Table 1. Antagonism of the potentiating action of reserpine by LSD. Adult male mice were given the various drugs intraperitoneally. In the hexobarbital experiments, reserpine (5 mg/kg) and LSD (10 mg/kg) were given 1 hr before hexobarbital. In the ethanol experiments, reserpine (5 mg/kg) was given 1 hr before and LSD (10 mg/kg) was given in two divided doses 1 hr before and simultaneously with ethanol (4 g/kg in 50-percent solution). The duration of hypnosis is defined as the time from the loss to the return of the righting reflex. Values for duration of hypnosis are means ± standard deviation. Figures in parentheses indicate the number of animals in each series.

Hypnotic	Duration of Hypnosis		
	Hypnotic alone	Hypnotic + Reserpine	Hypnotic + Reserpine + LSD
	(*min*)	(*min*)	(*min*)
Hexobarbital (100 mg/kg)	19 ± 6 (12)	68 ± 14 (7)	32 ± 12 (8)
Hexobarbital (150 mg/kg)	63 ± 11 (15)	140 ± 10 (15)	67 ± 14 (15)
Ethanol	40 ± 9 (9)	all > 300 (9)	54 ± 11 (12)

was found to be antagonized by lysergic acid diethylamide (LSD), a compound that produces profound mental disturbances in man (*4*). This paper describes experiments which indicate that LSD also antagonizes the potentiating action of reserpine on hexobarbital and ethanol and that reserpine induces the release of large amounts of serotonin from body depots.

The sleeping times of mice given reserpine and hexobarbital were compared with those of mice given reserpine, hexobarbital, and LSD. Animals given hexobarbital alone served as controls. It was found that reserpine exerted a marked potentiation on the effects of hexobarbital but that LSD antagonized this potentiating action (Table 1). Similar experiments, using ethanol as the hypnotic, again showed that reserpine exhibited a strong potentiating action that was blocked by LSD (Table 1). No effect on the hypnosis produced by hexobarbital or ethanol was observed when LSD was given alone.

The observed similarities of reserpine and serotonin suggested the possibility that some actions of reserpine might be mediated through the release of serotonin normally present in body depots. To test this possibility, reserpine was administered to dogs and the resultant urinary excretion of 5-hydroxyindoleacetic acid (5HIAA), a major metabolite of serotonin (*5*), was measured.

Eleven animals each received, intraperitoneally, 3 mg of reserpine per kilogram of body weight. Urine was collected over a number of 2-hr periods and 5HIAA was determined by the method of Udenfriend *et al.* (*6*). This method involves extraction of the 5HIAA into ether, reextraction of the material into buffer, *p*H 7, and the formation of a colored derivative by reaction with nitrosonaphthol and nitrous acid. In each animal the rate of excretion of 5HIAA-like material markedly increased following the administration of reserpine, remained elevated for 8 hr or more, and gradually dropped to below the normal value (Fig. 1, typical experiment).

The apparent 5HIAA in urine following the reserpine administration was identified by paper chromatography with two solvent systems as described by Udenfriend *et al.* (*6*). Additional evidence for the identity of the material in urine was provided by comparing the absorption spectra of the chromophores resulting from the reaction between the nitrosonaphthol-nitrous acid reagent and the apparent and authentic 5HIAA. These were found to be identical. Finally, the distributions of apparent and authentic 5HIAA between ether and water at various *p*H values were compared according to the procedure of Brodie and Udenfriend (*7*). Both compounds were found to have the same partition ratios.

The excretion of 5HIAA in three of the dogs was determined after a second dose of reserpine was administered the next day (Fig. 1). An increase in 5HIAA excretion did not reoccur. This suggests that the first dose of reserpine had depleted the serotonin depots in the body and that they had not yet been replenished.

Serotonin and reserpine exert a common central potentiating action that is antagonized by LSD. This suggests that certain actions of reserpine may be mediated through the liberation of serotonin. In accord with this hypothesis, there is a marked increase in the urinary excretion of 5-hydroxyindoleacetic acid in dogs following administration of reserpine.

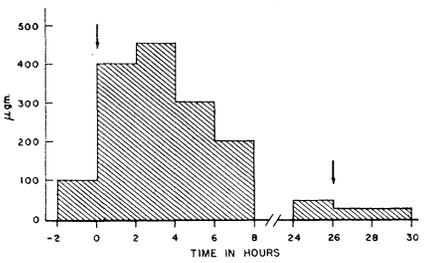

Fig. 1. Effect of reserpine on urinary excretion of 5-hydroxyindoleacetic acid (5HIAA) in the dog. Arrows depict times at which reserpine, 3 mg/kg, was injected intraperitoneally.

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8 April 1955

Osmotic Pressure

In his recent communication J. H. Hildebrand (*1*) has given an interesting account of the role played by osmotic pressure in the theory of solutions. He has omitted, however, to point out the dual nature of the common conception of osmotic pressure; for that reason it is perhaps worth while to expand his explanation with some additional remarks.

Hildebrand uses the term *osmotic pressure* (*1*, p. 117) in the sense of the "... pressure of the solute against a membrane permeable only to the solvent." The pressure of the solute arises from the thermal motions of the solute molecules and, as originally pointed out by van't Hoff, is analogous to the pressure of a gas. In this sense, the osmotic pressure is a measure of the tendency of the solute to expand.

The second picture of osmotic pressure arises from the classical osmotic experiment in which a solution is separated from pure solvent by a semipermeable membrane. From this experiment, the osmotic pressure is defined as the hydrostatic pressure that must be applied to the solution in order to stop the flow of solvent through the membrane. This is the original and, as I shall demonstrate, the exact definition of osmotic pressure.

It has been customary to assume that the pressure of the solute against the membrane is intimately related to the hydrostatic pressure of the classical experiment. In fact, it has often been implied that, in some obscure way, the solute pressure is the cause of osmosis. Haldane (*2*), in 1928, pointed out that it was illogical to assume that the pres-

sure of solute molecules could be responsible for the flow of solvent molecules through a membrane. Nevertheless, this view has persisted and undoubtedly has contributed to the confusion which, according to Hildebrand, still exists. Current ideas on diffusion through membranes (*3, 4*) give a simple and satisfying picture of the classical osmotic experiment; I hope, by a slight expansion of Hildebrand's explanation, to show how the hydrostatic pressure of the osmotic experiment is related to the pressure exerted by the solute molecules.

Osmotic pressure as the hydrostatic pressure required to prevent diffusion of solvent molecules through a membrane. It was suggested some time ago—see, for instance, Glasstone (*5*) or Guggenheim (*6*)—that the selective action of a semipermeable membrane arose from the fact that the membrane was permeable to those molecules that dissolve in it and impermeable to those that do not. This hypothesis assumes that the molecules of the solvent do not diffuse through the membrane as free molecules in pores within the membrane but as molecules attached in some way to the solid. The exact relationship between solvent molecules and the membrane is immaterial for an understanding of osmosis, and it is sufficient to record that the movement of the solvent molecules takes place by some sort of activated diffusion. If this diffusion is through the solid structure of the membrane, the relationship between solvent and membrane can be considered as a solution; if the diffusion is along internal surfaces, then the relationship between solvent and membrane can be considered as an adsorption.

Whether the relationship between solvent molecules and membrane is a solution or an adsorption, it is obvious that at the external surfaces of the membrane the interaction can be most accurately described as an adsorption (*3, 4*); it is the interaction at the surfaces that is important for osmosis. At one surface we have an adsorption of molecules from the solvent and at the other a desorption into the solution. The molecules of the solvent will continue to be adsorbed as long as the activity (or the vapor pressure) of the solvent is greater than that of the adsorbed molecules. Similarly, at the surface in contact with the solution, the molecules will desorb as long as the activity of the adsorbed molecules is greater than that of the solvent molecules in the solution. The transfer of solvent will stop only when the activity of the solvent molecules is the same throughout the whole system. In the typical osmotic experiment the equalization of activities arises through the effect of hydrostatic pressure; the hydrostatic pressure increases until it is just sufficient to raise the activity of the solvent molecules in the solution to that value which they possess in the pure solvent. At that point there is no further transfer of solvent and the system is in osmotic equilibrium.

On the basis of this picture it is easy to obtain an expresison for the osmotic pressure. From thermodynamics,

$$\frac{\partial \log \lambda_1}{\partial P} = \frac{V_1}{RT}, \qquad (1)$$

where λ_1 is the activity of the solvent molecules in the solution, P the hydrostatic pressure and V_1 the partial molar volume of the solvent. By integration we find that the osmotic pressure Π is given by

$$\Pi\, V_1 = -RT \log \frac{\lambda_1}{\lambda_1^0} = -RT \log \frac{f_1}{f_1^0}, \qquad (2)$$

where λ_1^0 is the activity of pure solvent and f_1 and f_1^0 are the fugacities of solvent molecules in solution and in pure solvent respectively. Equation 2 is the same as Eq. 1 of Hildebrand.

Osmotic pressure as the pressure of solute molecules against a membrane. We see, from the previous discussion, that there is a simple and satisfying explanation for the build-up of pressure in a solution separated from pure solvent by a semipermeable membrane. The problem is to relate this picture to the concept of osmotic pressure advanced by Hildebrand.

Following Hildebrand, we write van't Hoff's law in the form

$$P_2 = c_2 RT, \qquad (3)$$

where we have written P_2 instead of Π so that we may distinguish the solute pressure from the hydrostatic pressure described in the first part of this communication.

Hildebrand has shown how, with the help of Raoult's law, Eq. 3 can be derived from Eq. 2. In order, however, to demonstrate the relationship that exists between the solute pressure P_2 and the osmotic pressure Π we shall use a slightly different approach. By definition (*6*) we know that

$$\mu = RT \log \lambda, \qquad (4)$$

where μ is the chemical potential and λ is the absolute activity. Since

$$\left(\frac{\partial \mu}{\partial p}\right)_T = V, \qquad (5)$$

we have for the pressure P the relationship

$$P = \frac{RT}{V} \log \lambda. \qquad (6)$$

The pressure exerted by the solute molecules in a solution should accordingly be given by the expression

$$P_2 = \frac{RT}{V_2} \log \lambda_2. \qquad (7)$$

Equation 7 is the relationship between the pressure P_2 of the solute and the activity λ_2 of the solute molecules. For comparison with Eq. 2, P_2 must be expressed in terms of the activity of the solvent molecules. To do this it is only necessary to remember that the role played in solutions by the pressure P is analogous to that played by the partial pressure in a mixture of gases. Thus, we have, in an ideal binary solution, the relationship

$$P_1 + P_2 = P^0 = P_1^0 = P_2^0 \qquad (8)$$

where P^0 is the total pressure exerted by all the molecules in the solution and P_1^0 and P_2^0 are the pressures that would be exerted by the pure solvent and pure solute, respectively. We find, accordingly, that

$$P_2 = P_1^0 - P_1. \qquad (9)$$

Since

$$P_1^0 = \frac{RT}{V_1^0} \log \lambda_1^0$$

and

$$P_1 = \frac{RT}{V_1} \log \lambda_1,$$

we can obtain for P_2 the expression

$$P_2 = \frac{RT}{V_1} \log \frac{\lambda_1^0}{\lambda_1} \qquad (10)$$

if we assume that the molar volume of the solvent in the solution is identical with V_1^0, the molar volume of pure solvent. By comparing Eq. 10 with Eq. 2 we see that the pressure P_2 is equal to the osmotic pressure Π.

It is clear from this analysis, however, that P_2 and Π are in fact two separate pressures, and it remains to show how it is that they have the same value. P_2 is the pressure exerted by the solute molecules at the beginning of the osmotic experiment, that is, when the hydrostatic pressure on the solution is atmospheric. At the end of the osmotic experiment, when the hydrostatic pressure is Π, the pressure P of the solute molecules would be increased according to Eq. 1.

From Eq. 9 we see that P_2 is the amount by which the pressure P_1 of the solvent molecules in the solution must be increased in order that it will be equal to the pressure of the molecules in the pure solvent. P_2 is equal to the pressure deficit of the solvent molecules, and since in the osmotic experiment this pressure deficit is eliminated by application of the osmotic pressure Π it becomes obvious why P_2 and Π have the same value. Since they are not the same pressures, however, it is unfortunate that the term osmotic pressure has been indiscriminately applied to both. The pressure P is analogous to the partial pressure of a gas. As pointed out by Hildebrand (*1*, p. 117), in a solution the pressure P ". . . is primarily a consequence of the tendency of two different liquid species, under the

impulse of thermal agitation, to achieve a state of maximum disorder by any available path. . . ." P is, in fact, a measure of the tendency of a component to diffuse (7) and is most aptly described by the term *diffusion pressure.*

One other way of looking at these pressures is enlightening. If, in the preliminary stage of the osmotic experiment, the solvent and the solution were brought into immediate contact by the removal of the semipermeable membrane, then the solute molecules would diffuse into the solvent and the driving force for the diffusion would be the solute pressure P_2. At the same time solvent molecules would diffuse from the solvent to the solution and the force driving this diffusion would be the diffusion pressure deficit of the solvent molecules in the solution, which, as has been demonstrated in the previous paragraphs, is also equal to P_2. With the membrane in place, however, the diffusion of solute molecules is prevented by the resistance of the membrane, and the diffusion of solvent molecules through the membrane takes place by the mechanism outlined in this paper, namely, adsorption at one surface, diffusion through the membrane and desorption at the second surface.

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15 April 1955

Eradication of Screw-Worms through Release of Sterilized Males

Although the sterilizing effect of ionizing radiations has been known for years, it is only recently that entomologists have attempted to take advantage of the phenomenon for insect control. Knipling (*1*) has theorized on the effects of releasing sterilized males among a normal insect population. In 1947, on a visit to the Kerrville, Tex., laboratory, he proposed investigaitons on the mating habits of the screw-worm, *Callitroga hominivorax* (Cqrl.), and experiments with sterilized males. In such experiments Bushland and Hopkins (*2*) found that screw-worms were easily sterilized by exposing pupae to x-rays or gamma rays. They showed that under laboratory conditions male screw-worms mated repeatedly but females only once. If a female mated with a sterilized male it did not mate again and laid eggs that did not hatch. When mixed populations of normal and sterilized insects were observed in cages, the sterilized and normal males competed about equally for mates.

The U.S. Department of Agriculture conducted field tests with sterilized screw-worms in Florida from 1951 to 1953. Laboratory-reared irradiated males were found to mate with females of the normal population and to cause them to lay nonviable eggs. Continued releases of sterilized males greatly reduced the number of insects in nature, but a conclusive eradication experiment could not be made because of lack of a suitably isolated population of normal flies in Florida.

In 1954 an experiment was conducted on the island of Curaçao, which is beyond the flight range of screw-worms from the mainland of South America. On this island screw-worms were abundant and active throughout the year. The experiment was conducted by the Entomology Research Branch, USDA, in cooperation with the Veterinary Service of the Government of the Netherlands Antilles (Baumhover *et al., 3*). The work was conducted by two teams. A. H. Baumhover and W. D. New of the USDA branch, with B. A. Bitter of the Netherlands Antilles Veterinary Service, released sterilized flies on the island and made observations on the effects of the releases. At the branch's Orlando, Fla., laboratory A. J. Graham, D. E. Hopkins, and F. H. Dudley reared and sterilized screw-worms by irradiating 5-day-old pupae with 7500 r of gamma rays from cobalt-60, using the source described by Darden *et al.* (*4*). Immediately after irradiation the pupae were shipped by air freight to Curaçao, where the flies emerged and were distributed by airplane.

The density of the screw-worm population on Curaçao was estimated from the number of egg masses deposited on wounded goats held in pens suitably distributed over the island. In March, prior to the release of sterilized flies, native screw-worms deposited a total of 133 egg masses the first week and 155 the second week at 10 goat pens. All the egg masses were fertile. A weekly collection of 15 egg masses per goat pen represents a high population. This rate of oviposition was seldom equaled in any experiments in Florida.

Sterilized flies were released at the rate of approximately 100 males and 100 females (incapable of oviposition) per square mile each week over the 170-mi² island. Some of the released males mated with native females, for 15 percent of the egg masses deposited by native flies failed to hatch, but the percentage of sterile matings was not sufficient to reduce the population, because screw-worms increased during the release period. In May, after 7 wk of releases, 200 egg masses were collected, and after another week the collection was 240 egg masses.

During the next few weeks a lack of rainfall made conditions less favorable for screw-worms, and the number of egg masses deposited on the goats declined. After 1 July rains were frequent and the weather favored screw-worm increase. From 12 July to 8 Aug. half of the island was treated with sterilized flies at the rate of 100 males per square mile, and the other half at about 400 per square mile each week. The lower release rate caused approximately 31-percent sterility of egg masses, and the higher rate 49 percent.

Investigations up to this time were designed to determine the best method of release and the rate of release that would most likely lead to elimination of the insect. The rate decided upon was approximately 400 males per square mile each week over the entire island, although the number varied because of difficulties in rearing. The egg masses on the wounded goats were counted daily.

The releases during the first 4 wk, which averaged less than 400 per square mile each week, caused about 70-percent sterility. This rate of sterility caused a marked decline in the number of insects in the subsequent generation. This population depression in turn resulted in a higher ratio of sterile to fertile flies during the next few weeks. The percentage of sterile egg masses increased as the total number of egg masses declined.

Only two egg masses were collected after 3 Oct. One nonviable mass was collected on 4 Nov. and another on 11 Nov. The goat pens were maintained through 6 Jan. 1955, in order to be certain of eradication, but no more egg masses were observed. Releases of sterilized flies had been continued without interruption all this time but were discontinued when goat-pen records were stopped.

The U.S. Department of Agriculture personnel came home on 10 Jan., but Bitter continues to seek evidence of screw-worm activity on the island. From 1 Oct. 1954 to 1 July 1955, he had found no cases of screw-worms in the domestic animals on the island.

Under weather conditions prevailing on Curaçao the average time for a complete life-cycle should be about 4 wk. Most of the adult flies released would die of old age in about 3 wk. Because 9 mo have elapsed since the last evidence of normal fly activity, it appears that screw-worms have been eradicated.

The immediate significance of the Curaçao experiment is that it lends sup-