials. Each animal was kept in the maze until it reached criterion. Table 1 shows that the triploids, without exception, took more trials and made more errors than the diploids in order to reach criterion. The means are significantly different between the 2- and 5-percent levels of confidence.

These data demonstrate that the normal salamander larvae were capable of faster learning than the triploid. The slower learning of the latter cannot be attributed to an inability to respond properly to the tactual stimulus or to swim in normal fashion, as was shown by a test of their swimming ability in a technique devised by Detwiler (*10*).

It is not possible to decide at this time whether the difference in learning ability between diploid and triploid salamander larvae is connected with the difference in the total number of neurons and connections in the brain or with the difference in size of the individual neurons. Also, the possibility that differences in the number and size of peripheral nervous elements are partly concerned with the difference in performance has not been completely excluded.

Our experiments are somewhat related to those of Rensch (*11*) and his students who studied the effects on learning of differences in the size of the forebrain of closely related species or of races differing markedly in body size (mammals, fowl, and cyprinodont fish). In general, the brains of larger species or races contain more and larger ganglion cells. Actual tests of the learning ability revealed that races of small size learned easy tasks more quickly than larger animals, but that the latter could learn more difficult tasks and showed better retention. The interpretation of the results is complicated by the fact that the differences in learning performance are in part related to the greater liveliness and higher rate of metabolism of the smaller animals. Our experiments with salamanders of different chromosome numbers offer less complex conditions since the diploids and triploids are of the same size and do not seem to differ markedly in general reactivity and motility.

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Production of Catalase Changes in Animals with 3-Amino-1,2,4-Triazole

In connection with some work on the effect of 3-amino-1,2,4-triazole (AT) (*1*) on chlorophyll synthesis in plants, we have observed that, in addition to the depressing effect that AT has on chlorophyll, it also causes a great decrease in the catalase activity of plant tissue. This observation prompted us to study the effect of AT on catalase in animals (*2*).

That a malignancy, anywhere in the animal body, causes a marked depression of liver and kidney catalase has been well established. It has recently been reported (*3*) that injection of extracts of tumor tissue also affects the liver catalase of animals. We have now been able to reproduce all the catalase changes occurring in a cancer host by the use of AT on a normal animal.

Adult female rats of the Long-Evans strain were used throughout this experiment. The AT was injected intraperitoneally as a sterile aqueous solution of 50 mg/ml. The dose varied between 250 and 1000 mg/kg of body weight. Control rats were injected with equal volumes of NaCl solutions adjusted to the same F.P. depression as the AT solution. Both injecting and sacrificing were done under light ether anesthesia. The animals were exsanguinated prior to removal of the liver and kidney. Catalase activity and nitrogen were determined by methods previously described (*4*).

Table 1 shows the levels to which catalase activity of the liver and kidney was lowered at various times after the injection of 1 g AT per kilogram of body weight as a percentage of control animals.

The blood catalase and the hemoglobin of the treated animals remained normal, and there were no evident toxic effects. The failure of AT to depress blood catalase activity makes its effect similar to that of malignant growth, since tumors also do not affect blood catalase. This observation lends further support to the views (*4, 5*) that blood catalase differs in origin and, perhaps, in nature from that of liver and kidney catalase.

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References and Notes

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Table 1. Effect of 3-amino-1,2,4-triazole on catalase activity of rats. (Dose: 1 g/kg of body weight.)

Hours after injection	*Liver*			*Kidney*		
	No. of animals	Units*/mgN	% of control	No. of animals	Units*/mgN	% of control
3	5	0.46 ± 0.06†	10.9	4	0.17 ± 0.03	12.8
6	6	0.50 ± 0.05	11.5	4	0.14 ± 0.04	10.3
12	6	0.72 ± 0.17	16.9	2	0.25 ± 0.02	18.4
24	6	1.51 ± 0.34	35.6	2	0.49 ± 0.11	35.7
48	6	2.74 ± 0.25	58.5	4	0.94 ± 0.16	71.6
72	6	3.64 ± 0.416	84.6	3	1.10 ± 0.23	85.5
Control	23	4.24 ± 0.50		18	1.36 ± 0.19	

* A unit of catalase is the amount that will liberate 1 ml of oxygen per second from a 1.0*N* H_2O_2 solution at 0°C. † ± Standard deviation.

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Determination of Housefly Succinic Dehydrogenase with Triphenyltetrazolium and Neotetrazolium Chloride

The tetrazolium salts are being used with increasing frequency as convenient indicators of biological redox reactions and enzymatic activity (*1*). However, certain peculiarities associated with the reduction of the various derivatives have been noted. Brodie and Gots (*2*) and Throneberry and Smith (*3*) have observed that the rate of reduction of 2,3,5,-triphenyltetrazolium chloride (TPTZ) is increased by incubation in a vacuum, as have other authors, and various workers have suggested that there is a direct competition between the indicator and naturally occurring aerobic hydrogen carrier systems, a decrease in dehydrogenase activity in air, an elevation of the redox potential of the system in air to an unfavorable level for reduction of the tetrazolium salt, an alternate mechanism of reduction of the indicator in air as opposed to the anaerobic mechanism, or a toxicity manifested by the indicator per se. The rapidity of reduction of the tetrazolium derivatives by such enzymes as succinic dehydrogenase varies considerably; Glock and Jensen (*4*) found neotetrazolium [*p,p'*-diphenylenebis-2-(3,5-diphenyltetrazolium chloride)] to be a more sensitive indicator of plant succinic dehydrogenase than TPTZ.

In the course of an investigation (*5*) to determine the inhibition by DDT and related compounds of muscle succinic dehydrogenase of the housefly, *Musca domestica* L., with TPTZ and neotetrazolium, an interesting variability in performance was observed. This difference was also demonstrated by a comparison of the effect of sodium malonate, which is a competitive but weak inhibitor of succinic dehydrogenase, and sodium cyanide, which inhibits the cytochrome carriers strongly but not succinic dehydrogenase (*6*).

The determinations were made by incubating aliquots of homogenized thoraxes (8 per milliliter) of female adult houseflies in ignition tubes with 0.05*M* phosphate buffer (*p*H 7.4 final concentration) and the inhibitor for 30 minutes at room temperature, then adding 0.067*M* sodium succinate and 200 μg of indicator in aqueous solution, bringing the volume to 3.0 ml, and incubating at 35°C until measurable reduction of the indicator was observed. In anaerobic determinations the tubes were placed in a 3-lit filter flask, which was evacuated through a stopcock attached to the side arm. The formazan was extracted from each incubation mixture with 3.5 ml of water-saturated *n*-butanol (*4*) by shaking 50 times, followed by brief centrifugation to separate the layers. The optical density of the formazan extract was determined spectrophotometrically at 480 (TPTZ) or 520 (neotetrazolium) mμ. The amount of indicator reduced was read from standard curves prepared with known amounts of formazan.

Table 1. Effect of two indicators on the inhibition of housefly-muscle succinic dehydrogenase by sodium cyanide and sodium malonate. TPTZ determination, incubated 115 minutes; neotetrazolium determination, incubated 30 minutes

Inhibitor	Concn. (*M*)	Indicator reduced (μg)			
		TPTZ		Neotetrazolium	
		Aerobic	Anaerobic	Aerobic	Anaerobic
		35.4	39.2	50.4	87.8
Cyanide	6.7×10^{-4}	0	3.9	19.4	26.5
Cyanide	1.7×10^{-4}	0	15.2	20.6	32.8
Malonate	0.05	6.3	9.6	2.3	5.5
Malonate	0.017	21.2	24.5	2.8	7.3

Table 2. Influence of cytochrome *c* on cyanide inhibition, and of increased substrate concentration on malonate inhibition of housefly-muscle succinic dehydrogenase ($5 \times 10^{-4}M$ $CaCl_2$ added throughout; incubated in a vacuum 45 minutes)

Inhibitor	Concn. (*M*)	Reagent added	Concn. (*M*)	Indicator reduced (μg)	
				TPTZ	Neotetrazolium
				36.5	143.4
Cyanide	3.3×10^{-4}			4.0	156.0
Cyanide	3.3×10^{-4}	Cytochrome	1.7×10^{-5}	11.7	143.4
Malonate	0.017			20.0	58.0
Malonate	0.017	Succinate (2x)	0.067	26.0	125.1

TPTZ reduction (Table 1) was found to be more strongly inhibited by cyanide and less strongly inhibited by malonate than neotetrazolium, both aerobically and anaerobically. Furthermore, as is shown in Table 2, cyanide inhibition of TPTZ reduction was somewhat lessened by added cytochrome *c* (*7*). The effect of malonate was partially counteracted by increasing the substrate concentration, as would be expected. The addition of calcium chloride appeared to stimulate reduction but did not alter the relative effect of the inhibitors. The action of cyanide and malonate on neotetrazolium reduction is in agreement with the hypothesis that this tetrazolium compound is reduced directly by succinic dehydrogenase, while the results with TPTZ may indicate the mediation of a cyanide-sensitive hydrogen carrier. It is also possible that the difference in reactivity is an artifact arising not from differences in mode of reduction by the enzyme but from differences in solubility or reactivity of the tetrazolium derivatives themselves, which influence the availability of the indicator.

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