Table 1. Data for the brackish water–seawater example (Fig. 2).

Item	Value
Volume of the liquid under pressure	$V = 2000$ cm³
Volume of water charged	$V_a = V_b = 1000$ cm³
Area of membranes	$A_1 = A_2 = 100$ cm²
Original osmotic pressures	$\pi_a(O) = 22.5$ atm; $\pi_o = \pi_b(O) = 2.5$ atm
Compressibility of the liquid under pressure	$\beta = 48 \times 10^{-6}$/atm
Membrane constant	$K = 6 \times 10^{-6}$ cm³/(cm² sec atm)*

*Corresponds to a rate of production of water by reverse osmosis of 40 liter/(0.1 m²/day) from seawater at an operating pressure of 102 atm (gauge).

that during the first few seconds the water-production rate is negative as water flows back through membrane *2* into the brackish water. Influx of water by normal osmosis (Q_1 and Q_2) quickly raises the pressure in the closed system until the osmotic pressure of the brackish water is overcome. In the example (Fig. 2) hydrostatic pressure rises 2.5 atm, equalling the osmotic pressure of the brackish water, in 20 seconds. At this point, the rate of water production becomes positive and rises to a level controlled by the osmotic pressure of the seawater; meanwhile very little dilution or concentration has occurred in the chambers, and the exponential terms in Eqs. 5 and 6 have fallen to zero. If the osmotic pressures were constants, the pressure and production rate would remain constant at their maximum values.

However, the osmotic pressures are not constants, varying with time. If one assumes the osmotic pressure of seawater solutions to be linearly proportional to the molal concentrations, the osmotic pressures are

$$\pi_o = \pi_o$$

$$\pi_a = \pi_a(t) = \frac{\pi_a(O)}{1 + \frac{Q_1}{V_a}}$$

$$\pi_b = \pi_b(t) = \frac{\pi_b(O)}{1 - \frac{Q_2}{V_b}} \qquad (7)$$

where π_o is assumed to be maintained constant, either by circulation past the membrane or by adequate stirring in a very large volume; $\pi_a(O)$ is the original osmotic pressure of the seawater; and V_a is the volume of water in the original charge of seawater; $\pi_b(O)$ and V_b are defined similarly.

To account vigorously for changes in osmotic pressures, we should substitute Eqs. 7 into Eqs. 2–4. The resultant differential equation is quite complex. A very approximate indication can be obtained by substitution of Eqs. 7 directly into Eqs. 5 and 6, as was done for the dashed portion of the curves in Fig. 2. As the brackish water under pressure becomes concentrated the rate of water production gradually falls to zero. About 75 percent of the water in the original charge of brackish water can be ultimately desalted; this operation requires nearly 2000 hours. However, 50 percent of the water can be recovered in only 62 hours, so that optimization of the cycle time should be possible.

Although the example presented refers to the seawater–brackish water system, it should be understood that the process is applicable to any couple in which a net difference of osmotic pressures exists and where the solute is rejected by the membrane. So, for example, processing effluents of the food or chemical industry may be concentrated to the point where they may be treated with a view to reclamation of valuable constituents. Low concentrations of thermolabile solutes also may be conveniently concentrated. The system tends to show particular merit when one contemplates concentrations of marine waste products such as stick water and whale blood; in such instances the method does with a loss in efficiency what can be achieved directly by either osmotic dehydration (*10*) or reverse osmosis (*8*); the advantage is, however, the safety factor of guarding against rupture of the membrane and contamination in the former instance, and economy of energy in the latter.

For straightforward desalination of seawater it is quite possible to couple this system with a solar-still brine or distillation-blowdown brine and effect additional desalting. Coupling with a solar still may be of substantial benefit, especially in view of the relatively high cost of the still superstructure (*11*), because the capacity of the still will be at least doubled.

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References

1. G. W. Murphy, R. C. Taber, H. H. Steinhauser, *Saline Water Research and Development Progr. Rep. 9*, (U.S. Dept. of Interior, Washington, D.C., 1956).
2. C. B. Ellis, *Fresh Water from the Ocean* (Ronald, New York, 1954); K. S. Spiegler, *Salt Water Purification* (Wiley, New York, 1962); O. Girelli, Ed., *Fresh Water from the Sea* (Pergamon, Oxford, 1965).
3. K. Popper, R. J. Bouthilet, V. Slamecka, *Science* **141**, 3589 (1963); K. Popper and V. Slamecka, U.S. patents 3,073,725 (1963) and 3,239,460 (1966).
4. G. W. Murphy, *Ind. Eng. Chem.* **50**(8), 1181 (1958).
5. D. Farrington, *Direct Use of the Sun's Energy* (Yale University Press, 1964), pp. 167–195.
6. G. C. Kennedy, "Geothermal energy, nuclear energy and sea water conversion," presentation, Univ. of California, Berkeley, 1965.
7. Reid and Breton (1959).
8. J. S. Johnson, L. Dresner, K. A. Kraus, in *Principles of Desalination*, K. S. Spiegler, Ed. (Academic Press, New York, 1966), p. 345 *et seq.*; A. I. Morgan, Jr., E. Lowe, R. L. Merson, E. L. Durkee, *Food Technol.* **19**, 1790 (1965).
9. U. Merten, *Ind. Eng. Chem. Fundamentals* **2**, 229 (1963).
10. K. Popper, W. M. Camirand, F. S. Nury, W. L. Stanley, *Food Eng.* **38**(4), 102 (1966).
11. A. Melamed, Tahal, Water Planning Board for Israel, personal communication, 1966.

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Ribonucleic Acid Control of Steroid Synthesis in Human Adrenals and Testes

Abstract. *The pattern of steroid synthesis in human fetal testes and adrenals was altered by prior exposure, in organ culture of the explants, of one gland to the ribonucleic acid extracted from the other gland. The new pattern reflected the origin of the RNA.*

Preparations of ribonucleic acid from adrenals of human fetuses altered the pattern of steroid synthesis in explants of testes of the same fetus in organ culture. The new pattern resembled that of human fetal adrenals. The reverse phenomenon could be demonstrated with explants from human fetal adrenals and with ribonucleic acid from mouse testis.

Ribonucleic acid was extracted from adrenals of human fetuses (*1*). Testes from three human fetuses were maintained in organ culture for 24 hours in a chemically defined medium (Parkers 1066). Control tissues were cultured in medium alone. Experimental tissues were cultured in medium that contained preparations of ribonucleic acid from adrenals of the same fetus. After 24 hours in organ culture in 95 percent oxygen and 5 percent carbon dioxide at 26°C, the explants were removed and

homogenized in phosphate buffer at *p*H 7.4. Pregnenolone-7α-^{3}H (0.33 μc per milligram of testis), nicotinamide-adenine dinucleotide, nicotinamide-adenine dinucleotide phosphate, and glucose-6-phosphate were added in excess to the incubation vessel. After the vessels were incubated at 37°C for 1 hour (with constant shaking), carrier steroids were added; the mixture was then extracted exhaustively with twice-distilled methanol. The extract was dried in a vacuum, and the steroid products in the residue were separated by column partition chromatography on Celite (*2*). Radiochemical homogeneity of the radioactive products and carrier steroids was achieved by their crystallization, subsequent formation of a derivative, and finally crystallization of the derivative to constant specific activity.

Table 1. Radioactive steroid products, expressed as the percentage of the total radioactivity extracted from the incubation medium, isolated from cell-free incubations of human fetal testes previously cultured in Parkers 1066 medium alone or in medium containing RNA extracted from the adrenals of the same fetus. The substrate was pregnenolone-7α-^{3}H (0.33 μc per milligram of testis). P, progesterone; D, dehydroepiandrosterone; A, androstenedione; T, testosterone; C, corticosterone.

RNA in medium (μg/4 ml)	Testis explants (mg)	Radioactivity					
		Steroids (%)					Total (count/min)*
		P	D	A	T	C	
			9.5-cm fetus				
None	16.5	0.9	5.7	22.4	6.4	0.8	4220
190	18.3	1.1	13.6	26.6	3.8	1.3	4220
			11-cm fetus				
None	13.0	2.8	5.6	11.4	21.1	0	3200
720	13.5	3.0	14.9	14.2	9.5	0.3	3620
			12-cm fetus				
None	15.3	0.8	4.0	12.4	5.1	0	3600
614	10.6	1.2	10.0	7.5	3.8	0.3	3220

* Radioactivity extracted from incubation medium $\times$ 10^{-3}.

Table 2. Radioactive steroid products, expressed as counts per minute per milligram of adrenal, isolated from cell-free incubations of human fetal adrenals previously cultured without or with RNA extracted from mouse testis. The substrate was pregnenolone-7α-^{3}H (0.10 to 0.36 μc per milligram of adrenal). D, dehydroepiandrosterone; 17OH, 17-hydroxyprogesterone; C, corticosterone; CL, cortisol; A, androstenedione; T, testosterone; 4-3, total Δ 4-3 ketones. Increase in Δ 4-3 ketones (%) with RNA in medium was: 14 (for 4.8-cm fetus); 42 (for 5.8-cm fetus); and 69 (for 15-cm fetus). Increase in androstenedione plus testosterone (%) with RNA in medium was: 49 (for 4.8-cm fetus); 30 (for 5.8-cm fetus); and 51 (for 15-cm fetus).

RNA in medium (μg/4 ml)	Adrenal explants (mg)	Radioactive steroids (count/min)						
		D	17OH	C	CL	A	T	4-3
				4.8-cm fetus				
None	14.5	129,600	3,450			3,030	551	7,040
440	12.0	Sample lost	2,690			4,940	395	8,030
				5.8-cm fetus				
None	14.0	132,400	3,540	183		2,400	3,930	10,000
440	15.0	64,500	4,800	1250		2,290	5,960	14,300
				15-cm fetus				
None	99.0	43,500	0		144	1,320	245	1,710
680	131.5	27,500	101		512	1,890	480	2,880

Table 3. Radioactive steroid products, expressed as counts per minute per milligram of adrenal, isolated from cell-free incubations of human fetal adrenals previously cultured without or with RNA extracted from mouse testis. The substrate was progesterone-4-^{14}C (0.025 to 0.70 μc per milligram of adrenal). 17OH, 17-hydroxyprogesterone; C, corticosterone; CL, cortisol; 16OH, 16-hydroxyprogesterone; A, androstenedione; T, testosterone. Increase in testosterone (%) with RNA in medium was: 210 (for 4.8-cm fetus); 216 (for 5.8-cm fetus); and 264 (for 15-cm fetus).

RNA in medium (μg/4 ml)	Adrenal explants (mg)	Radioactive steroids (count/min)					
		17OH	C	CL	16OH	A	T
			4.8-cm fetus				
None	14.5	35,900				5,480	429
440	12.0	46,300				12,800	1,300
			5.8-cm fetus				
None	14.0	49,200	796		23,160	9,140	2,130
440	15.0	37,200	803		16,400	8,590	6,720
			15-cm fetus				
None	99.0	3,780		6,380		3,590	94
680	131.5	550		6,540		2,760	342

The major product of the metabolism of pregnenolone in the adrenal of the human fetus is dehydroepiandrosterone (*3*). Explants of human fetal testes previously exposed in organ culture to RNA from the fetal adrenal subsequently formed approximately two and one-half times as much dehydroepiandrosterone-^{3}H from pregnenolone-7α-^{3}H as the control testes did (Table 1). Exposure to adrenal RNA also produced a marked decrease in the amount of tritiated testosterone that could be isolated from the incubation mixture. Usually the testis produced little or no corticosterone; however, exposure to adrenal RNA resulted in an enhanced conversion of pregnenolone-7α-^{3}H to corticosterone-^{3}H in the testis. Thus, the fetal testis exposed to fetal adrenal RNA shifted to a pattern of steroid synthesis which resembled that of the fetal adrenal.

Comparable experiments were performed with explants of human fetal adrenals and RNA extracted from mouse testis. The lack of sufficient tissue precluded the use of human fetal testes as the source of RNA. Adrenal explants from a fetus 15 cm in length (crown to rump) and from two very early fetuses were exposed for 24 hours in organ culture to RNA (110 to 170 μg/ml) from the testes of adult mice. The explants were subsequently incubated in a cell-free system with both pregnenolone-7α-^{3}H and progesterone-4-^{14}C as substrates. The radioactive steroid products were isolated by reverse isotope dilution. The adrenal of the young fetus forms little or no cortisol or corticosterone from progesterone, whereas the older fetal adrenal (after gestation for about 8 weeks) readily forms them. The early fetal adrenal tends to accumulate 17-hydroxyprogesterone and 16α-hydroxyprogesterone as products of progesterone. Fetal adrenals at all stages of gestation have a relative lack of 3β-hydroxysteroid dehydrogenase and therefore can form only small amounts of the Δ 4-3 ketone steroid hormones.

From a study of the products of pregnenolone-7α-^{3}H (Table 2), it appears that exposure to RNA from mouse testis increases the activity of the 3β-

hydroxysteroid dehydrogenases (E.C. 1.1.1.51) in fetal adrenal explants. Less tritiated dehydroepiandrosterone accumulated in two of the experimental vessels, and in all vessels more of the Δ 4-3 ketone steroids were formed. Adrenal explants exposed to testis RNA also formed more androgen (androstenedione plus testosterone) than control explants did. This effect may be due in part to the increased activity of the 3β-hydroxysteroid dehydrogenase; however, examination of the products of progesterone-4-^{14}C (Table 3) reveals that synthesis of testosterone-^{14}C was increased in all three vessels. There was no effect of testis RNA on the synthesis of glucocorticoids (corticosterone and cortisol) from progesterone. Thus, in these experiments adrenal explants from human fetuses shifted to a pattern of steroid synthesis that more closely resembled the source of the RNA to which they had been exposed.

We have already shown (*1*) that ovaries and adrenals of rats and mice are susceptible to the influence of ribonucleic acid introduced into the organ-culture medium of the explants. We have extended these observations to the testis and adrenal of the human fetus. Our experiments with two isotopically labeled substrates suggest that the influence of RNA is on several enzymes and not merely on a single one.

These results must be interpreted with caution because the contamination of the RNA with a small amount of protein cannot be ruled out. Experiments with hydrolyzed RNA [20 μg of ribonuclease (from Worthington) per milligram of RNA] and with RNA extracted from kidney suggest that intact RNA from a steroid-producing gland is required for the biologic effects previously noted. However, our experiments do not provide conclusive evidence of template activity of the RNA added to the culture medium. Both testes and adrenals can synthesize most of the steroid hormones; therefore, a shift in pattern of steroid metabolism may be mediated by influences which may not necessarily involve the function of an RNA template.

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References and Notes

1. D. B. Villee, *Science* **158**, 652 (1967).
2. ———, *Advances Enzyme Regulat.* **4**, 269 (1966).
3. ——— and S. G. Driscoll, *Endocrinology* **77**, 602 (1965).
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Tetraethylammonium Chloride as an Antidote for Certain Insecticides in Mice

Abstract. *In tests on mice, tetraethylammonium chloride (TEAC) was superior to atropine sulfate as an antidote for some carbamate insecticides and nicotine; it did not produce the traumatic and sometimes fatal reactions caused by atropine sulfate, although injections of the two antidotes provided equal protection against lethal oral doses of the carbamates Zectran, NIA-10242, and Lannate. The effects of TEAC were not evaluated against the carbamates Sevin and Baygon because acute oral toxicity values could not be determined. Results with Matacil were inconclusive. Tetraethylammonium chloride was not an effective antidote against the organophosphate Parathion, but its use fully eliminates the effects of nicotine intoxication.*

The carbamate insecticides Sevin (*1*) (1-naphthyl methylcarbamate) and Zectran (*2*) (4-dimethylamino-3,5-xylyl methylcarbamate) inhibit a chlorinesterase in the blood serum of mice (*3*). We tried to determine the value of tetraethylammonium chloride (TEAC) as an antidote for mice treated with carbamates.

Pellets of pesticides were made with a hydraulic press, and lethal doses were fed orally to 9-week-old female Swiss-strain mice weighing 25 g. Ten minutes after such feeding, TEAC at 20 mg/kg was injected intraperitoneally, intravenously, or intramuscularly. The antidotal effects of TEAC were ascertained after intravenous injection. Ten mice were used in each series of tests, and the surviving animals were killed and autopsied 30 days after treatment.

Within 10 to 30 minutes of receiving a lethal dose of Zectran [LD_{50} (lethal dose, 50 percent effective), 30 to 50 mg/kg], mice exhibited characteristic response: tachypnea, fasciculation of the back, excessive salivation, acute conjunctivitis, gasping, convulsions, and death. Although the survival rates of Zectran mice subsequently treated with either TEAC or atropine sulfate were comparable, those treated with atropine showed symptoms attributable to either atropine or Zectran. However, mice subsequently treated with TEAC not only were free from the Zectran syndrome, but were quiet and relaxed.

The control mice treated with TEAC only were quiet and relaxed for about 1 hour after injection and then resumed normal activity. The control mice treated with atropine sulfate alone were irritable and hyperactive, showing an accelerated respiration rate for at least 4 hours after treatment. These effects made it difficult to establish a satisfactory antidotal dosage. The dosage of atropine sulfate was 8 or 80 mg/kg orally; 8 or 80 mg/kg intramuscularly; or 4, 8, 40, or 80 mg/kg intraperitoneally. Whether TEAC was injected intramuscularly or intraperitoneally, there was no apparent difference in its effect as an antidote on mice fed Zectran at 80, 160, or 320 mg/kg.

Sodium barbital alone gave no protection against a lethal oral dose of Zectran, nor did it enhance or antagonize the effect of TEAC. Tetramethylammonium hydroxide, betaine hydrochloride, hyoscyamine sulfate, 3,3-dimethyl butyl acetate, ethyl acetoacetate, and isoamyl isovalerate were each ineffective as antidotes after a lethal oral dose of Zectran.

A lethal oral dose of the carbamate insecticide NIA-10242 (*4*) (2-3-dihydro-2,2-dimethyl, 7-benzofuranyl *N*-methylcarbamate) caused in mice symptoms similar to those of Zectran; death occurred within 10 to 15 minutes. Mice fed a lethal dose of NIA-10242 and later treated with TEAC survived; they showed occasional slight tremors and appeared quiet but ill for about 3 hours.

Mice fed a lethal dose of the carbamate Lannate (*5*) [*S*-methyl *N*-(methylcarbamoyloxy)thioacetimidate] showed symptoms resembling those in mice fed Zectran or NIA-10242. Lannate, however, was unlike NIA-10242 in that the antidotal effect of TEAC was immediate: the animals resumed normal activity within 10 minutes of injection.

The antidotal effect of TEAC on mice fed Sevin could not be determined since the mice tolerated an acute oral dosage of 2.0 g/kg. Baygon (*6*) (2-isopropoxyphenyl methylcarbamate) likewise was not a satisfactory test insecti-