The pure acid was tested on strains of *Escherichia coli* and *Micrococcus pyogenes* by incubating both organisms in antibiotic assay media fortified with serial dilutions of the acid. Comparison with penicillin (*M. pyogenes*) and chlortetracyclne (*E. coli*) indicated that this fatty acid was less than one-fourth as active as penicillin and less than one-fifth as active as chlortetracycline. In this procedure, the activity cannot be attributed solely to any change in *p*H produced by incorporating the acid into the medium, because each serial dilution was adjusted to *p*H 6.7 after the substance was added.

When 10-hydroxy-Δ^2-decenoic acid was evaluated by the paper disc method, neither of these organisms was able to grow in the presence of an impregnated disc during a 14-day period. However, when the salt was tested by the same procedure, the activity was considerably less than that of the free acid. Conversion of the acid to the salt may explain why royal jelly loses its bactericidal activity when neutralized or made slightly alkaline (*9*).

10-Hydroxy-Δ^2-decenoic acid also inhibited the growth of *Neurospora sitophila* as well as several unidentified molds. The antibiotic activity of this acid merits attention because it is active against both bacteria and fungi.

Townsend *et al.* (*10*) reported that admixture of royal jelly with tumor cells before inoculation completely suppresses the development of a transplantable mouse leukemia and the formation of ascitic tumors in mice. Fractionation showed this antitumor activity arises from the main fatty acid of royal jelly: 10-hydroxy-Δ^2-decenoic acid.

This growth inhibiting fatty acid is related to another natural product, the wound hormone of plants, traumatic acid (Δ^1-decene-1, 10-dicarboxylic acid) (*11*). Growth-inhibiting properties have also been demonstrated for other fatty acids (*12*), particularly for undecylenic acid against bacteria (*13*) and fungi (*14*).

It is not surprising that royal jelly contains a substance inhibiting the growth of microorganisms, since it would otherwise be an excellent substrate for bacterial growth. The origin of this fatty acid in royal jelly is now being investigated.

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Inhibition of O-Methyltransferase by Catechol and Sensitization to Epinephrine

Abstract. Catechol at equimolecular concentration inhibits the inactivation of adrenaline by O-methyltransferase in vitro about 50 percent. The inhibition is probably responsible for the sensitization of smooth muscles to epinephrine by various ortho di- or triphenols.

One of us (Z.M.B.) described in 1936 a remarkable potentiation of the effects of epinephrine on various smooth muscles of the cat (nictitating membrane, spleen, uterus, blood vessels) after injection of catechol or pyrogallol, but not of resorcinol (*1*).

The interpretation, at that time, was that the sensitizing phenols slowed down the oxidation of epinephrine by virtue of their well-known antioxidant action.

A new interpretation becomes highly probable after the demonstration by Axelrod *et al.* that epinephrine is inactivated by an O-methyltransferase which methylates the phenolic OH in position 3 (*2*). It seems that epinephrine is not a specific substrate, that other ortho-diphenols are also methylated by this enzyme. Thus, logically, catechol might sensitize in vivo by competitive inhibition of the methyltransferase.

The following simple test strongly suggests that such an inhibition occurs in vitro. In the reaction studied by Axelrod, the methyl donor is S-adenosylmethionine—that is, methionine activated by adenosine triphosphate (ATP). We have prepared a solution of enzymes containing the methyltransferase and the methionine-activating enzyme by precipitating a particle-free supernatant of rat liver homogenate (1 hour at 105,000 *g*) with ammonium sulfate (90 percent saturation) and dissolving the precipitate in $KHCO_3$. This solution, in the presence of a sufficiently high concentration of methionine and ATP, inactivates epinephrine.

The conditions are as follows: 0.6 to 0.4 μmole of adrenaline is incubated at 37°C for 45 minutes with 1 ml of the enzyme solution, in the presence of $MgCl_2$ (10 μmole/ml) in phosphate buffer (*p*H 7.6) (100 μmole/ml). The final volume is 2 ml. The quantities of ATP and methionine necessary to inactivate 70 percent of the epinephrine are about 10 μmole each; greater quantities do not increase the percentage of inactivation and interfere with biological titration. After incubation, proteins are precipitated by trichloracetic acid (5 percent) and centrifuged. Epinephrine is titrated by fluorescence (*3*) and by its action on the blood pressure and nictitating membrane in the cocainized cat.

During incubation, part of the epinephrine is inactivated, probably by auto-oxidation. If one adds an equimolecular amount of catechol to the system, the percentage of enzymatic inac-

Table 1. Influence of catechol on the inactivation of epinephrine by incubation with soluble enzymes of rat liver in the presence of ATP and methionine.

Experimental conditions				After incubation		
Tube No.	Epinephrine (μmole)	Catechol (μmole)	ATP and *l*-methionine (μmole)	Fluorescence (%)	Epinephrine (μmole/ml)	Inactivation (%)
1	0.4			61.5	0.08	0
2	0.4		10	18.5	0.024	71
3		0.4		2		
4		0.4	10	4		
5	0.4	0.4		62.5	0.081	0
6	0.4	0.4	10	36	0.047	42
7		0.8		2		
8	0.4	0.8		58	0.075	7.5
9	0.4	0.8	10	53	0.069	15

tivation falls from 71 to 42 percent; with double the concentration of catechol, only 15 percent of the epinephrine is inactivated (Table 1). Neither ATP nor catechol interferes with chemical titration; ATP interferes with biological titration only at high doses. Biological tests have confirmed the results obtained with the fluorescence method.

Many plant phenolics with a catechol nucleus sensitize smooth muscle to epinephrine (*4*); inhibition of methyltransferase is probably the responsible mechanism.

The facts (i) that, in vivo, iproniazid or any inhibitor of amino oxidase does not sensitize to epinephrine or adrenergic nerve stimulation and (ii) that inhibitors of O-methyltransferase do sensitize in vivo are powerful arguments in favor of the opinion that methyltransferase (and not amino oxidase) is the enzyme which normally inactivates the bulk of catecholamines in mammals (see *5*).

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Prolactin, a Factor in Promoting Survival of Hypophysectomized Killifish in Fresh Water

Abstract. The naturally occurring corticosteroids, cortisol and aldosterone, failed to promote survival of hypophysectomized *Fundulus heteroclitus* in fresh water. Extracts of *Fundulus* interrenal tissue, carp corpuscles of Stannius, and hog renin were ineffective. Injection of whole rat-pituitary brei was partially successful. Highly purified prolactin maintained survival, although the recipients did not eat normally. A synergic action of prolactin with some unidentified pituitary hormone is suspected.

Burden (*1*) showed that the hypophysectomized and normally euryhaline killifish, *Fundulus heteroclitus,* is unable to survive in fresh water. Death results from progressive asthenia, correlated with hypochloremia, which becomes severe after 6 to 7 days at 15° to 17°C. Replacement therapy with *Fundulus* pituitary brei was completely successful; perch pituitary was partially effective, but pollack pituitary gave negative results. Mammalian pituitary hormones (TSH, ACTH GH, and posterior lobe powder) were ineffective. Lack of participation of the thyroid was confirmed by administration of thyroxin. More recently, Harris (*2*) found that hypothyroidism, resulting from treatment with I^{131}, had no detrimental effect on the survival or blood chloride levels of this species in fresh water. Burden suggested that a special agent, regulating adaptation to fresh water, was present in the pituitary of teleosts living in this medium.

In Burden's experiments lack of participation of the adrenal, indicated by failure of natural stimulation with exogenous ACTH, was partially confirmed by negative results with DOCA. Recent studies by one of us (*3*) showed that the circulating glucocorticoid of *Fundulus* is cortisol. Moreover, incubation of the head kidney, containing the adrenocortical (interrenal) tissue, with tritiated progesterone results in the synthesis not only of cortisol but also of other steroids, including aldosterone (*4*). An investigation was therefore undertaken to study the possible participation of these natural steroids in the survival of killifish in fresh water.

Hypophysectomized fish, pretested for failure to withstand fresh water, were rescued at the onset of severe symptoms and allowed to recover in salt water. Each experimental group contained four such fish. Hormones or extracts dissolved in 0.6-percent NaCl were administered by intraperitoneal injection on alternate days, at constant volume (0.01 ml/g wt. of fish). The temperature was 15° to 16°C except at the higher dose of cortisol, which was tested at 20°C. It was noted that failure in fresh water is accelerated at the higher temperature. Negative results were obtained with cortisol (2.5 and 0.0025 μg/g wt.), aldosterone (24 and 2.4 μμg/wt.) and extracts of right and left head kidney of *Fundulus* [20 mg (wet wt.)/g wt.] The lower doses of cortisol and aldosterone were considered to be at physiological levels. The right head kidney contains the major part of the adrenal tissue in this species (*5*).

These results, and those of Burden, indicate that the adrenal plays no direct role in regulating survival of *Fundulus* in fresh water. This is in accordance with recent work of Holmes (*6*), who found that in rainbow trout cortical steroids promoted renal salt retention, under a salt load, but that this effect was offset by an enhanced loss of salt through the gills and inhibited re-uptake. Different results have been reported, however, for the goldfish (*7*).

Other hormones that might be concerned with teleostean osmoregulation were considered. The gonadotropins and sex steroids can presumably be excluded, since fish of either sex, juvenile, mature, or in seasonal regression, can move freely from salt to fresh water. On the other hand, the corpuscles of Stannius may play some role in osmoregulation (*8*), although there is no evidence that these glands are regulated by the pituitary (*5*); however, a brei of carp corpuscles of Stannius [1 mg (wet wt.)/g wt.] gave negative results. Renin, said to be present in the kidneys of fresh-water but not in those of marine teleosts (*9*), was tested at two dosages (50 μg/g wt. on alternate days; 5 μg/g wt. daily) without beneficial action.

The possible participation of an unidentified mammalian hypophysial hormone was tested with whole rat-pituitary brei [1 mg (wet wt.)/g wt.]: two fish out of four survived for 20 days but refused to eat, and neither recovered in salt water.

Prolactin, which is known to be present in the teleostean hypophysis (*10*) and which initiates the "water drive" in efts of *Triturus* (*11*), was tested (10 μg/g wt.) with unexpectedly successful results. All four recipients survived for 20 days and remained lively, although they ate little. A combination of prolactin with some other pituitary hormone may be necessary for complete adaptation to fresh water, and investigations of this aspect of the problem are in progress. The synergic action of prolactin is well known in mammalian endocrinology, and its function in potentiating the melanophore-proliferating effect of intermedin in *Fundulus* has been established (*12*). The ineffectiveness of pollack pituitary brei, reported by Burden, may be correlated with the low prolactin content of this material (*10*). Rat pituitary, on the other hand, evidently, contains sufficient prolactin to be partially effective (*13*).

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