Table 2. Distribution of tritium (μg/g) in various organs of *Busycon canaliculatum* after injection of 114 μg of tritiated reserpine. Data are expressed as micrograms of reserpine equivalent to the count per minute of 1 g of wet tissue or liquid.

Hours after injection	Blood	Muscle	PG*	Ganglia	Liver	Kidney	Recovered from sea water (μg)
2	0.77	0.10	3.79	2.83		7.63	35.4
4	.34	.03	2.26	1.84	0.41	16.19	83.2
6	.28	.12	1.09	1.30	.83	12.63	107.5
10	.15	.06	0.91	1.86	.39	9.23	131.5
24	.12		.10	1.01	.59	4.54	146.8
67†	.09		.17	0.94	.42	3.50	182.3
120†	.06	.05	.16	.22	.59	2.10	196.7
168†	.10		.36	.70	1.21	2.71	214.1
264†			.07	.96	1.40	1.50	234.6
336†				.73	1.11	2.18	249.0

* Mixed sample of salivary glands, esophagus, and connective tissue surrounding the ganglia.
† Water changed in the aquariums.

present in the selected organs was measured with a scintillation counter at progressively greater intervals of time. In order to determine which organs participated in excretion of the drug, the investigation was extended to the kidney, the liver, and the water of the aquariums (*3*).

The radioactive background of the organs investigated was high, probably because of large amounts of K^+ in the tissues. The total activities of known quantities of tritiated reserpine added to samples prepared according to the procedure used in the experiment are low; the error in determination is of the order of 10 percent (Table 1).

The total amount of label recovered from the water of the aquariums increased rapidly during the first 3 days to a value equivalent to 4/9 of the reserpine injected (Table 2); thus a significant fraction of the drug was retained in the bodies of the animals. After the initial peak of activity the label quickly disappeared from the blood and muscles and more slowly from a mixed sample (PG) of salivary glands, esophagus, and connective tissue; readings from none of these parts were significant after 7 days. The label in the ganglia was at its highest concentration 2 hours after the injection; later it decreased very slowly and was still detectable after 14 days.

The rate of disappearance of tritium from the ganglia and from the other parts examined was not proportional to the amount present initially. Thus 2 hours after the injection more radioactive material was present in the PG sample than in the ganglia. Yet after 7 days labeled material was no longer detectable in the PG sample, whereas activity of the ganglia remained much greater than the experimental error. It appears, therefore, that a substantial fraction of the injected drug is bound in the nervous tissue in a highly stable form that is only slowly removed by metabolic processes. However, the data are insufficient to establish whether this binding is specific for the nervous tissue.

A certain amount of labeled material may have been present in the other organs also but only in quantities that were undetectable above the highly radioactive background of the tissues investigated; in this case the only real difference between the ganglia and the other organs would be in the relative number of binding sites. Although not conclusive, an argument in favor of a specific binding site for reserpine in the ganglia is that the PG sample, which contained traces of labeled material much longer than the muscles and blood, contained the initial tracts of the major nerves, and therefore a sizable amount of nervous tissue. Our results indicate that reserpine acts primarily on the mollusk's nervous system; this agrees with what is generally known of the pharmacology of the drug (*4*). In mollusks as in mammals, the effects of reserpine do not outlast the presence of the drug or of its metabolities in the nervous system (*5*).

The large amount of tritium in the kidney throughout the experiment and the increase in radioactivity of this organ after the first 2 hours indicate that the drug, or its metabolites, that is removed from other organs, is excreted mainly by way of the kidney. This is not, however, the only route of excretion. In fact, changes in the amount of radioactivity in the liver show that a large portion of the labeled material slowly concentrated in this organ from other body spaces. Furthermore, the gills, mantle, and skin were not investigated; it is conceivable that these parts also participate in the process.

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3. Two animals were used for each determination; the ganglia of both were pooled and samples of other organs were taken from either animal. Samples were weighed, hydrolized with concentrated HCl in a glass vial free of K^+, brought to alkalinity, and bleached with H_2O_2. After addition of 15 ml of dioxane with fluorophor, a compact gel was obtained by addition of Cabosil.
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Actinomycin D: Inhibition of Protein Synthesis Unrelated to Effect on Template RNA Synthesis

Abstract. *Incubation of sarcoma-37 ascites cells in vitro with actinomycin D resulted in inhibition of synthesis of nuclear and cytoplasmic proteins. The overall inhibition could be prevented or relieved by glucose; it is thus unrelated to breakdown of template RNA.*

The actinomycins have become widely used for investigation of the stability of protein-forming templates in mammalian cells as well as in bacteria. In studies of the effects of these antibiotics in intact mammalian tissue (*1*) or in acellular systems (*2*) that incorporate amino acids, the synthesis of protein was shown to be inhibited. It is generally assumed that this inhibitory ef-

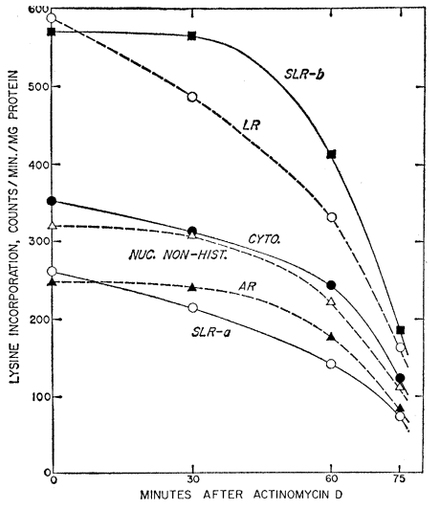

Fig. 1. Inhibition of incorporation of L-lysine-C^{14} into protein fractions of sarcoma-37 ascites cells by actinomycin D. Each flask contained 2 ml of packed cells in 13 ml of lysine-free Eagle's spinner culture medium (*11*). Pyruvate was present at a concentration of 5 m*M* and glucose was absent; the gas phase was oxygen. Incubations were carried out with actinomycin D (25 μg/flask) for the indicated time intervals; 1.25 μc of L-lysine-C^{14} was then added from the side-arms, for a 10-minute labeling period. Nuclei were isolated by the procedure of Fisher and Harris (*12*). The protein fractions are *CYTO,* total cytoplasmic protein; *AR*, arginine-rich histone; *SLR-a* and *-b*, slightly lysine-rich histones; *LR*, lysine-rich histone; and *NUC. NONHIST.*, nuclear protein after extraction of the histones (*13*).

fect reflects (i) the degradation of short-lived RNA, which serves as a template in protein synthesis, and (ii) a block by actinomycin in further synthesis of the RNA. This conclusion is based principally on the finding that in intact mammalian cells these antibiotics promptly suppress RNA synthesis, with a delayed effect on protein synthesis (*3*), and on the hypothesis that templates for protein synthesis consist of RNA species (messenger RNA) which require continuous production for maintained function (*4*).

Some doubt was raised whether this was the only mode of action of actinomycin when Acs *et al.* (*5*) showed that, in both bacteria and mammalian cells, the antibiotic exerted a direct degradative effect on cellular RNA in addition to its inhibition of RNA synthesis. That the activity of actinomycin is not limited to inhibition of the synthesis of template RNA is also clear, since moderate doses of the drug are lethal (*6*) in whole animals prior to times at which inhibition of cell division or protein synthesis alone would be expected to cause significant morbidity. Revel *et al.* (*7*) found that administration of actinomycin to rats led to an inhibition of protein synthesis in liver homogenates and a decrease in the polyribosome content. In tissue slices from the same animals, however, these changes were not seen. The protein inhibitory effect was observed prior to a significant suppression of RNA synthesis, a further demonstration that it is not a consequence of the latter effect.

In studies of the effects of actinomycin D on protein synthesis by sarcoma-37 cells in vivo (*8*), it was found that after injection of the antibiotic, the synthesis of histones was inhibited before that of the other protein fractions. In an attempt to define more completely the characteristics of this inhibition, we have studied the effect of actinomycin D on synthesis of the nuclear and cytoplasmic protein fractions in vitro. Actinomycin D (1.7 μg/ml) inhibited lysine incorporation into all the protein fractions to approximately the same extent (Fig. 1). In contrast with the results in vivo (*8*), the lag in the onset of inhibition was similar for each of the fractions; in general there was little inhibition before 60 minutes, with a major inhibitory effect occurring shortly thereafter. Some degree of variation in the length of the lag period was observed from one experiment to another; it approached 90 minutes in some cases. The similar effect of actinomycin on synthesis of nuclear and cytoplasmic proteins suggested that the inhibition may be related to total cellular metabolism rather than to a fortuitous similar requirement for messenger RNA among the different protein-forming systems.

Since the inhibitory effect of actinomycin D was similar in all the protein fractions studied, synthesis of total cellular protein was measured in subsequent experiments. The addition of glucose to the incubation medium prevented the inhibition of protein synthesis by the antibiotic. There was complete recovery from the inhibition when the glucose addition was delayed until the end of the incubation period (Fig. 2).

Tumor cells generally can maintain normal biosynthetic activity in the presence of inhibitors of oxidative phosphorylation if a glycolyzable substrate is also present (*9*). But glucose may not prevent the inhibition in other cell types which have lower glycolytic capacities.

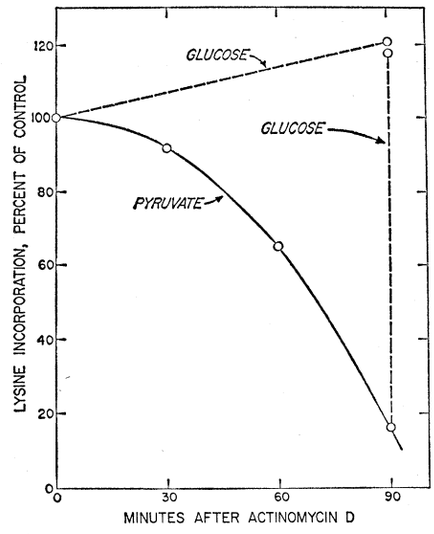

Fig. 2. Inhibition of incorporation of L-lysine-C^{14} into total protein of sarcoma-37 ascites cells by actinomycin D, and relief by glucose. Incubation conditions were as described for Fig. 1. The cells were incubated with actinomycin D for varying time periods, followed by a 10-minute treatment with uniformly labeled L-lysine-C^{14}. Addition of glucose (final concentration 5 m*M*) was made either at the beginning of the incubation or after 90 minutes. In the latter case it was added together with the radioactive lysine. Incorporation is expressed as percentage of the specific activity of corresponding controls which were incubated without actinomycin D.

Inasmuch as the inhibitory effect of actinomycin D on protein synthesis could be immediately relieved by glucose addition at the end of the incubation period with actinomycin, this inhibition cannot be related to breakdown of template RNA. As with other mammalian cells (*10*), RNA synthesis in sarcoma-37 cells was inhibited by actinomycin D whether or not glucose was a constituent of the medium. After a 10-minute incubation period with actinomycin D in glucose-containing medium, the rate of incorporation of uridine and adenine into RNA was reduced to less than 10 percent of the control value. Thus, glucose could not have affected the inhibition of RNA synthesis by actinomycin D in our studies. Therefore, great caution must be exercised in interpreting the inhibitory effects of actinomycin solely in terms of template stability.

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2 August 1965

Uncrossed Visual Pathways of Hooded and Albino Rats

Abstract. *The uncrossed visual pathways to the principal primary optic centers of hooded rats are substantially larger than those of albino rats. This provides an anatomical basis for the results of a recent study on behavior which showed differences in interocular transfer in the two varieties.*

There are differences in the visual systems of gray and albino rats. The eyes of the albino rat are larger (*1*), the optic nerves are smaller and have more unmyelinated fibers (*2*), and the visual cortex is thinner (*3*). In anatomical studies on the central projection of the optic nerves, no differences have been described between varieties of rat. The anatomical study of Hayhow *et al.* (*4*) on both albino and hooded rats revealed no difference between them. However, in his recent work on interocular transfer, Sheridan (*5*) found that albino rats showed less successful transfer after monocular training on brightness and pattern problems than hooded rats. He interpreted the difference as being due possibly to a reduced uncrossed pathway in the albino rat compared with the hooded. My study on the uncrossed pathway stems from some earlier data which confirm this suggestion.

One eye was removed from each of 4 hooded rats and 12 albinos of the strains Glaxo, Sprague-Dawley, and Wistar. After 8 days the animals were perfused with 10 percent neutral formal-saline; they were then cut either transversely or parasagittally and were stained either by the Nauta-Gygax method (*6*) or with its paraffin modification (*7*).

The pattern of uncrossed degeneration in the hooded rats largely confirms that described by Hayhow *et al.* (*4*). There is a substantial amount in the lateral geniculate nucleus pars dorsalis, largely restricted to the dorsomedial part, and there is a smaller amount in the pars ventralis. A small amount occurs in the pretectal region, and a significant band is found in the intermediate region of the stratum opticum of the superior colliculus (Fig. 1). Comparing this with Lashley's map of crossed retinotectal fibers, it would appear that the binocular area of distribution of fibers is from the central region of the retina, extending medially and laterally in a band.

In the albino rats studied, the pattern of ipsilateral degeneration is different, particularly in the Glaxo strain which was studied in most detail. A small amount of passage degeneration can be detected in the optic tract and the brachium of the superior colliculus. None of this can be traced into the ventral part of the lateral geniculate body, but in the dorsal part there are a few degenerating fibers in some animals. There is no indication of an organized uncrossed terminal distribution of the kind found in the hooded rat. No uncrossed degeneration can be found in the pretectum. In most of the brains cut in a parasagittal plane, a few degenerating fibers can be traced into the stratum opticum of the superior colliculus. As in the geniculate, these are few in number in comparison with the uncrossed degeneration in the hooded rats, and again show no definite area of termination (Fig. 2). The three Wistar rats studied showed a pattern similar to that found in the Glaxo rats, but slightly more degeneration was found in the Sprague-Dawley strain. However, this is still much less than that found in the hooded rats.

The results, while showing the pattern (suggested by Sheridan, *5*) of reduced uncrossed input in albinos, do not agree with the results of Hayhow *et al.* (*4*). It is possible that the strain of albino rat that they used may resemble the hooded rat more closely in the central projection of its optic fibers. However, Wistar rats were used by Sheridan (*5*), and my results are, therefore, relevant to his work. It would appear, then, that the corpus callosum and other midline commissures of rats are not as effective in establishing a bilateral memory trace, or using one established on one side only, as they appear to be in cats and monkeys (*8*). A direct pathway from the retina to the visual center used in training, whether crossed or uncrossed, appears important for the rat to show good retention.

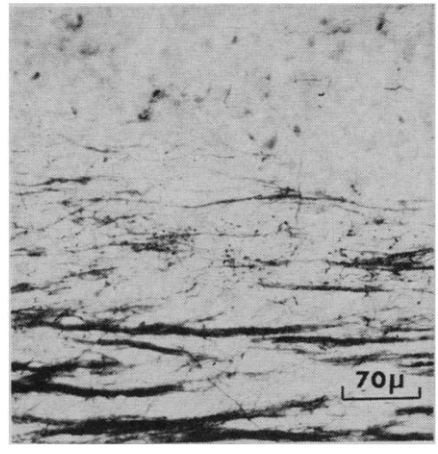

Fig. 1. Area of maximum degeneration in the stratum opticum of the superior colliculus on the side ipsilateral to the lesion in a hooded rat; stained by the Nauta-Gygax method.

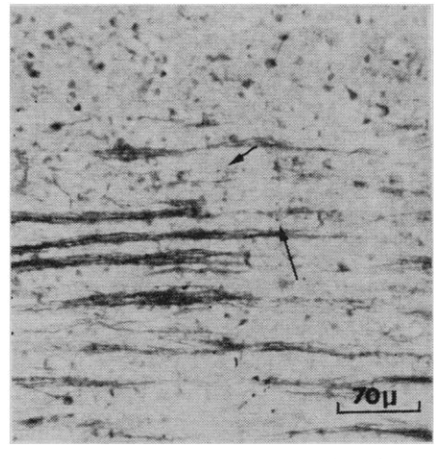

Fig. 2. Region similar to that in Fig. 1, but in an albino rat, showing the maximum density of uncrossed degeneration. The few degenerating fibers are indicated by arrows; stained by the Nauta-Gygax method.

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