lateralis; certainly in this viviparous fish it would be reasonable to assume that the function of a fetal hemoglobin would be the same as that accepted for the fetal hemoglobins of mammals (*9*).

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Effect of Digestion on Distribution of Blood Flow in the Rat

The belief that splanchnic blood flow increases at the expense of flow in other organs during digestion was challenged by Herrick *et al.* (*1*) in 1934. By use of thermostromuhrs, dogs were found to show similar increases in carotid, femoral, and superior mesenteric arterial blood flow postprandially. Abramson and Fierst (*2*) found that the blood flow to the human hand, forearm, and leg tended to increase after eating. A meal increases the splanchnic blood flow of a human subject (*3*). However, the cardiac output also increases (*4*). When the experimental values are adjusted for surface area, it is found that the absolute postprandial increase in splanchnic blood flow in a 1.73 m^2 man is about 710 ml/min. A 24 percent increase in the cardiac output of such a man (*5*) represents 1300 to 1400 ml/min. From this it may be concluded that the nonsplanchnic blood flow is increased by digestion.

The subject has been reinvestigated (*6*) with the aid of a newly developed method (*7*). The method is based on the observation that all organs other than the brain have, during the first minute after a single intravenous injection of $K^{42}Cl$, substantially the same extraction ratios for K^{42}. The fractional distribution of K^{42} among the organs during the first minute therefore corresponds to the fractional distribution of the cardiac output. The anomalous behavior of the brain has been shown to be of minor consequence in the measurements of the blood flow to other organs. Values obtained by this method describe the fractions of the cardiac output directed to each organ. A knowledge of the cardiac output permits the calculation of the blood flow to each organ.

One hundred and seventeen rats were used. Control animals were starved for 24 to 72 hours but were permitted to drink water *ad libitum*. "Fed" animals were allowed to eat and drink *ad libitum* up to the time of the experiment. The gastrointestinal tract of the "fed" animals always contained 10 to 15 g of food at autopsy. The animals were anesthetized with Nembutal (40 mg/kg intraperitoneally). The cardiac output was determined by dye dilution, with Evans blue as the indicator; the blood was sampled at a rate of 90 collections per minute (*8*). Other similarly treated animals of the same stock were used for the fractional distribution studies with K^{42}; the details of the method have been described previously (*7*).

The cardiac output of 17 control animals averaged 172 ± 38 ml/kg min. Eleven fed animals had a cardiac output of 223 ± 59 ml/kg min. Determinations of fractional distribution were made on 49 control and 40 fed animals. The fractions found for each organ were multiplied by the cardiac output value in animals of the same group (adjusted for body weight) to give blood flow values to the various organs.

Table 1 shows the blood flow values obtained in the organs of the two groups. For simplicity all values have been adjusted for the body weight and are presented as the blood flow to the organs of a 250-g rat.

It is clear from these results that, during digestion, there is a uniform increase in the blood flow to all organs of the rat. The splanchnic organs do not gain their increased blood supply at the expense of the blood supply to other organs; on the contrary, all organs benefit from the increased cardiac output associated with digestion.

These results, though obtained in anesthetized rats, are similar to those reported in conscious dogs and men; they do not conflict with any reported findings. In the absence of contrary evidence, it is suggested that the prevailing concept that digestion results in diversion of blood flow from other organs to the digestive tract be critically re-examined.

Table 1. Blood flow values in fasting and fed rats (all values have been adjusted to 250-g rats; blood flow is given in milliliters per minute).

Organ	Blood flow	
	Fasted	Fed
Liver (arterial)	3.2	4.3
Gut and spleen	7.1	9.4
Myocardium	1.1	1.3
Skin	3.2	4.2
Kidneys	6.6	8.7
Carcass	21.8	27.9

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Activation of Enzymatic Hydrolysis of Benzoylcholine by Tryptamine

During an investigation of the anticholinesterase activity of indole derivatives (*1*) it was found that tryptamine accelerates the enzymatic hydrolysis of benzoylcholine by plasma cholinesterase (*2*). It has also been reported that analgesics (*3*) and other compounds (*4*) activate plasma cholinesterase, and in certain cases, red cell cholinesterase (*5*). Some authors attributed this activation to an interference wth the partial inhibition of the enzyme (E) by the excess

substrate (S) molecules at high substrate concentrations (*6*). In agreement with this, Kalow (*7*) found that benzoylcholine inhibits plasma cholinesterase at high concentrations. He obtained optimal activity with benzoylcholine concentrations of about $10^{-4}M$. On the other hand, Hardegg and Schaeffer (*8*) assumed that cholinesterase is partially inhibited by the substrate at all concentrations. It seemed worth while to investigate whether the activation of plasma cholinesterase by tryptamine was due to the prevention of the combination of enzyme-substrate complex with a second molecule at higher than the optimum substrate concentrations or whether the activation resulted from a competition between the substrate and the activator for the active site of the enzyme at all concentrations.

In the present experiments, the hydrolysis of benzoylcholine by concentrated, purified, human plasma cholinesterase (Cholase, *9*) was investigated by two different methods. With relatively low initial concentrations of benzoylcholine ($5.10^{-5}M$), Kalow's ultraviolet spectrophotometric method was employed (*10*). With concentrations of $2.10^{-3}M$ or higher, Ammon's modification of Warburg's manometric technique was applied (*11*). All experiments were carried out at *p*H 7.4 and 37°C. The spectrophotometric determination of the hydrolysis of benzoylcholine was made at 240 mμ. In these studies, the plasma cholinesterase activity was accelerated 62 percent when the tryptamine concentration was $2.10^{-4}M$. Because of optical reasons, higher tryptamine concentrations could not be used. In the manometric experiments, when the concentrations of benzoylcholine and tryptamine were $2.10^{-3}M$, the activation was 83 percent. In the Warburg studies, a fourfold change in the enzyme concentration (from 1/8000 to 1/2000 by volume) had little effect on the acceleration of hydrolysis of $5.10^{-3}M$ benzoylcholine caused by $2.10^{-3}M$ tryptamine. The increase in activity in the presence of the highest and the lowest enzyme concentrations was 51 and 62 percent, respectively.

The effect of other indole derivatives on the hydrolysis of benzoylcholine by plasma cholinesterase was also investigated. Those compounds, which had an OH group attached to the indole nucleus or a carboxyl or a dimethyl group substituted in the side chain, or both (for example, 5-hydroxytryptamine, tryptophan, 5-hydroxytryptophan, dimethyltryptamine, gramine, or bufotenine) did not activate plasma cholinesterase. Some of these compounds inhibited the enzyme (*12*). The hydrolysis of other substrates (for example, acetylcholine, butyrylcholine, procaine, and murexine) by plasma cholinesterase was not accelerated but inhibited by tryptamine. Some of these substrates, similarly to benzoylcholine, have a low ($\approx 10^{-6}M$), and others (for example, acetylcholine and butyrylcholine) a high ($\approx 10^{-3}M$), Michaelis constant (K_m). Consequently, it is unlikely that the hydrolysis of benzoylcholine was accelerated because of its low K_m. It is of interest that the enzymatic breakdown of PDMI-669 (the triethyl derivative of benzoylcholine) was neither accelerated nor inhibited by tryptamine. The hydrolysis of acetylcholine by red cell cholinesterase was also inhibited by tryptamine.

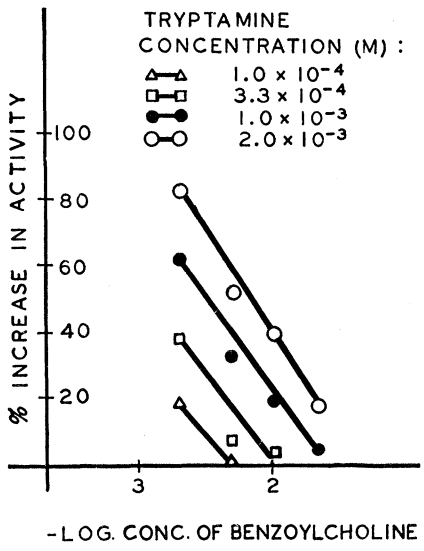

Fig. 1. Effect of increasing the substrate concentration on the acceleration of benzoylcholine hydrolysis by tryptamine.

It has been suggested that excess substrate inhibits the enzymatic hydrolysis by the attachment of a second substrate molecule to the enzyme with the formation of an ES.S complex (*13*). Since it was found that tryptamine also accelerated the hydrolysis of benzoylcholine at suboptimal substrate concentrations ($5.10^{-5}M$), where the formation of an ES.S complex is unlikely to occur, it is therefore not probable that the activating effect of tryptamine is due to the prevention of the formation of this complex. It is also conceivable that the accelerating effect of tryptamine might be caused by the formation of a complex of tryptamine and benzoylcholine. This would decrease the concentration of excess substrate and would interfere with its inhibitory effect. Because tryptamine also activates at suboptimal substrate concentrations, where the binding of benzoylcholine by tryptamine would decrease the rate of enzymatic hydrolysis, this hypothesis is not likely to be correct. A further argument against the above assumption is that, while there is less than 10 percent increase in the rate of enzymatic hydrolysis of benzoylcholine, when the substrate concentration is decreased from 10^{-2} to $10^{-3}M$ (*7*), more than 20 percent acceleration could be obtained at these substrate concentrations by tryptamine (see Fig. 1). Finally, there was no change in the ultraviolet absorption spectrum of a $5.10^{-5}M$ benzoylcholine solution after the addition of $10^{-4}M$ tryptamine. A more likely explanation of the accelerating effect of tryptamine is that, in its absence, the quaternary N of benzoylcholine may be attached to the anionic site of the enzyme in such a way that the resulting configuration is unfavorable for the reaction between its ester group and the esteratic site of the enzyme. Tryptamine, which is a cation at *p*H 7.4, may compete with benzoylcholine for the anionic site and thereby facilitate the attachment of benzoylcholine to the functionally important esteratic site. The curve obtained by plotting the increase in activity caused by various tryptamine concentrations against the negative logarithm of substrate concentrations (see Fig. 1) indicates that the acceleration is indeed a competitive process. The rate of hydrolysis increased with decreasing concentrations of benzoylcholine or with increasing concentrations of tryptamine. Preliminary studies with other pharmacologically active cations seem to corroborate this hypothesis (*2*).

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Role of Polyphenolase in Streptomycin-Induced Resistance to Phytophthora in Potato

Some information has been obtained about the biochemical mechanisms of resistance to plant diseases from study of the potato, *Phytophthora infestans* de By. complex. Rubin and co-workers (*1*) and the Göttingen school (*2*) succeeded in demonstrating that the activation of polyphenolases (tyrosinase) at the site of infection may lead to the accumulation of polyphenol derivatives. The increase in these highly fungitoxic substances being more intense in resistant than in susceptible varieties, their accumulation may be regarded as an important factor contributing to disease resistance.

Recently Müller *et al.* (*3*) were able to show that, if streptomycin is absorbed by potato or tomato plants through their roots, these plants become resistant to *Phytophthora*. The effect is indirect, for the fungus is known to be highly insensitive to streptomycin *in vitro*. Similar data that pertain to other host-parasite complexes have been described (*4*).

This study was undertaken to shed some light on the mechanism of streptomycin action. As is shown, streptomycin absorbed by the potato tissues greatly enhances their polyphenolase activity. It seems, therefore, that both the natural and streptomycin-induced resistance of potato depend on the same biochemical mechanism.

Whole potato sprouts or detached leaves, or both, were placed in streptomycin sulfate solutions (100 ppm in tap water). Controls were treated similarly but were placed in pure tap water. Samples were taken for the assays every two days for a week. The leaves used for the experiments were cut into halves. One half was used for the determination of polyphenolase activity and the other for the assay of the streptomycin content. Enzyme activity was measured in homogenates by the use of conventional manometric procedures. Catalytic amounts of catechol were used as substrate, and hydroquinone was chosen as a suitable reductant (*5*). Streptomycin was assayed according to the method of Pramer (*6*), with *Bacillus subtilis* as a test organism.

In several consecutive experiments, 20 assays of polyphenolase activity were carried out; as a result of treatment with streptomycin, strong stimulation was found in each case (30 to 110 percent). Slight stimulation was found in the early stages of treatment, when streptomycin was present only in traces in the tissues. Higher streptomycin content was generally correlated with higher polyphenolase activity. Representative data are shown in Table 1. Autooxidation and trace-element catalysis of substrates was estimated by use of boiled controls. The data in the tables have been corrected for autooxidation values.

Table 1. Polyphenolase activity and streptomycin content in potato leaves treated with streptomycin. Enzyme activity is expressed as the increase in oxygen uptake upon addition of substrates (0.02 percent catechol and 0.6 percent hydroquinone) in cubic millimeters of oxygen per milligram (fresh weight) of tissue homogenate, per hour.

Hours after treatment	Polyphenolase activity		Streptomycin content (μg/g fresh wt.)
	Control	Treated	
24	0.81	1.02	Traces
72	0.90	1.61	40

Table 2. Respiratory rate and polyphenolase activity in potato disks treated with streptomycin. Respiratory rate is expressed as cubic millimeters of oxygen per gram (fresh weight) per hour. Enzyme activity is expressed as increase in oxygen uptake in cubic millimeters under identical conditions upon addition of substrates.

Hours after treatment	Respiratory rate		Polyphenolase activity	
	Control	Treated	Control	Treated
3	68	65	28	30
24	64	40	32	102

The effect of streptomycin on the tissues of tubers is very similar. Small disks (5 mm in diameter) of cortex tissues were placed in streptomycin solution or in water. The polyphenolase activity was measured by adding the phenolic substrates from the side bulb to the disks that were suspended in buffer solution in the main compartment of Warburg vessels. As may be seen (Table 2), the respiration of streptomycin-treated disks was strongly decreased. Simultaneously, the polyphenolase activity was considerably enhanced. The activation of polyphenolases was shown also by the quick blackening of the treated disks, in contrast to the modest discoloration of the controls.

The effect of streptomycin is indirect, for the antibiotic was shown to be totally inactive when tried directly as a "substrate" in the assay of polyphenolase activity.

The results reported provide strong evidence for the idea that streptomycin exerts its protective effect via the polyphenol-polyphenolase system of the host plant. Further support for the validity of this suggestion is delivered by the recent observation of McNew (*7*) which indicated a synergistic effect of copper and streptomycin. The relation of this finding to our results is evident: polyphenolases are copper enzymes, and their activity is greatly dependent on the copper supply of the plant.

Results similar to those described above were obtained with tomato plants.

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Effect of Gibberellic Acid on Breaking of Rest Period in Elberta Peach

Gibberellic acid, which is produced from the fungus *Gibberella fujikuroi,* has been reported to exhibit profound growth-regulating properties when applied to plants. Rappaport found a 60-percent increase in fresh weight 10 days after an application of this material to the first expanded leaf of the young tomato plant (*1*). Kahn reported that gibberellic acid replaces the red light required for proper germination of lettuce seed (*2*). Harrington's investigations revealed that gibberellic acid induces flowering in nonvernalized endive plants (*3*).

The multitude of effects that gibberellic acid has induced in a number of plants led to our investigation, in which gibberellic acid was used as a chemical activator for breaking the rest period of the peach. The rest period, as referred to in this report, is a state of dormancy during which a plant will not produce visible growth even though environmental conditions are favorable. In order to overcome or "break" the rest period in peaches, a period of chilling is necessary. According to Weinberger, the chilling